Diseases of Fruits and Vegetables

Diagnosis and Management

Volume I

Kluwer Academic Publishers

Diseases of Fruits and Vegetables
Volume I

Diseases of Fruits and Vegetables

Diagnosis and Management

Volume I

Edited by

S.A.M.H. Naqvi

National Research Centre for Citrus (Indian Council of Agricultural Research), Nagpur, Maharashtra, India

Created in the United States of America

CONTENTS

PREFACE

Among the Horticultural Crops, Fruits and Vegetables (FV) are of primary importance as the key source of essential components in an adequate and balanced human diet. FV have supported largely the daily food requirement of mankind since ages and even before man learned to grow cereal crops systematically. Over the years, growing FV has been the mainstay of rural economy and has emerged as an indispensable part of agriculture world over, offering farmers a wide range of crops in varied topography and climate. In certain parts of the world, FV are the major dietary staple. Apart from being a rich source of vitamins and minerals, this sector also contributes significantly in economy of the region or the nation. The increased income from per unit area of FV is far ahead and can not be compared with that of cereal crops.

A recent survey by the Economist revealed that the world population has increased by 90 % in the past 40 years while food production has increased only by 25 % per head. With an additional 1.5 billion mouth to feed by 2020, farmers worldwide have to produce 39 % more. Looking at the load of the future food requirement, the global increased production of FV during last few years has absorbed the additional food requirement and accordingly the eating habits are also changing and shifting towards more consumption of these commodities worldwide. During 2002, world fruit production excluding melons was recorded 471.377 million metric tons and that of vegetables including melons 772.71 million metric tons and thus a total world production of FV to the tune of 1244.377 million tones has substantially absorbed the additional food requirement needed for the increasing population (FAOSTAT ,2002).

Unlike cereal crops, there is a wide range of diversity available to farmers to select suitable FV crops. However, the cultivation of these crops for optimum yield and quality produce, is highly technical and needs improved technological support. Management of perennial fruit crops requires further close monitoring especially for the management of diseases that can affect production significantly and subsequently the post-harvest life of these highly perishable commodities. In given favourable conditions, even a single pathogen or disease may cause catastrophe and complete failure of the crop. The famous Irish potato famine is a well-known example where single disease could devastate the whole socio-economic fabric of the country and in fact laid a strong foundation of Plant Pathology.

Obviously with area and population to feed, China stands first in production of FV with 68.43 million tones of fruits and 368.57 million tons of vegetables in the world followed by India with 48.57 million tons of fruits and 68.06 million tons of vegetables. India leads the world in production of banana, mango, sapota and acidlime and among vegetables it is the largest producer of pea and cauliflower and second largest in onion, brinjal (egg plant) and cabbage. However, there has been huge gap in production per unit area in developing nations and developed nations though the area under cultivation is far ahead in developing nations. Hence there is an ample scope and potential in increasing production per unit area in developing nations and in certain developed nations. The low productivity and short productive life of fruit plants has been

mainly attributed to the unavailability of disease free planting stocks and among vegetable crops, inadequate plant protection measures and quality seeds. For example, China is the third largest producer of Citrus after Brazil and USA, having maximum harvested area under Citrus (1.42 million ha) with production only 8.45 MT/ha as compared to Citrus productivity 33. 33 MT / ha of USA from 0.441 million ha during 2001-2002 (FAOSTAT, 2002). Like Irish potato famine, the citrus tristeza virus has been highly destructive and this single pathogen could ravage citrus industry of many countries like Argentina, Brazil etc. amounting to billions dollars. Budwood certification programme developed to get rid of these viruses is very effective but still in countries where it has not been adopted fully are facing the problem of low productivity and short productive life of Citrus plants. Thus, effective disease management plays a key role in successful quality production of fruits and vegetables. In favourable environment conditions, the pathogen attack may reduce the productivity significantly and may also become the cause of total crop failure.

There has been a drastic deviation in global weather pattern under El Nino effect. Black Sigatoka and weak to moderate El Nino weather pattern could affect banana production. Rampant eruption of new races of banana pathogens and their rapid resistant development to new fungicides has posed a threat to banana industry in Africa that produces around 30 million tons of bananas yearly, which is mostly consumed locally. But production is already being reduced and may very well cease entirely within ten years, Scientists warn in a report published in British Weekly 'New Scientist' magazine. Because existing banana plants are reproduced from cuttings, there is little genetic diversity. Diseases, in particular fungus, rapidly can wipe out entire production regions.

With the imposition of WTO conditions in export and import of fresh fruits and vegetables, now it has become more relevant to produce disease free quality produce in order to comply strict phytosanitary conditions laid by certain countries. Recent molecular advancement in our knowledge to detect and diagnose the pathogens in commodities even at very low level made it rather mandatory to produce exportable commodities free from the pathogens.

The new millennium promises excitement and hope for the future by new advancement in eco-friendly technologies in integrated disease management of fruits and vegetables. During past twentieth century, Plant Pathology has witnessed a dramatic advancement in management of fruits and vegetable diseases through in-depth investigations of host-pathogen interactions, development of molecular diagnostic tools, integration of new concepts, principles and approaches.

My effort in bringing out this edited book is to update the achievements of twentieth century in diagnosis and management of diseases of fruits and vegetables of international trade and some under-exploited minor fruits which otherwise are widely dispersed in various scientific journals and to develop future strategies for the new millennium. The book includes latest diagnostic tools and management strategies of

almost all the economically important temperate, tropical and subtropical fruits and vegetables at one place which would be easier to refer by the students, research workers, planners, administrators, policy makers and other end users like grower of fruits and vegetables world-wide. The chapters on individual crop on various aspects of diseases like geographical distribution of disease, diagnosis, disease forecast, approaches to eliminate difficult sytemic pathogens, production of disease free planting material and integrated disease management at nursery, orchard and post-harvest level are contributed by leading Plant Pathologists having authority and significant contributions in respective fields at international level.

The diseases of economic importance caused by fungi, bacteria, viruses and virus like organisms, Phytoplasma and nematodes of each crop are covered, describing their history, distribution, losses incurred, symptoms, latest diagnostic tools, epidemiology and integrated applied management approaches including cultural, chemical, genetic resources, use of bio-control agents being adopted world-wide. The layout of each chapter includes a brief abstract, introduction and pathogen-wise description of the diseases. Each chapter is vividly illustrated with photographs of typical symptoms, graphs, tables and line drawings to make the subject more interesting and easy to understand for students, Scientists, planners, administrators, growers and other end users with latest pertinent references.

In volume I, diseases of Apple, Citrus, Grapes, Mango and Pineapple among fruits and Carrot, Celery and Cucurbits among vegetable crops with special reference to integrated diseases management practices have been included. Volume II covers Avocado, Banana, Grapes, Guava, Papaya, Passion fruit, Strawberry, Stone fruits and Minor tropical and subtropical fruits. Among vegetables, Lettuce, Pea, Pepper, Potato, Onion and Garlic have been included in this volume besides role of mycorrhiza and biocontrol agents in disease management. I am sure that these two volumes will be of immense help and use to the fruits and vegetables growers world over, students, re-search workers, planners, administrators, teachers and other end users engaged in diag-nosis and management of fruits and vegetables diseases.

I am grateful and indebted to all the learned galaxy of contributors who have spent their considerable time in contributing the chapters on various internationally important fruits and vegetables crops. I thank them for their cooperation and support during this project.

I dedicate this work to all those great Scientists who have spent their life time in diagnosis and management of diseases of fruits and vegetables world over in order to improve the quality and productivity of fruits and vegetables, to uplift the nutritional status of human diet and fight against hunger. I am thankful to my wife Dr Nikhat Sarwar Naqvi, for her constant encouragement and help in various ways while editing the book.

25th September, 2003 **S.A.M.H. Naqvi**

List of Contributors

1. Arora, J.K.
   Department of Plant Pathology
   Punjab Agricultural University
   Ludhiana – 141 004, INDIA

2. Bhatia, Alka
   University of Florida, IFAS,
   Plant Pathology Department,
   Citrus Research and Education   Cen
   ter, 700 Experiment Station Road,
   Lake Alfred, FL, USA, 33850

3. Da Graça, J.V.
   Texas A and M University,
   Kingsville, Citrus Center, 312 N.
   International Blvd.,
   Weslaco TX 78596, USA
   Tel. 1-956-969 2132
   Fax 1-956-969 0649;
   email j-dagraca@tamu.edu

4. Davis, R. Michael
   Department of Plant Pathology,
   University of California,
   Davis 95616, USA
   Phone 530-752-0303,
   FAX 530-752-1199,
   email rmdavis@ucdavis.edu

5. Korsten, L.
   Department of Microbiology and
   Plant Pathology, University of
   Pretoria, Pretoria, 0002,
   SOUTH AFRICA
   Tel. 27-12-420 3295;
   Fax 27-12-420 4588;
   email lkorsten@fabi.up.ac.za

6. Lee, Richard F.
   University of Florida, CREC
   700 Experiment Station Road
   Lake Alfred, FL 33850, USA
   Phone: 863 956 1151 ext 295
   Fax: 863 956 4631
   e-mail: rfl@lal.ufl.edu

7. McGrath, Margaret Tuttle
   Cornell University, Department of Plant
   Pathology, Long Island Horticultural
   Research and Extension Center, 3059
   Sound Avenue, Riverhead, NY, USA
   Phone: 631-727-3595;
   FAX: 631-727-3611
   e-mail: mtm3@cornell.edu

8. Mohan, C.
   Department of Plant Pathology
   Punjab Agricultural University
   Ludhiana – 141 004, INDIA

9. Mondal, S.N.
   University of Florida, IFAS,
   Plant Pathology Department,
   Citrus Research and Education
   Center, 700 Experiment Station Road,
   Lake Alfred, FL, USA, 33850;

10. Muller, Gerd W.
    Centro de Citricultura Sylvio Moreira,
    Instituto Agronômico,
    13490-970 Cordeirópolis, SP,
    BRAZIL
    Phone (19) 546-1399.
    e-mail: gerd@centrodecitricultura.br
       gwmuller@uem.br

11. Naqvi, S.A.M.H.
National Research Centre for Citrus,
Indian Council of Agricultural
Research, PO Box 464, Amravati
Road, NAGPUR 440 010,
Maharashtra, INDIA
Fax:91-712-2500813
Phone:91-712-2500249; 2500518
E-mail naqvi_ngp@sancharnet.in

12. Peres, N.A.R.
University of Florida, UNIEMP
Project, Instituto Biológico,
São Paulo,
BRAZIL

13. Prakash,Om
Department of Crop Protection,
Central Institute for Subtropical
Horticulture, Rehmankhera,
P.O.- Kakori, Lucknow-227 107,
INDIA

14. Prem Raj
Department of Plant Pathology
Punjab Agricultural University
Ludhiana – 141 004, INDIA

15. Raid, Richard, N.
Everglades Research and Education
Center, University of Florida,
3200 East Palm Beach Road,
Belle Glade, FL 33430, USA.
Fax:561-993-1582
Phone:561-993-1564
e-mail: rnr@mail.ifas.ufl.edu

16. Rezende, Jorge A.M.
Departamento de Entomologia,
Fitopatologia e Zoologia Agrícola,
ESALQ/USP, 13418-900 Piracicaba,
SP, BRAZIL
Phone: (19) 3429-4124.
e-mail: jamrezen@esalq.usp.br

17. Roistacher, C.N.
Department of Plant Pathology,
University of California,
Riverside, 92521,California.USA
e-mail: chester.r@worldnet.att.net
FAX: USA (909) 684 4324
Phone: USA (909) 684 0934

18. Singh, S.J.
Indian Agricultural Research
Institute,Regional Station, Agricul
tural College Estate, Shivajinagar,
Pune 411005, Maharashtra, India
Phone/ Fax:91-20-25537601

19. Skaria, Mani
Texas A&M University, Kingsville
Citrus Center, 312 North International
Blvd., Weslaco, TX 78596, USA
Fax:956/ 969.0649
Phone:956/ 968.2132
e-mail:m-skaria@tamu.edu

20. Thind, T.S.
Department of Plant Pathology
Punjab Agricultural University
Ludhiana – 141 004, India
e-mail : tsthind@pau.edu

21. Timmer, L.W.
University of Florida, IFAS,
Plant Pathology Department,
Citrus Research and Education
Center, 700 Experiment Station Road,
Lake Alfred, FL, USA, 33850;
email: lwt@lal.ufl.edu

22. Turechek, William W.
Cornell University: New York State
Agriculture Experiment Station,
Department of Plant Pathology,
Geneva, NY 14456 USA
Phone: (315) 787-2472
Fax: (315) 787-2389
email: wwt3@cornell.edu

Apple Diseases and their Management

William W. Turechek

Cornell University: New York State Agriculture Experiment Station
Department of Plant Pathology, Geneva, NY 14456 USA

Abstract: In this chapter the world's most important apple diseases are described and common approaches for managing them are reviewed. The chapter does not provide a description of every disease that occurs on apple, a function that is better fulfilled by the APS Compendium of Apple and Pear Diseases (Jones and Aldwinckle 1990). Nor is this a comprehensive literature review on the selected diseases. I have attempted to include information on the geographic distribution of the disease, as well as providing a brief historical aspect for most diseases covered. I have also attempted to include the most recent references and scientific work related to each disease.

The chapter is divided into nine sections. The first section is an introduction to apple production and pest management. Section two covers major diseases caused by fungi, including the ever important apple scab and powdery mildew. The next section covers diseases of the roots and crown with particular emphasis on Phytophthora root, crown, and collar rot. Sections four and five cover canker diseases, such as Nectria canker, and the summer fruit rotting diseases, respectively. The summer fruit rots include diseases such as black rot and white rot which could have been included in sections other than the one chosen; however, their importance as fruit rots takes precedence over their ability to cause leaf spotting or cankers. A small section on minor fungal diseases precedes an important section 7 on bacterial diseases. Here the management of fire blight is covered in detail. Section 8 focuses on the important and difficult-to-manage post harvest diseases such as blue mold. The last section addresses virus and virus-like diseases of apple.

1. Introduction

1.1 Apple production

Apples are members of the rose family (*Rosaceae*) in the genus *Malus*; the domestic apple is *Malus* H *domestica* Borkh. The genetic origin of the apple is still debated but it is likely to have originated from either *M. pumila* Mill., a small-fruited species found in Eastern Europe and southwestern Asia around the Black and Caspian seas or *M. sieversii* (Ledeb.) M. Roem., a larger and sweeter apple found in the mountains of central Asia.

The apple has been cultivated for nearly 2000 years in Europe with records dating back to Greece as early as 325 B.C. (Smock and Neubert 1950). The domestic apple is grown throughout the temperate zones of both the northern and southern hemispheres, *i.e.*, at latitudes north and south of 35 degrees, respectively. The apple was brought to North America as seed by the early settlers of England and dissemi-

1

nated westward by Indians, traders, missionaries, and the legendary Johnny Appleseed (Childers *et al.,* 1995). The Europeans were also responsible for introducing the apple to Australia. The geographical distribution of apple is limited by its chilling requirement of greater than 1000 hours under 5°C and its susceptibility to winter kill at temperatures below –30°C. The primary apple growing countries in the world are the Soviet Union, the United States, China, France, East and West Germany, and Italy each producing over 2 million metric tons of apples annually. Turkey, Korea, Japan, Iran, Poland, Hungary, Argentina, and Spain each produce approximately 1 million metric tons per year. In the United States over 90% of commercial apples are produced in the Northeastern, Central Atlantic, and Pacific Coast States with Washington, New York, Michigan, California, Pennsylvania, and Virginia being the leading producing states. In Canada, most apples are grown in the provinces of British Columbia and Ontario. Apple production in Mexico is primarily confined to the Sierra Madre Mountains in the states of Chihuahua and Cuauhila. In the former USSR, apples are grown in Moldavia, Ukraine, and northern Caucasia. China, a particularly important future producer, grows apples in the Liaoning province in southern Manchuria and in the provinces of Shansi, Shensi, and Kansu. In the Southern Hemisphere, apples are produced in the Neuquen (largest), Mendoza, and Buenos Aires-Sante Fe areas of Argentina. In Chile, most apples are produced north of Santiago and in Peru the limited production is centered around Lima. In Australia, most apples are grown south of Perth and east of Adelaide up to Brisbane, whereas production in Tasmania has decreased due to the cost of oceanic shipping rates. In India, apples are grown in the states of Himachal Pradesh, Jammu and Kashmir, and Uttar Pradesh. Finally, South Africa and Morocco are the primary regions of apple production on the African continent (Childers *et al.,* 1995).

There are thousands of cultivars of apple, yet only a small proportion of these are grown in commercial production. A few of these varieties, such as 'Delicious' and 'Gala', are grown throughout the world, whereas others are grown almost exclusively in a single location. The leading varieties are 'Delicious' and 'Golden Delicious'; these two varieties are grown in nearly every region in the world. A number of other important varieties including 'Granny Smith', 'Jonagold', 'McIntosh', 'Rome', 'Cox's Orange', 'Fuji', 'Jonathan', 'York', 'Morganduft', and 'Gala' are produced on a considerable amount of acreage throughout the world. What makes a variety successful depends on a number of characteristics. Childers *et al.,* (1995) lists as desirable traits: (i). reliable annual bearer, (ii). good to very good in dessert quality, (iii). attractive in appearance, (iv). good pest resistance, (v). productive, and (vi). hardiness. Furthermore, if the fruit stores and processes well, and has good handling qualities the value of the variety increases.

Propagation of apples is primarily through grafting since seedlings are very variable. In the US, most of the oldest orchards are grafted on seedling rootstock. Over the last 40 years, a number of clonal rootstocks have been developed in breeding programs throughout the world and are used extensively in modern-day apple production. To name a few, the East Malling Experiment Station in England developed the Malling series rootstock (*e.g.*, M.9, M.26) and the Malling-Merton rootstocks (*e.g.* , MM.111, MM.26). The New York Agricultural Experiment Station in Geneva, NY has developed the Geneva series of rootstocks (*e.g.*, G.7). These clonal rootstocks impart

characteristics to the tree such as dwarfing, early flowering, resistance to fire blight, crown rot, and the wooly apple aphid.

Apple production systems have changed extensively over the years. High density plantings on dwarfing or semi-dwarfing rootstocks, supported by elaborate trellising systems are replacing widely-spaced seedling rooted trees. High density plantings are 5 to 10 times denser than older plantings; for example, it is not uncommon to find 1000-2000 dwarf trees planted per hectare in many of the leading commercial production regions in Europe and North America. These new plantings, however, require intensive and careful management. Pruning is critical to maintaining productivity and, for some varieties, fruit coloring. Pest management has become increasingly important because the dense tree canopy has created conditions that tend to favor pest development.

1.2 Pest management

There are many strategies to managing pests. Which strategy is chosen depends upon many factors including the marketing objective, (*i.e.*, organic, processing, conventional fresh fruit, or direct marketing), which diseases dominate in the region, consumer preference, and, perhaps most importantly, economics. Managing diseases of tree fruit is not inexpensive. If fungicides are used routinely, then the cost of the fungicides, application equipment, labor, and costs associated with adverse non-target effects (*e.g.*, detrimental effects on predaceous mites) all play important roles in determining which products are used, when they are used, and how often they are used. A grower must be able to justify why he or she is willing to spend over 3 times more for one fungicide over another. As will be discussed below, many of the newer chemistries provide excellent levels of control, have curative or kickback activity, and can be used less frequently than standard protective fungicides. The drawback is that most of these new chemistries are expensive and prone to losing efficacy due to the development of fungicide resistance in the population.

1.2.1 Fungicides

The history of fungicide use on apple centers on the management of apple scab. In the United States, the first experiments on chemical control of apple scab were conducted by Galloway of the United States Department of Agriculture (USDA) beginning in 1886. At this time, Bordeaux mixture (*i.e.*, a 100-8-8 solution of water, copper sulfate and hydrated spray lime) was found to be the most effective fungicide for the control of apple scab and was considered the standard for many years. In 1908, Cordley of Oregon State University reported on the use of lime sulfur for the control of scab and this replaced Bordeaux mixture as the standard in the Pacific Northwest. In the eastern US, the work of Wallace in New York and Scott in the USDA prompted the switch to lime sulfur.

By the mid-1900's more advanced chemistries began to emerge. In the 1940's, the carbamates thiram, ziram, and ferbam, and the ethylenebisdithiocarbamates (EBDC) maneb, mancozeb, and metiram were introduced. These fungicides offered a superior level of protection against apple scab and were less phytotoxic than lime-sulfur or

Bordeaux mixture. In the 1950's captan, captafol, and folpet were another class of fungicides that also offered excellent levels of protection against apple scab. Both classes of fungicides have activity against diseases caused by Oomycetes, Ascomycetes/ Deuteromycetes, but have little activity against powdery mildew. These fungicides are used routinely to this day.

The development of dodine in the 1950's was a major advancement in the management of apple scab because it was the first fungicide to provide after-infection activity. However, its usefulness was short lived because it was also the first fungicide to which apple scab developed resistance. In the late 1960's the benzimidazoles were introduced. This group includes the fungicides benomyl, thiophanate-methyl, and carbendazim. Benomyl was used heavily at first because of widespread resistance to dodine and, unlike dodine, benomyl controlled powdery mildew and many other fungal diseases including fly speck and sooty blotch. Unfortunately, resistance to the benzimidazoles began to develop only 4 years after their introduction. Benomyl is no longer manufactured.

The sterol-inhibiting (SI) fungicides (a.k.a. demethylation-inhibiting (DMI)) are a large class of fungicides that were introduced in 1980's. Fungicides in this group can be further subdivided into the piperazines (*e.g.* triforine), pyrimidines (*e.g.* fenarimol), imidiazoles (*e.g.* triflumizole), and a number of traiazoles (*e.g.* bitertanol, fenbuconazole, myclobutanil, propiconazole, tebuconazole, and triadimefon). Like benomyl and dodine, these too provided post-infection activity of apple scab. Also like benomyl and dodine, there have been documented cases of apple scab resistance to the SI's (Köller *et al.,* 1997). Nonetheless, the SI's also provide excellent activity against powdery mildew, sooty blotch and fly speck but, unlike the benzimidazoles, are effective against rust diseases.

Other important groups of fungicides used to manage diseases of apple (not necessarily apple scab) include the dicarboximides introduced in the early 1970's (*e.g.* iprodione and vinclozolin). The dicarboximides are generally mycelial-growth inhibitors and inhibit germ tube formation. Theses fungicides are used primarily against *Monilinia*, are not systemic and are applied as protectants. The anilino-pyrimidines (cyprodinil, mepanipyridesm, and pyrimethanil) have some activity against apple scab. The phenylpyrroles (fludioxinil) have activity against a broad spectrum of fungi, but it is primarily sold only as a mixture with cyprodinil and is marketed towards apple scab and gray mold on grape and strawberry.

The strobilurins represent the most recent class of significant fungicides for the management of apple diseases. The strobilurins, which include trifloxystrobin and kresoxim-methyl, are a class of fungicides that exhibit efficacy against a broad-spectrum of fungal diseases, possess significant after infection activity, and have a mode of action that is considerably different than the SI fungicides (Bartlett *et al.,* 2002). This combination of traits makes the strobilurins an excellent candidate for use in rotation with the SI fungicides in a resistance management program. Recently, however, there has been disconcerting evidence that the after infection activity of the strobilurins is compromised in SI-resistant populations. This, of course, can have significant impact on the value of these fungicides. If the after infection activity of strobilurin fungicides is lost, then these rather expensive fungicides lose their value for both disease control

and resistance management. Moreover, populations of the apple scab fungus highly resistant to the strobilurins are known to exist; although these are not widespread as of 2003.

There are several comprehensive treatments to fungicide chemistry and their use in disease management. For example, *Fungicides* (Torgeson 1967) and *Antifungal Compounds* (Siegel and Sisler 1977) are both two volume sets that provide detailed information of earlier fungicides. The most current treatment is Modern Selective Fungicides (Lyr 1995). An excellent introduction to modern fungicides and their use in managing plant disease, as well as recent literature review, was recently written by Köller (1999).

2. Major Diseases caused by Fungi

2.1 Apple Scab (Black Spot)

Apple scab is caused by the fungal pathogen *Venturia inaequalis* (Cooke) Wint. (anamorph *Spilocaea pomi* Fr.). Apple scab is the familiar name used throughout North America whereas in England, Australia, and South Africa the disease is commonly known as black spot. The disease was first described by Fries in Sweden in 1819. In the United States it was first described in 1834 by Schweinitz who collected it from cultivated apples in New York and Pennsylvania. The earliest report of the disease in England was by Berkeley in 1855.

Apple scab is the most economically important disease of apple in the world. The disease occurs in all countries where apples are grown, although it has less impact in semi-arid regions. For example, in the United States the disease is much more severe in the northeastern and midwestern states than in the semi-arid production regions in the state of Washington. In non-arid regions, apple scab causes greater losses than any other major disease. It causes yield losses directly from infections of the fruit and pedicel. When foliar infections are severe mid-summer defoliation is possible, weakening the tree and possibly leading to the failure of fruit bud formation (Thakur and Sharma 1999).

2.1.1 Symptoms

The earliest symptoms of the apple scab are usually evident on the underside of emerging cluster leaves. However, symptoms may first develop on the upper side of these leaves in cases where significant infection was delayed. Young lesions are velvety brown to olive green and have feathery, indistinct margins. Lesions expand with time and may coalesce with other leaf lesions (Fig. 1). The number of lesions can vary from very few to several hundred per leaf. Young leaves with significant infection often curl, shrivel, and fall from the tree. However, it is not atypical for infected leaves to remain on the tree for the entire season. The term "sheet scab" refers to the condition when the entire leaf surface is covered with the disease; when this occurs, leaves typically shrivel and fall to the ground. Eventually, fungal growth stops and the lesions develop distinct margins. The infected leaf tissue around lesions often becomes thickened and results in

a bulging of the infected area and a corresponding cupping of the area underneath the leaf lesion. Lesions on the petiole (leaf stem) extend along the length of the petiole and are similar in appearance to those on the leaf. Severe infection of the petiole typically leads to a yellowing of the infected leaf and eventual leaf drop.

On the fruit, young lesions appear similar to those on leaves. Although the entire surface of the fruit is susceptible to infection, lesions often cluster around the calyx end of the fruit (Fig. 2). As lesions get older they become brown and corky and take on a "scabby" appearance. Early infections kill the expanding tissue which often results in deformed fruit. As lesions age, they typically crack and provide sites that may serve as an opening to invasion by secondary pathogens. Infections late in the season are usually not detectable until after harvest when the fruit are in storage. This is referred to as "pin-point scab". The term "storage scab" refers to incipient infections

Figure 1: Apple scab lesions on leaves.

that were too small to see prior to fruit storage or may be the result of infections during storage that occur as a result of sporulation from older scab lesions.

2.1.2 Disease cycle

Many aspects of the disease cycle (Fig. 3) can be attributed to the discoveries of Aderhold in Germany and Clinton in Illinois in the mid to late 1890s. One of their most important discoveries was that the scab fungus overwintered in fallen leaves on the orchard floor. The fungus is also capable of overwintering in dormant apple buds

(Becker *et al.,* 1992) and, in maritime climates, as mycelium in twig lesions; the importance of these methods of overwintering and their contribution to epidemic development has not been well researched. The fungus is heterothallic, meaning that opposite mating types are required for the formation of ascospores. The formation of the pseudothecial initials occurs within four weeks after leaf fall. After a sufficient dormant period, pseudothecia develop and reach maturity with the formation of asci and ascospores. The length of and conditions needed to fulfill dormancy is still not well understood. In a 3 year field study, James and Sutton (1982) found no relationship between temperature, moisture and the length of the dormancy period. However, they found that the dormancy period lasts approximately 45 days, and this was usually met by the first of February in North Carolina, USA. Moreover, they believe that sufficient moisture is required for the maturation of pseudothecia. The optimum temperature for

Figure 2: Symptoms of apple scab on McIntosh fruit

the development of the ascogonium (*i.e.*, the structure bearing the asci and ascospores) is 8-12°C. The optimum temperature for ascospore maturation is 16-18°C.

Primary infection refers to infection resulting from ascospores. Ascospores are released by forcible discharge from the pseudothecia in response to wetting events. Although some ascospores can be detected after only 1 hour of wetting, the number of ascospores released increases dramatically after 2 to 3 hours of continuous wetting (Gilpatrick and Szkolnik 1978, Rossi *et al.,* 2001). The ascospores are disseminated to susceptible host tissue by wind. The first release of ascospores often occurs around budbreak and typically continues over a 5-9 week period (Fig. 4). This occurs because

both the asci and ascospores mature over a period of time allowing for an extended period of ascospore discharge. In seasons, where sufficient moisture and relatively warm temperatures precede budbreak, it is quite possible to have sufficient numbers of mature ascospores available to initiate infection as soon as the first susceptible tissue is exposed, *i.e.*, the underside of cluster leaves that become visible at bud-break. Conversely, in cooler and/or drier springs, pseudothecial maturity may be delayed resulting in an extended period of primary infection events. However, the peak period of ascospore discharge usually occurs between pink and the full-bloom stage.
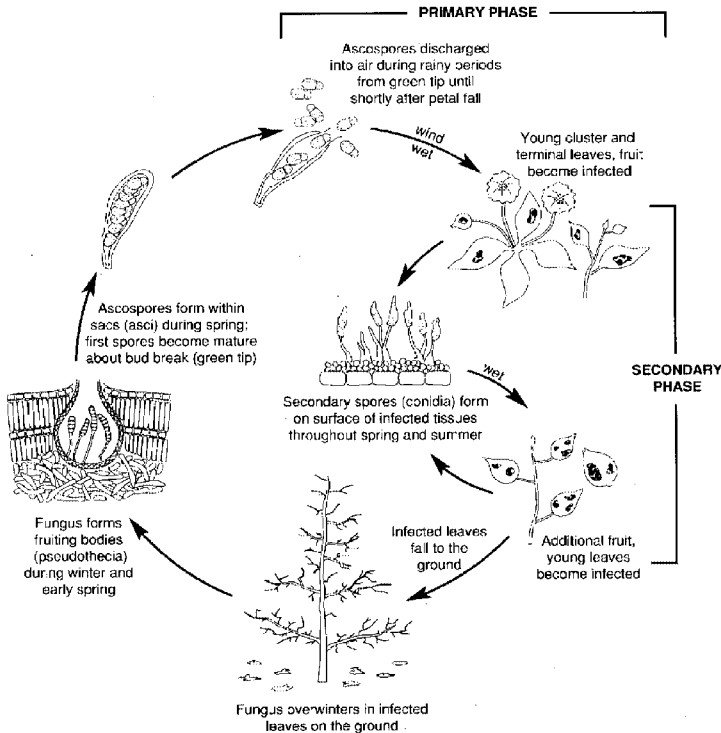

Figure 3: Apple scab disease cycle (Reproduced, with permission, from New York State IPM Fact Sheet Series, Cornell University, Geneva, NY).

Free moisture is required for the initiation of ascospore germination and it is usually present because wetting is also needed to trigger ascospore release. Once initiated, germination will continue as long as the relative humidity is above 95%. It is important to keep in mind that conditions that favor ascospore release and germination do not guarantee infection. Additional factors, such as inoculum availability, the inherent susceptibility of the variety, and particularly the length of the wetting period and temperature at which the wetting occurs have a much greater impact on disease severity than release and germination alone. Some of the earliest work demonstrating the

importance of the relationship between the wetting period and temperature was done by Keitt and Jones in 1926. Mills (1944), however, put these two factors together to develop the well-know Mills curves. Despite the success and wide use of the Mills curves a few refinements to the original curves have been necessary due to inconsistencies that were observed by various researchers (MacHardy 1996, MacHardy and Gadoury 1989, Stensvand *et al.,* 1997). For example, one factor that Mills did not take into account was that ascospore release occurs almost exclusively during daylight hours (MacHardy and Gadoury 1989). Taking this into account has allowed for a 3 hour reduction from Mills' original predictions. However, in an orchard study Rossi *et al.,* (2001) was able to trap ascospores at night when: (i). the cumulative proportion of ascospores already trapped represented 80% of the seasonal total; and (ii). greater than one-third of the total seasons ascospores was mature and were ready to be discharged.
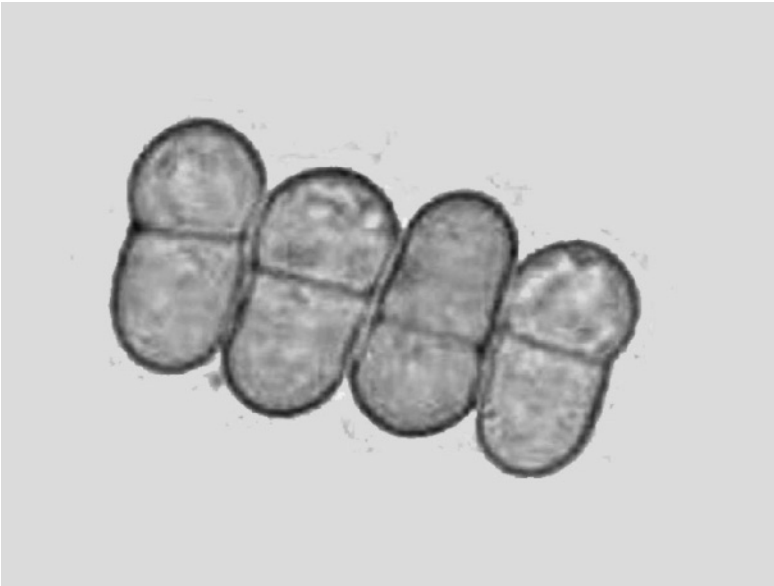

Figure 4: Mature ascospores of *Venturia inaequalis*

Once infection has occurred the fungus produces conidiophores and conidia. Sporulating lesions become visible approximately 9-17 days after infection depending upon the temperature and relative humidity. The minimum relative humidity needed for sporulation to occur is between 60-70%. Upon sporulation, conidia are dispersed to susceptible leaf and fruit tissue by wind and splashing rain. Conditions necessary for infection by the conidia are identical to those for ascospore infection (Stensvand *et al.,* 1997). However, when conidia are present, they are usually present in much higher numbers than are ascospores and therefore conidia may cause more severe infections during short wetting periods than would occur with ascospores. Several infection cycles can occur throughout the course of the season depending upon host susceptibility

and weather conditions. Under favorable conditions, these secondary cycles can cause tremendous losses and provide a substantial amount of overwintering inoculum.

2.1.3 Epidemiology

2.1.3.1 Disease Forecasting

The Mills curves, published in 1944, were the first attempt at using forecasting to help growers time the application of sulfur dusts for apple scab management (Mills 1944). The Mills curves relate the hours of leaf wetting and temperature during a wetting period to the likelihood of scab infection (Sutton 1996). The first fungicide spray is applied during the first predicted infection event and subsequent applications are applied relative to the residual activity of the pesticide and other predicted infection events. The Mills curves have been modified over the years as more was learned about the disease, but the overall premise behind the use of these curves remains the same. Jones' modification of the curves is referred to as the 'modified' Mills (Table 1) and MacHardy and Gadoury's modification are referred to as the 'revised' Mills curves.

    A number of automated or electronic apple scab predictors have been developed over the past 20 years. Most of these function by recording temperature, rainfall, and/or relative humidity to predict the occurrence of apple scab under the rules of the Mills table and, to varying degrees, the models discussed below. The most popular include a system developed by Jones *et al.,* (1980), Ventem (Butt *et al.,* 1992), ADEM (Butt and Xu 1996), Spraycheck (Stewart *et al.,* 1998), and most recently RIMpro (Trapman and Polfliet 1997) to name a few.

    One important aspect that is typically neglected when predicting infection events based on temperature and leaf wetness is the impact of discontinuous wetting on the survival of ascospores and conidia. Becker and Burr (1994) conducted the most detailed study to date to answer this question. In their approach, they asked if apple scab conidia could cause disease after exposure to various wet-dry-wet intervals at 10, 15, 20, or 25°C. Three initial wet intervals were tested, either: (i) 15 min., (ii) the time at each temperature required for ~50% of conidia to germinate, which turned out to be 7, 5, 4, and 5 hours at 10, 15, 20, and 25°C, respectively, or (iii) the time at each temperature required for ~20% of the conidia to also form an appresorium (*i.e.*, 20% of the spores penetrated the host) which was 12, 8, 7, and 8 hours at 10, 15, 20, and 25°C, respectively. After exposure to the initial wet interval, plants were exposed to 0, 0.25, 6, 12, 24, or 96 hours of drying at either 60% (low) or 90% (high) relative humidity. This was followed by a final wet interval of 24 hours. After exposure to the final wet period, they assessed the proportion of ungerminated conidia and germlings (*i.e.*, germinated conidia) with or without an appresorium that were killed.

    The viability of ungerminated conidia was not affected by exposure to drying intervals until drying exceeded 96 hours within the temperature range studied and at relative humidity's of 60% (low) or greater than 90% (high). Germlings with or without appresorium formation were more sensitive to drying. However, up to 85% of those that were viable after 96 hours of drying were able to produce an appresorium when exposed to a second interval of wetness of 24 hours. They proposed the following rule based on

their results: "If the interval of drying is less than 48 hours in length, the initial and subsequent intervals of wetting should be summed to calculate Mills infection periods,

Table 1: Approximate wetting period required for primary apple scab infection at different air temperatures and time required for development of conidia. (Reproduced, with permission, from Compendium of Apple and Pear Diseases, 1990, The American Phyto pathological Society, St. Paul, MN).

Average Temperature		Wetting Period (hr)			
°F	°C	Light Infection	Moderate Infection	Heavy Infection	Incubation Period (days)
78	25.6	13	17	26	—
77	25.0	11	14	21	—
76	24.4	9.5	12	19	—
63-75	17.2-23.9	9	12	18	9
62	16.7	9	12	19	10
61	16.1	9	13	20	10
60	15.6	9.5	13	20	11
59	15.0	10	13	21	12
58	14.4	10	14	21	12
57	13.9	10	14	22	13
56	13.3	11	15	22	13
55	12.8	11	16	24	14
54	12.2	11.5	16	24	14
53	11.7	12	17	25	15
52	11.1	12	18	26	15
51	10.6	13	18	27	16
50	10.0	14	19	29	16
49	9.4	14.5	20	30	17
48	8.9	15	20	30	17
47	8.3	15	23	35	—
46	7.8	16	24	37	—
45	7.2	17	26	40	—
44	6.6	19	28	43	—
43	6.1	21	30	47	—
42	5.5	23	33	50	—
41	5.0	26	37	53	—
40	4.4	29	41	56	—
39	3.9	33	45	60	—
38	3.3	37	50	64	—
37	2.7	41	55	68	—
33-36	0.5-2.2	48	72	96	—

but the regression equations (*see* Becker and Burr 1994) should be used to correct for the proportion of the initial inoculum that remains viable at the beginning of the second

wet interval."

Becker and Burr's rule is more conservative than the "typical" rule of 'summing wetting periods separated by less than either 8 hours of sunny weather or 12 hours of cloudy weather.' Where did this rule come from? In a review of the scientific literature MacHardy (1996) found no scientific basis for the establishment of this rule. Although there are inconsistencies in past studies, nearly all the research that was conducted shows that a high proportion of both ascospores and conidia survive drying periods of 24 hours or more whether it is sunny or not. The inconsistencies in past studies may have been eliminated and could have possibly resulted in better set of rules for combining successive wetting periods if the following factors were included within the studies (MacHardy 1996): (i). a measure of the amount inoculum in an orchard; (ii). the number of hours and the average temperature between the start of rain and when leaves dry; (iii). the time of day when wetting occurs (based on the knowledge that ascospore discharge occurs only during daylight hours); and (iv). differentiating between ascospore and conidia infection.

Based on the current literature MacHardy (1996) defines a less conservative but simpler rule to follow than Becker and Burr (1994) for combining successive wetting periods: "two successive wetting periods, the first started by rain, should be considered a single, uninterrupted wet period if the intervening dry period is less than 24 hours, regardless of weather conditions (sunshine, temperature, and RH) during the intervening dry period."

2.1.3.2 Quantifying Primary Inoculum Pressure

The second approach to timing initial fungicide applications relies on quantifying primary inoculum pressure in an orchard. Gadoury and MacHardy (1986) developed a model for determining the potential ascospore dose (*PAD*) based on a procedure to assess the severity of apple scab at leaf fall and leaf litter density at budbreak. *PAD* is expressed as the total seasonal production of ascospores per square meter of orchard floor and is written (theoretically) as $PAD = LD @ PD @ AD @ LLD @ n$; where *LD* is lesion density (lesion per square meter of leaf tissue at leaf fall), *PD* is pseudothecial density (mature ascocarps per visible lesion), *AD* is ascal density (asci per ascocarp), *LLD* leaf litter density (proportion of the orchard floor covered with leaf litter), and *n* is the number of ascospores per asci. The first fungicide spray is delayed if the *PAD* of the orchard is less than 100,000 ascospores/m³ leaf litter. The delay in days is determined by solving the equation $dt = 1/r(\ln X_o/X_{os})$; where *dt* is the delay in days, *r* is the infection rate (0.04 units/day), X_{os} is the *PAD* for the orchard in question, and X_o is 100,000.

MacHardy *et al.,* (1993) later offered an alternative approach for timing the first spray based on *PAD*. In orchards where *PAD* is low, the first apple scab spray can be delayed to coincide with the control of arthropod pests, usually occurring at the tight cluster or pink stage. A "low" *PAD* generally is defined as a *PAD* less than 1000 ascospores per square meter; this is approximately met if the severity of foliar scab at leaf fall is less than 0.5% just prior to leaf fall.

Estimating *PAD* in the orchard is accomplished by a relatively simple, but intensive, sampling strategy. The sampling strategy calls for sampling all terminal leaves on

10 shoots on 60 trees throughout the orchard prior to leaf drop. Selecting shoots is somewhat of an arbitrary process, but every attempt should be made to select shoots throughout the canopy. The number of diseased leaves is then tallied for all shoots. An orchard is considered a "low-inoculum" orchard if less than 53 infected leaves were found on the 600 shoots. This strategy is admittedly labor intensive and recently a sequential sampling strategy has been developed that allows users to rapidly classify the inoculum status of an orchard (MacHardy 2000).

Later research by Wilcox *et al.,* (1992) integrated the ideas of low inoculum pressure with the post infection activity of SI fungicides into a reduced-spray program. Wilcox *et al.,* (1992) defined a threshold for a low-inoculum orchard as one with less than 1% fruit infection at harvest. Once an orchard has been defined as a low-inoculum orchard, four sprays of DMI plus a protective fungicide are made at the tight cluster, pink, petal fall, and first cover phenological stages. This program has the advantage over the strict calculation of *PAD* in that it is simpler to execute and the four spray timings coincide with insecticide and miticide sprays (Sutton 1996). However, this program has the major disadvantage of relying solely on DMI tank-mixes and their after-infection activity for the management of scab which selects for fungicide resistant populations. This approach is no longer recommended in New York (Agnello *et al.,* 2002).

2.1.3.3 Measuring Ascospore Maturity

Ascospore maturity and/or discharge can be measured directly. Ascospore maturity can be measured via the squash mount technique (Gadoury and MacHardy, 1982a). Here, apple leaf litter that have visible scab lesions from the previous year are collected from the orchard floor and brought back to the laboratory for examination. Essentially, the pseuduthecia are ruptured, or *squashed*, on a microscope slide and the proportion of mature ascospores is enumerated by observation.

An ascospore discharge test (or tower shooting test) is usually conducted at the same time as squash mount assessments and provides an independent evaluation of spore maturity. In the tower shoot test, leaves are wetted and placed on a screen about 40 cm above a plenum through which air is drawn by a vacuum pump. Spores discharged from the wet leaves are trapped on greased slides just below the holes in the plenum. Counting the number of spores trapped provides an estimate of whether or not leaves are actually discharging spores. This test is qualitative test. That is, the number of spores discharged should only be used as an indication that spores are ready to be discharged and not as an absolute measurement of the ascospore potential. Although there is some correlation between the number of spores discharged in the test and the inoculum potential, the quality of the sample (*i.e.*, the number of lesions per leaf and the time of collection) has a large impact on the number of spores that can be observed. Empirical models for predicting ascospore maturity have also been developed. These models are based on the relationship between temperature and the rate of ascospore maturity. Massie and Szkolnik (1974) developed a regression model that predicts ascospore maturity based on the accumulated number of degree days (base temperature $0^{\circ}C$) and precipitation from 50% leaf fall. Gadoury and MacHardy (1982b) developed a

simple linear regression model based simply on the cumulative number of degree days from maturation of the first ascospores. The original degree-day model of Gadoury and MacHardy (1982b) has since been modified to use the phenological stage of green tip as the biofix rather than the date of first ascospore maturity based on observations that the first mature ascospores are produced typically within a few days of budbreak (Gadoury *et al.,* 1994). Furthermore, the green tip biofix has been chosen based on the rationale that any mature ascospore that has been discharged prior to green tip will not contribute to epidemic development since no susceptible host tissue is available for infection (MacHardy *et al.,* 2001). A more recent modification to the model is to account for leaf litter wetness (Rossi *et al.,* 1999, Stensvand *et al.,* 1997). There is variety of methods of accomplishing this, but no one model has gained complete acceptance. The use of the degree models has been criticized because of claims that these models are regionally dependent, cultivar specific, and that the biofix is too relaxed for use in commercial settings.

2.1.4 Disease management

Apple scab is managed primarily via the timely application of fungicides. This discussion focuses on the management of apple scab, although it is important to remember that pesticide programs developed to control apple scab are closely integrated with the management of other apple diseases such as powdery mildew, rust diseases, summer fruit rots, fly speck and sooty blotch, and also with the management of various insect and mite pests. The following discussion will take a seasonal perspective, first emphasizing strategies to manage primary inoculum, followed by means to manage secondary cycles of disease.

2.1.4.1 Managing primary inoculum

Primary inoculum refers to the spores that cause the first infections in spring. For apple scab, this almost exclusively refers to the ascospores. Ascospore discharge begins around the green tip (bud break) phenological stage. Their release can be delayed substantially or hastened depending on temperature and amount of rainfall. The fungus can overwinter as mycelium in some regions where it would produce conidia to serve as the source of primary inoculum. In general, however, conidia are considered an unimportant source of primary inoculum in commercial production.

   It is widely accepted that controlling primary infections is critical to successful disease management. Not because these early infections are any more damaging to the tree than those that occur later in the season, but rather the rapid or exponential increase of disease is dependent partly on having a sufficient length of time over the season to allow disease to reach damaging levels. Early infections provide the opportunity for this to occur, particularly when early infections are followed by a later lapse in fungicide coverage or protection. In general, diseases which have numerous infection cycles in a season are best managed by targeting the earliest cycles when there is much less inoculum to manage and thorough fungicide coverage is possible because the canopy has not fully developed.

One strategy for managing primary inoculum is through the destruction of leaf litter in the fall or spring via shredding, litter degrading compounds or biological control agents (Carisse *et al.,* 2000, Carisse and Dewdney 2002, Spotts *et al.,* 1997). The idea is to hasten the decomposition of leaf litter and, hence, the decomposition of overwintering inoculum in an effort to reduce the potential ascospore dose or *PAD* (*see* below) to a level that allows one to delay, reduce, or eliminate the need for fungicides to manage scab. In a study by Sutton *et al.,* (2000), it was shown that shredding leaf litter with a flail mower in November or April will reduce the risk of apple scab by 80-90% relative to the control if all the leaf litter is shredded.

The risk of scab is reduced by only 50-65% if 10-35% of the leaf litter can not be shredded because of the offset of the flail mower, uneven ground, or the location of fallen leaves within an orchard. The application of urea to the litter when 95% of the leaves have fallen will reduce the risk of scab by 50%; the risk of scab is reduced by 66% if the application is made in April before budbreak. Other approaches for reducing spore production in leaf litter include application of urea to fallen leaves in late fall or early spring, or application of lime to leaves in late autumn.
Targeting leaf litter is not meant to be a stand alone practice for the management of apple scab. The destruction of litter reduces the inoculum pressure and effectively delays (or possibly eliminates) the exponential increase in disease. Ultimately, this will result in fewer diseased fruit and/or a reduction in the number of fungicide applications needed to manage scab.

Fungicides are almost always needed to manage scab in a commercial setting. The first fungicide application is often applied between 1-2 cm green followed by another 7-14 days later (other options are explored in the next section). The first two applications are usually a contact fungicide, such as mancozeb or captan. These are used here because: (i). they are inexpensive relative to alternative fungicides; (ii). there is not enough leaf tissue to allow sufficient absorption of a strobilurin or SI fungicide; and (iii). fungicide labeling and fungicide resistance management restrict how often strobilurin and SI fungicides can be used in a single season (therefore, it is best to save these pesticides for later in the season when their chemistry can be put to better use). Because the peak period of apple scab activity occurs around pink, a fungicide with both protectant and post-infection activity is used at this time to prevent disease from establishing. In very wet years and/or in orchards with high inoculum pressure, an application of a strobilurin or SI may be timed at the tight cluster stage. If fire blight is a problem in the orchard an application of a fixed-copper product should be considered as the first application at green tip. Copper is also bactericide and will serve to knock back populations of the fire blight pathogen *Erwinia amylovora* that would have survived the overwintering cankers. Copper should be applied prior to 1 cm green, preferably at green tip, to avoid any possibility of fruit russetting. If applications are to be made beyond 1 cm green, then the lowest labeled rate should be used to prevent fruit russetting, unless heavy rains are predicted then the rates can be increased.

2.1.4.2 Timing initial fungicide applications

Much research has been devoted to learning how to precisely time the first fungicide

sprays under the premise that excellent early-season disease control is a prerequisite for good control throughout the season. In general, three approaches are used either in integration or independently. The first approach is classical disease forecasting where fungicides are applied according to predicted favorable weather conditions. The second strategy is another model-based approach in which an estimate of inoculum pressure is used to delay and/or eliminate the first one or two sprays. The third approach is based on estimating or physically inspecting the proportion of ascospores that are mature and available for discharge at the next wetting event.

2.1.4.3 Managing secondary inoculum

Secondary inoculum refers to the spores that initiate secondary cycles of disease. For apple scab, these are the conidia which develop from infections initiated by ascospores. Secondary infections result in exponential increase in disease (one scab lesion can produce more than 200,000 conidia!) and are responsible for terminal leaf and, more importantly, fruit infections. In seasons when weather conditions are particularly conducive for disease development, numerous secondary cycles can occur and lead to substantial terminal leaf infection and extensive defoliation. Furthermore, extensive foliar infection can lead to a large overwintering population of the fungus and this in turn can result in an abundance of primary inoculum the following season, particularly if sanitation practices are not used.

The impact of secondary cycles can be minimized if primary infections are well managed; particularly if environmental conditions after petal fall are not favorable for disease development. When conditions are conducive for disease development it is the use of fungicides which prevents economic losses. However, it is important to realize that fruit and leaves gradually become more resistant to infection as summer progresses. This is because the heat of summer has adverse effects on the viability of conidia and the amount of susceptible tissue (*i.e.*, new leaves) drops substantially after the spring growth flush. The use of fungicides after petal fall must be considered in concurrence with the management of other diseases, such powdery mildew, sooty blotch and flyspeck, and rusts (Gadoury *et al.,* 1989). These will be covered in greater detail below.

2.1.4.4 Fungicide Selection

There are many fungicides to choose from for the management of scab (see Table 2). Decisions of which ones to use and when to use them can often be confusing. Protectant or contact fungicides can provide excellent season-long control of scab as long as adequate and continued coverage is maintained, particularly in a drier year. The SI fungicides can be used to provide an added level or protection, or can be used in a "rescue" mode if an infection event occurred when coverage lapsed. This is also true for the strobilurin fungicides. SIs or strobilurins can be applied effectively (in some cases) 48-96 hours after the infection event.

In a comprehensive disease management program, the best time to consider the first use of an SI fungicide is tight cluster; especially when powdery mildew is of concern (Rosenberger 2001a, 2001b). Tight cluster is approximately the phenological

stage when the vegetative buds begin to break and terminal powdery mildew infections become active. SI fungicides should always be used at the full labeled rate and in tank-mix with a contact fungicide for resistance management (Köller and Wilcox 1999).

Table 2: Efficacy ratings of several fungicides against the most serious foliar and fruit rotting diseases of apple.

Fungicide	Apple scab	Powdery mildew	Rusts	Sooty blotch & flyspeck	White rot	Black rot	Bitter rot	Brown rot	Bull's eye rot[2]
Benzimidazoles[1]	E-G	E-G	n/a	E	E	E	S-P	G	G
Captan	G	n/a	S-P	G	E	E	G	G	G
Copper	S	n/a	n/a	n/a	n/a	n/a	n/a	?	?
Cyprodinil	S	n/a	n/a	n/a	n/a	n/a	n/a	?	?
Dodine[1]	E-G	n/a	P	F-S	n/a	n/a	n/a	?	?
Fenarimol[1]	E	E	E	n/a	n/a	n/a	n/a	G	?
Ferbam	F	n/a	G	G-F	F	F	E-S	?	?
Iprodione	?	?	?	?	?	?	?	E	?
Kresoxim-methyl	E	G	G-F	E	E-G	E	E-G	?	?
Mancozeb	G	n/a	G	G	F-S	F-S	E-G	?	G
Metiram	S	n/a	G	G	F-S	F-S	E-G	?	?
Myclobutanil[1]	E	E	E	n/a	n/a	n/a	n/a	G	?
Sulfur	G-F	G	P	S	n/a	n/a	n/a	?	?
Thiram	F	n/a	G-F	G-F	F	F	G-F	?	?
Triadimefon	S-P	E	E	n/a	n/a	n/a	n/a	G	?
Trifloxystrobin	E	G	G-F	E	E-G	E	E-G	?	?
Triflumizole[1]	E	E	E	n/a	n/a	n/a	n/a	G	G
Vinclozolin	?	?	?	?	?	?	?	E	?
Ziram	S-P	n/a	P	G	F-S	F-S	G-F	?	G

E=Excellent; G=Good; F=Fair; S=Slight; P=Poor; n/a=no activity
[1] Dodine, the benzimidazole, sterol-inhibiting, and strobilurin fungicides can be ineffective against apple scab alone or in tank-mixes where pathogen resistance exists. Therefore, the ratings given work under the assumption that these fungicides are still effective.
[2] Bull's eye rot is managed primarily in storage; preharvest applications alone are insufficient for management of the disease.

Strobilurin fungicides offer an alternative to the SI's. For apple scab management, using an SI-mancozeb (or captan) combination at tight cluster and pink followed by a strobilurin at petal fall and first cover is no better than reverse. Yet, the following should be taken into consideration. The SI-mancozeb combination will not be as effective as the strobilurin against black rot (*see* below) if used at petal fall and first cover. Substituting captan for mancozeb could improve this mix against black rot but would weaken it against fly speck. Use of a strobilurin fungicide at petal and first cover, however, will provide excellent control of both black rot and fly speck. SI fungicides

have slightly better efficacy against powdery mildew than the strobilurins, but if either of these were applied prior to petal fall then either can be applied again at petal fall. However, if only contact fungicides have been used up to petal fall, then an SI fungicide should be applied at petal fall on mildew susceptible varieties because they typically have better kickback activity (against earlier apple scab infections) than the strobilurin fungicides. Where rusts are a concern, realize that strobilurins have little activity against them. SIs should be used at pink and tight cluster to prevent fruit rust, and between petal fall and 2nd cover to prevent terminal leaf rust infections.

2.1.4.5 Fungicide resistance

Most fungicide programs rely on the fact that SI and strobilurin fungicides can control apple scab effectively (Creemers and Vanmechelen 1998). It is widely known that apple scab has developed resistance to dodine, the benzimidazoles, and, to a lesser degree, the SI fungicides. Apple scab has not developed resistance to any of the contact fungicides such as the EBDC fungicides, captan, ferbam, ziram, or sulfur. The contact fungicides are multi-site inhibitors of numerous metabolic pathways of the fungus making it difficult for the fungus to overcome the fungicidal activity. The SI and strobilurin fungicides, on the other hand, act on a few or a single metabolic pathway and, as we have learned, fungicide resistance develops when the fungus modifies or utilizes alternative metabolic pathway to the one inhibited.

If SI resistance is prevalent in an orchard then serious thought must be put into designing an appropriate fungicide program (Rosenberger 2001c). The strobilurin fungicides are still very effective against scab but must be used cautiously. A recent study showed that resistance to SI fungicides developed to significantly higher frequencies in populations of *V. inaequalis* resistant to dodine than in populations sensitive to dodine (Köller and Wilcox 2001). This suggests that fungicide resistance is likely to occur more rapidly in populations resistant to an unrelated class of fungicides than in those populations which are sensitive to all known fungicides. This model deviates from the current line of thinking that fungicide resistance occurs independently across unrelated chemistry classes.

With this in mind, the use of strobilurin fungicides, or for that matter any class of fungicide where the threat of resistance looms, should not be used routinely in a "kickback", "rescue" or "clean up" mode. Thus, in SI-resistant orchards the strobilurins should always be used in tank mixes with a good contact fungicide, and should always be applied at the full labeled rate. Relative to timing, the strobilurins are very effective against scab and powdery mildew and should be applied during peak activity. An application at pink (replacing an SI here) and first cover is essential. If apple scab pressure is high, an application at tight cluster and/or petal fall may be necessary. In the worst case scenario, four sequential applications will be made. If applied on a protective schedule at full labeled rates, this use pattern should preserve the effectiveness of this fungicide.

Unfortunately, determining if fungicide resistance exists at a level which compromises the efficacy in the field is often learned the hard way. That is, a grower will find that the particular fungicide is providing an insufficient level of control in the orchard.

Of course, resistance can be determined by evaluating isolates of the pathogen in the laboratory, however, this is a time-consuming and laborious task and is usually done only after one suspects that resistance is the culprit. The industry could benefit tremendously with the development of rapid and reliable test to evaluate fungicide resistance in the field.

2.1.4.6. Resistant varieties

There are a number of apple varieties that have high levels of resistance to apple scab (Carisse and Dewdney 2002). Currently, there are six major genes that are known to confer resistance to apple scab: Vf (*Malus floribunda*), Vr (Russian apple seedling), Vbj (*M. baccata* Jackii), Vb (Hansen's baccata), Va (Antonovka), and Vm (*M. micromalus* susceptible to race 5). Each of these genes, except for Vm, confer resistance to all known races of the pathogen. Nearly all resistant commercial varieties contain the Vf gene. Resistant varieties include 'Prima', 'Priscilla', 'Macfree', 'Florina', 'Liberty', 'Jonafree', and 'Pioneer' to name a few. These varieties are planted primarily in organic orchards and not widely planted in many commercial orchards.

The role of cultivar susceptibility has received little attention in disease management, particularly in forecasting. The original Mills curves were developed for the highly susceptible cultivar 'McIntosh'. Aldwinckle (1974) ranked 51 varieties but made no attempt to adjust Mills' curves based on his findings. Olivier (1984) ranked cultivars into susceptibility groups for the selection of an appropriate infection curve but does not seem to have verified his results to confirm his classification. Schwabe (1980) in South Africa tested commercial varieties for differences in leaf wetness required for infection and found that all cultivars required between 3-6 hrs of wetness for ascospore infection but made no mention of the relative susceptibility of the cultivars.

In a 3 year study, Ellis *et al.,* (1998) evaluated the efficacy and economics of using an inorganic (primarily sulfur) and conventional spray program to manage apple scab on the scab-resistant variety 'Liberty' versus the scab-susceptible variety 'McIntosh' in Ohio. Over the three year period, an average of 5 and 9 applications of fungicide were applied under the conventional program and 7 and 12.6 applications under the inorganic program on 'Liberty' and 'McIntosh', respectively. The reduction in the number of sprays on 'Liberty' was associated with the elimination of all pre-petal fall applications which are usually targeted for apple scab. This resulted in a cost savings of 73% and 57% for the inorganic and conventional, respectively, for disease management on 'Liberty' compared to 'McIntosh'. Despite the savings, scab resistant varieties are not widely grown as there is virtually no consumer demand for these varieties.

2.2 Powdery Mildew

Powdery mildew is caused by the fungus *Podosphaera leucotricha* (Ell. & Ev.) E. S. Salmon (anamorph *Oidium farinosum* Cooke). The disease was first reported by Bessey in Iowa (USA) in 1871 where it found infecting apple seedlings. The fungus was first named *Sphaerotheca leucotricha* by Ellis and Everhart in 1888. In 1892, Burrill changed the name to *S. mali* (Duby), but in 1900 E.S. Salmon established the name definitively as

P. leucotricha.

   The disease occurs wherever apples are grown but is particularly problematic in semiarid regions or in nursery production. Losses from the disease vary depending upon the inherent level of susceptibility of the cultivar, environmental conditions, and management practices. Immature tissues (*e.g.* young expanding leaves) are particularly susceptible to pathogen attack, hence the problem in nursery production. Galloway in 1889 was the first to initiate detailed studies on the management of the disease in the eastern United States. In 1914 Ballard and Volck reported powdery mildew as a serious disease on the west coast. Although the lack of detail makes it difficult to establish reports exactly, it is likely that powdery mildew was first described in Germany by Sorauer in 1889, in Australia by Cobb in 1892, and in New Zealand by Cunningham in 1923.

Figure 5: Powdery mildew symptoms on Cortland leaves

2.2.1 Symptoms

The disease infects leaves, blossoms, green shoots, and the fruit. On the leaves, the fungus can appear as felt-like patches or as a solid mat on the surface, particularly the undersurface of the leaf (Fig. 5). Initial infections on the underside of the leaf may cause chlorotic patches or spots to occur on the upper side of the leaf. This symptom, however, is not unique to powdery mildew so inspection of the underside of the leaf is necessary to confirm that powdery mildew is the cause of these symptoms. Under favorable environmental conditions, the disease will spread over the entirety of the leaf and progress down the petiole and on to young, green shoots. Infected leaves tend to

crinkle, curl, or roll upwards along the edges giving them a narrow appearance. If the infection is severe, infected leaves will usually drop prematurely during the summer.

Infected terminal shoots are stunted and the leaves along the shoots appear as described above. Shoot infections are the typical result of overwintering infections of the fungus in buds. When the terminals push in early spring, the fungus grows along with the new succulent growth and infects this tissue immediately. These shoots may be killed outright in the spring, or may survive throughout the season and die in late fall or winter (Fig. 6). The initial growth of the fungus on newly-infected twigs appears powdery white but eventually turns a darker brown. Small black fruiting bodies, called cleistothecia, can form in the mycelial mat and function as the source for sexual reproduction and the eventual production of ascospores.

The blossoms, petals, sepals, receptacles, and peduncles may become infected

Figure 6: Terminal shoot infected with powdery mildew

and covered with the fungus. Blossom infections are less common but are important because infected blossoms will either fail to set fruit or produce small, stunted and/or russetted fruit. These fruit are unmarketable for fresh market consumption.

2.2.2 Disease cycle

P. leucotricha overwinters as mycelium in infected buds or as cleistothecia on the surface of infected twigs. Infected terminal buds are more susceptible to winter injury than healthy buds, typically bud break in the spring is 5-8 days later than healthy buds, and are more susceptible to spring frost than healthy buds. In fact, healthy buds may

survive at temperatures 2-10°C colder than infected buds. As a result, many infected buds will not survive through a cold winter. This is important to keep in mind because a hard winter can dramatically reduce both disease pressure and the need for control measures during the subsequent season. The survival rate of infected buds is less than 5% when temperatures drop below –24°C and, although not well studied, it appears that temperatures around -12 °C will kill the mycelium in infected buds and allow the bud to produce healthy leaves. The cleistothecia, which form the sexual stage of the fungus and produce ascospores, apparently do not play an important role in the disease cycle (Fig. 7).

Mycelia in infected buds produce conidia to initiate primary infections and these infections can be found as early as the tight cluster stage. There is often an
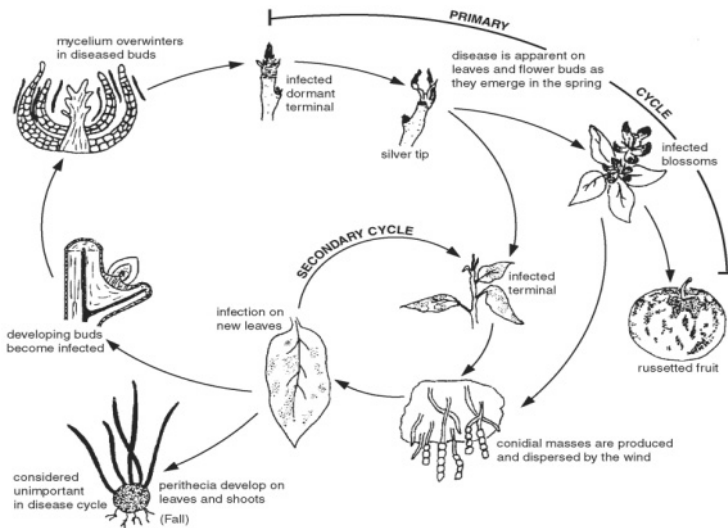
Figure 7: Powdery mildew disease cycle (Reproduced, with permission, from New York State IPM Fact Sheet Series, Cornell University, Geneva, NY).

abundance of susceptible tissue for the conidia to infect because infected buds break later than healthy buds. Conidia are disseminated by wind and can infect young leaf tissue, blossoms, and fruit. Leaves become increasingly resistant to infection as they age and become nearly immune once they have matured, although infection can occur through injuries on older leaves. Conidia germinate easily on glass slides at temperatures in the range of 10-25 °C at 96-100% relative humidity, although the optimum range for germination is between 20-22°C(Coyier 1968). At cooler temperatures germination occurs but is greatly reduced and is virtually non-existent when conidia are exposed to low temperatures for greater than 6 hours. In laboratory studies, Xu and Butt (1998) studied the growth of colonies on young apple leaves in relation to temperature and

water vapor pressure deficit. They found that colony growth responded non-linearly within the range of 13-28°C and optimum temperature for growth occurred at 22°C. The vapor pressure deficit had little effect on colony growth relative to temperature within the range of 1.6-10.4 mmHg.

Symptoms may develop as early as 5 days after infection. Numerous secondary cycles can occur under favorable conditions and, like many powdery mildews, cooler temperatures rather than relative humidity drive secondary infections. Like other powdery mildews, *P. leucotricha* exhibits a diurnal periodicity in that the highest concentration of airborne conidia is found from midday to early afternoon (Xu *et al.,* 1995). Infections that result in fruit russet occur primarily during the pink stage of bud development (Daines *et al.,* 1984).

Apparently, infection of lateral and fruit buds occurs within 1 month after they are formed. The infections remain latent until budbreak the following spring where they will serve as the initial source of inoculum. The lateral buds are susceptible to infection longer than the terminal buds, however, it is the terminal buds that are the likely source of overwintering of the fungus as infection can be greater than 50% by terminal bud set.

2.2.3 Disease management

Powdery mildew is managed by two basic strategies: resistance and fungicides. Powdery mildew resistant varieties exist, however, this resistance has to balanced with other factors such as market demand for that particular apple (Aldwinckle 1974, Jeger and Butt 1986). Moderately resistant varieties will require fewer fungicide applications to manage the disease than more susceptible varieties. Yoder (2000) recently showed the long-term economic benefits of controlling mildew under various fungicides programs for the cultivar 'Ginger Gold' in Virginia. The least effective fungicide treatment was six applications of sulfur and this yielded twice that of the unmanaged check. Several other programs, primarily those which incorporated SI or DMI fungicides, yielded twice that of the sulfur treatment.

In regions where apple scab must be considered, it can be relatively easy to integrate a powdery mildew program with that of apple scab. The SI fungicides are very effective at controlling both mildew and apple scab if timed appropriately. The concern, however, and this has received very little attention, is fungicide resistance. The widespread and perhaps injudicious use of these fungicides has led to widespread resistance (certainly for apple scab). This of course is not unique to powdery mildew, but growers must be made aware that failed control may be the result of more than just bad application timing.

Several fungicides are effective against powdery mildew. One of the most economical of these is sulfur. Sulfur is very effective at reducing the development of powdery mildew, however, it primarily serves as protective spray so it has to be applied frequently as its residual activity is approximately 5 days under the best of conditions. Mono-potassium phosphate (MKP) has been shown to provide good levels of control, particularly when used in alternation with SI fungicides (Reuveni *et al.,* 1998). Various mineral and plant oils are known to be efficacious against powdery mildew (Northover and Schneider 1993). Oils have been used traditionally to combat insect and mite pests

as well as serving as a carrier or adjuvant for copper fungicides. Oils have limited kickback activity, about 24 hours, and their protective activity is very much dependent upon the type of oil used and, not surprisingly, the amount of rainfall. Oils, though, have very little activity against apple scab and most of the other major pathogens of apple and captan and sulfur can not be used in combination with or within at least 5-7 days of an oil spray. Therefore, in area where apple scab is problem, oils are typically not incorporated into fungicide schedules.

The SI fungicides (*e.g.*, fenarimol, mycobutanil, tebuconazole) are very effective at controlling powdery mildew. The SI fungicides have suitable kickback activity and, when applied on a regular schedule, can effectively control the disease. Applications should begin at tight cluster and continue until terminal growth stops in mid-summer. The spray interval is generally 10 days from tight cluster through petal fall, when leaf tissue is developing rapidly, and is lengthened to 14 days after petal fall. To prevent the development of resistance it is recommended that all SI fungicides be applied at the full labeled rates. Reduced rates will allow for a more rapid build up of pathogen strains with moderate levels of resistance.

Like all pathogens, suitable environmental conditions are needed in order for the fungus to infect and for disease to develop. Unfortunately, secondary cycles of powdery mildew are essentially continuous because infection does not require water (Xu 1999). A number of models have been developed to describe various aspects of disease development. Xu (1999) listed these to include: the relationship between disease incidence and severity (Seem and Gilpatrick 1980), disease progress and the cumulative number of spores trapped (Jeger 1984), primary inoculum and fungicide control (Lalancette and Hickey 1986), the effect of leaf age and fungicide control (Lalancette and Hickey 1985), the effect of weather on spore dispersal (Sutton and Jones 1979, Xu *et al.,* 1995), sporulation (Stephan 1988), and the length of the incubation period (Xu 1996). Although not specifically targeted for apple powdery mildew, the Gubler-Thomas model (Gubler *et al.,* 1999) for timing fungicide applications for the management of powdery mildew of grape has been used with success for managing powdery mildews on other crops (usually with slight modifications). The model is based simply on temperature and is widely used in California and the Pacific Northwest, USA where powdery mildews are serious diseases of numerous crops. The model has adapted for other powdery mildews, including cherry and hops, and has been applied in other regions of the world such as Germany and France.

Xu (1999) recently developed a model to simulate powdery mildew epidemics. The model, named Podem™ (short for Podosphaera, East Malling), has been incorporated into Adem™ (Apple Diseases, East Malling), a more comprehensive forecaster for assisting growers in managing apple disease. Adem™ also contains forecasters for apple scab, fire blight, and Nectria fruit rot and canker. Podem simulates powdery mildew epidemics on vegetative shoots on a daily time step from vegetative budbreak (assumed to occur 1 week before full bloom) through the end of shoot expansion. The model consists of a series of submodels to generate daily forecasts of the severity of new infections and the total amount of infectious disease. To do this, the model calculates the percentage of susceptible host tissue, the percentage of infectious disease, the latent period, the rate of infection, and uses a number of weather variables (tempera-

ture, relative humidity, and rainfall). Podem will also generate risks of infection based only on the weather factors vapor pressure deficit and temperature (current and past) and tree phenology.

The model, although complicated in its construction, is simple to use because it has been programmed for use on a PC. Model validation occurred over the course of 4 years in two unsprayed research orchards and performed well under these conditions. As Xu points out, the model does not incorporate the effects of disease management practices on disease development and this limits Podem's use in commercial operations. Clearly, fungicides have a tremendous impact on many aspects of disease development and these must be considered when modeling the development of disease in commercial orchards. However, the Podem submodel that incorporates weather indices to predict the favorableness of the environment for powdery mildew development is still valuable, and this is the part of the model that has been incorporated into Adem.

2.3 Sooty Blotch and Flyspeck

Sooty blotch and flyspeck are two of the most important diseases of apple in temperate climates. The diseases do not result in direct losses in yield in terms of number of fruit, rather they cause a reduction in fruit quality which can lead to significant economic loss in fresh market fruit. Losses can exceed 25%, especially in much warmer climates such as in the southeastern United States. Both flyspeck and sooty blotch have been reported throughout North America, Europe, Australia, and regions of Africa.

Until recently, sooty blotch was thought to be caused by the fungus *Gloeodes pomigena* (Schwein.) Colby. However, recent studies have shown that sooty blotch is a disease complex caused by at least 3 different fungi: *Peltaster fruticola* Johnson, Sutton & Hodges sp. nov., *Leptodontium elatius* (Mangenot) de Hoog, and *Geastrumia polystigmatis* Batista & M.L. Farr. (Johnson *et al.,* 1996). All three fungi grow superficially and do not penetrate the apple cuticle and all three are not necessarily present at all times. The disease is thought to be indigenous to North America as it is described on numerous wild hosts. One of the earliest accounts of the disease is in 1832 where Schweinitz described this fungus on apples in Pennsylvania. In 1834, either flyspeck or sooty blotch or perhaps both, it is uncertain which, was reported in France. However, it was not until 1910, when the English pathologist E. S. Salmon reported sooty blotch on apple, that concerns arose about this new disease apparently imported from America. Flyspeck is caused by the fungus *Schizothyrium pomi* (Mont. & Fr.) Arx (anamorph *Zygophiala jamaicensis* Mason)(Nasu *et al.,* 1985). Sooty blotch and flyspeck were often thought to be caused by the same pathogen because the two diseases often occurred together. By 1940, however, descriptions by Baines (1940) and Baines and Gardener (1932) conclusively showed that the two diseases were caused by different pathogens.

2.3.1 Symptoms

Sooty blotch appears as various shades of olive-green on the surface of the fruit. Colonies range in shape from nearly circular colonies with distinct margins to rather

large, amorphous colonies with diffuse margins. The variation in shapes and color can be attributed to the interaction between the different fungi causing the disease and environmental conditions, specifically temperature and relative humidity. Flyspeck colonies appear as distinct groupings of shiny, black fungal bodies (called thyriothecia) on the surface of the fruit (Fig. 8). The number of colonies range from a few to over fifty. Although flyspeck colonies appear to exist individually, close examination reveals mycelium connecting the individual colonies. As mentioned, both diseases exist within only the first few cellular layers of the fruit and are quite superficial. Rubbing the fruit with a cloth will often be enough to "clean-up" the apple.

2.3.2 Disease cycle
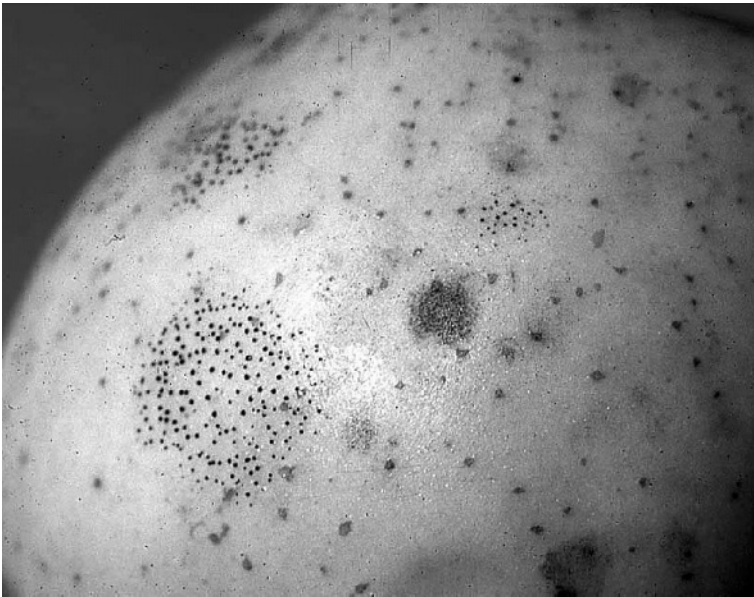
Figure 8: Symptoms of sooty blotch and flyspeck on Golden Delicious (Courtesy of David Rosenberger).

The full details of the life cycle of the sooty blotch fungi *P. fruticola*, *L. elatius*, and *G. polystigmatis* are not specifically known because they have only recently been identified as the cause of sooty blotch. However, the disease cycle is assumed to be similar to that as when it was thought to be caused solely by *G. pomigena*. The fungi overwinter on infected twigs on apple and on its numerous wild hosts. Conidia are formed in late spring and early summer and dispersed to developing fruit by wind and splashing rain. Fruit infection typically occurs from late-April to mid-May in the southeastern United States and in June in the northern and northeastern United States. The first symptoms

are generally apparent 20 to 25 days after infection, but can be visible in 8 to 12 days under optimal conditions.

In a study conducted in Pennsylvania, the development of sooty blotch was found to be highly correlated to the amount rainfall received in July, and to a lesser degree in August and September. In laboratory studies, conidia of *P. fruticola* germinated between 12-24°C and for *L. elatius* between 12-32°C at relative humidities greater than 95%. The optimum temperature range for fungal development was between 12-24°C and 16-28°C for *P. fruticola* and *L. elatius*, respectively. The production of conidia
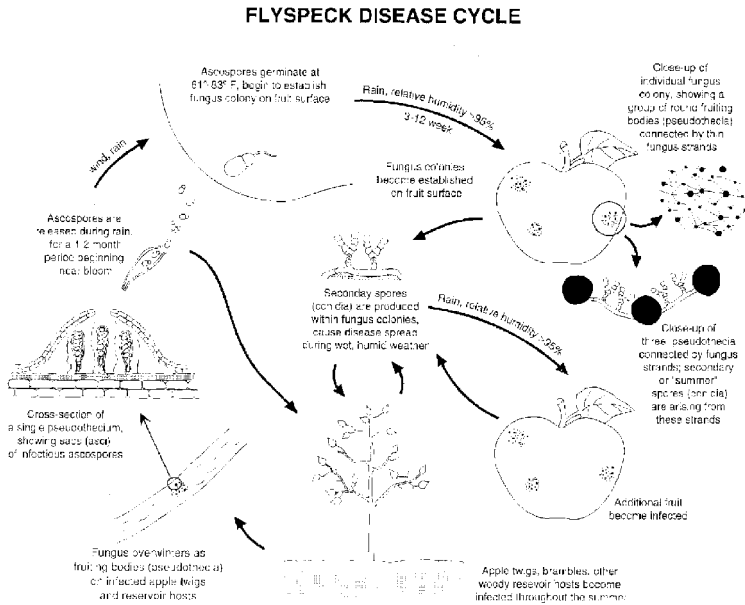
Figure 9: Flyspeck disease cycle (Reproduced, with permission, from New York State IPM Fact Sheet Series, Cornell University, Geneva, NY).

of both fungi was greatest when the relative humidity exceeded 97%.

Flyspeck overwinters as thyriothecia on apple twigs, culled apple fruit, and on numerous wild hosts (Fig. 9). Ascospores mature and are discharged in a single discrete period and initiate infection (Lerner 1999, Williamson and Sutton 2000). The time of discharge varies from region to region and in relation to environmental factors. Symptoms are visible 10-12 days after infection under optimal conditions, but may not occur for 1 month under less than ideal conditions. Initial infections will give rise to conidia which initiate secondary infection throughout the remainder of the season. Numerous observations in the field have shown that warm and wet or humid conditions are needed for the development of disease. Laboratory studies have shown that conidia

can germinate within the range of 8-24°C, colony development occurs over the range 5-28°C, and spore production between 12-24°C (Ocamb-Basu *et al.,* 1988a, Williamson and Sutton 2000). All three processes require that the relative humidity exceed 96%. The development of asci was initiated at temperatures between 4-6°C and ascospore maturation occurred at various temperatures between 9 and 21°C. Again, both processes required a high relative humidity.

2.3.3 Disease management

Management of sooty blotch and flyspeck truly requires an integrated approach. Perhaps the single most important practice to reducing the damage caused by these diseases, outside of the use of fungicides, is to assure that orchard sites and horticultural practices promote rapid drying of fruit surfaces. New orchards should be planted in areas that have good air circulation and long, direct exposure to the sun. Outside of site selection, pruning is the next important practice. In a field study conducted in Alabama, it was shown that the incidence of sooty blotch and flyspeck could be reduced by an average of 30% by "severe pruning" (Latham and Hollingsworth 1973). In a later study, dormant pruning in a non-sprayed orchard reduced the incidence and severity of sooty blotch in 2 out of the 3 years, but the results were inconsistent with respect to flyspeck (Ocamb-Basu *et al.,* 1988b).

In a 2-year study conducted in Massachusetts, Cooley *et al.,* (1997) showed that summer pruning could reduce the incidence of flyspeck by nearly 50% in an unsprayed orchard. In the same study, they indicated that the number of fruit downgraded from USDA Extra Fancy was reduced when summer pruning was practiced in commercial orchards. They concluded that summer pruning helped to decrease the incidence of flyspeck by reducing the number of hours of relative humidity >95% and allowing increased penetration of pesticides to the upper two-thirds of the canopy when applications were made with an airblast sprayer.

The primary means of managing sooty blotch and flyspeck is through the scheduled use of fungicides. In the northeastern United States, fungicides are applied to apples from mid-June through August primarily to control sooty blotch and flyspeck. Four or five summer fungicide applications may be needed to control these diseases in wet years, whereas only two or three well-timed applications are needed in dry years. Omitting summer fungicide sprays is risky because gaps in fungicide protection during critical periods in summer can result in the sudden appearance of numerous flyspeck infections just before harvest.

Field research conducted in the Hudson Valley of New York in the late 1980's and though the 1990's was used to develop a model for timing apple fungicide sprays during the summer (Rosenberger 1994). The model targets flyspeck simply because fungicide programs geared to managing flyspeck will nearly always control sooty blotch in New York, USA. The concepts used to develop the N.Y. Flyspeck Model are outlined below. Before using this model in commercial orchards or in regions where environmental conditions are dissimilar to New York, it is recommended that this approach for timing summer fungicides be tested in a trial orchard. Omitting fungicides is always risky because potential losses from disease on fruit can quickly eliminate any savings

that accrue from withholding sprays.

The first step in constructing the N.Y. Flyspeck Model was the development of a table of estimated residual activities for various summer fungicides (Table 3). This table was developed using data from small-plot field trials conducted by Rosenberger in the Hudson Valley from 1987-1996. Residual activities shown in the table are shorter

Table 3: Suggested fungicides, rates, and spray intervals for controlling sooty blotch and fly speck in orchards considered at moderate risk for these diseases. Adapted from Agnello *et al.,* (1999).

Fungicides grouped by effectiveness	Rate/378 L dilute spray	June/July		From last spray until harvest	
		Spray interval (days)	Maximum rainfall (cm)	Total number of days	Maximum inches of rain allowed before Aug. 30 without respray
Benomyl	85 g	21	8.9	50	4.0
or Kresoxim-methyl	35.4 g				
or Mancozeb	454 g				
or Ziram/sulfur	454+454 g				
Thiophanate-methyl	85 g	21	6.4	45	3.0
or Trifloxystrobin	28.34 g				
or Ziram 76W	681 g				
or Captan 50W	908 g				
Ziram 76W	454 g	21	5	45	2.5
Captan 50W	454 g	14	5	30	2.5

during summer than for the last spray before harvest because cooler conditions in the fall slow development of sooty blotch and flyspeck, and also because late infections will fail to develop symptoms before harvest and therefore are of no concern.

In addition to the residual activity of fungicides shown in Table 3, research has shown that the benzimidazoles (*e.g.* benomyl, thiophanate-methyl) provide limited eradicant activity against sooty blotch and flyspeck. Their eradicant activity decreases as the time between infection and fungicide application increases. Benomyl has reasonable eradicant or suppressive activity against flyspeck infections that have accumulated fewer than 100 hours of wetting after infections occurred. In North Carolina, Brown and Sutton (1986) showed that sooty blotch and flyspeck appear on fruit only after fruit are exposed to 275-300 hours of accumulated wetting following infection. This suggests that benomyl will provide eradication of flyspeck and sooty blotch provided the infections are less than one-third of the way through the incubation period, with "incubation period" defined as 275-300 hours of accumulated wetting after infection. The model was developed using benomyl, but the strobilurin fungicides have post-infection activity equivalent to or better than that of benomyl (Rosenberger *et al.,* 2002). Therefore, a strobilurin can be substituted for benomyl.

By taking advantage of both the residual and post-infection activities of fungicides, it may be possible to eliminate one or two summer fungicide sprays after the last scab spray was applied in early to mid-June. This is possible because it is assumed that the last spray for apple scab (usually first or second cover spray) will provide the residual activity noted in Table 3. If mancozeb is used for the last scab spray, then fruit will be protected for the shorter of either 21 days or through 8.9 cm of accumulated rain following the mancozeb application. After the residual activity from the last scab fungicide spray is exhausted, a "protection gap" of up to 100 hours of leaf wetting (including dew periods) can be tolerated if benomyl or a strobilurin is used as an eradicant later in the season. A means to record leaf wetness, such as a leaf wetness recorder, will be required to monitor hours of leaf wetting. During the protection gap, fruit will not be protected by fungicides, so sooty blotch and flyspeck infections will occur on fruit if inoculum is present in the vicinity of the orchard.

At the end of the protection gap, a strobilurin must be applied to eradicate infections. To be conservative and allow for unexpected rains that might intervene before sprays are completed, the strobilurin should be applied after the accumulated wetting during the protection gap reaches 80 hours. A minimum of two strobilurin applications should be used following the protection gap and prior to harvest to ensure complete suppression of incubating flyspeck infections. The sprays should be 14-21 days apart and, in dry years, will most likely coincide with insecticide applications timed to control apple maggot. Including a strobilurin or a benzimidazole in later summer applications should also control black rot infections that may develop in fruit lenticels as the fruit begin to ripen.

In orchards with dense canopies (*e.g.,* if left unpruned) or clustered fruit, complete fungicide coverage will almost certainly be impossible during late summer when the canopy reaches maximum density and the clustered fruit prevent fungicide from reaching the center of clusters. In such orchards, a strobilurin should be applied during July when the likelihood of good coverage is possible. Even a very tight fungicide program may fail to control flyspeck during wet seasons in orchards with dense canopies.

2.4 Rust diseases

There are several species of rusts that attack apple. Rusts are unusual diseases in that many require two hosts (*i.e.*, two different plant species) to complete their life cycle. Consequently, the geographic distribution and the natural density of the alternate host can have a significant impact on how important a particular rust disease is in any given region. The most commonly encountered and commercially important rusts are caused by fungi in the genus *Gymnosporangium*. Cedar-apple rust, caused by *G. juniperi-virginianae* Schwein., is the most important rust in eastern North America. The fungus attacks both the fruit and leaves of apples and can cause serious losses through a direct reduction yield or diminished fruit quality. The alternate host for this fungus is the red cedar (*Juniperus virginiana* L.). Quince rust, caused by *G. clavipes* (Cooke & Peck) Cooke & Peck, is also widely distributed in eastern North America. Unlike cedar-apple rust, the fungus attacks only the fruit of apple. The alternate hosts are trees in the

genus *Juniperus*. Hawthorne rust, caused by *Gymnosporangium globosum* (Farl.) Farl., is less common than quince rust but, unlike quince rust, hawthorne rust infects only leaves and not the fruit (Fig.10 and 11).

Cedar apple rust was first described on red cedar by Schweinitz in 1822 and on wild crab apple shortly thereafter. In 1889, the association between the so-called cedar gall fungus and apple rust was made by Thaxter. The disease was apparently not a major concern in commercial apple production until the end of the 1800's. The first attempts at managing this disease with fungicides, particularly the use of ammoniacal copper carbonate, were ineffective. In Vermont (USA), Jones demonstrated that adequate control of the disease could be attained by removing cedar trees. In the early 1900's the disease was severe in many apple-producing states, particularly between 1910-1915. This prompted the enactment of the Cedar Rust Law in Virginia and West
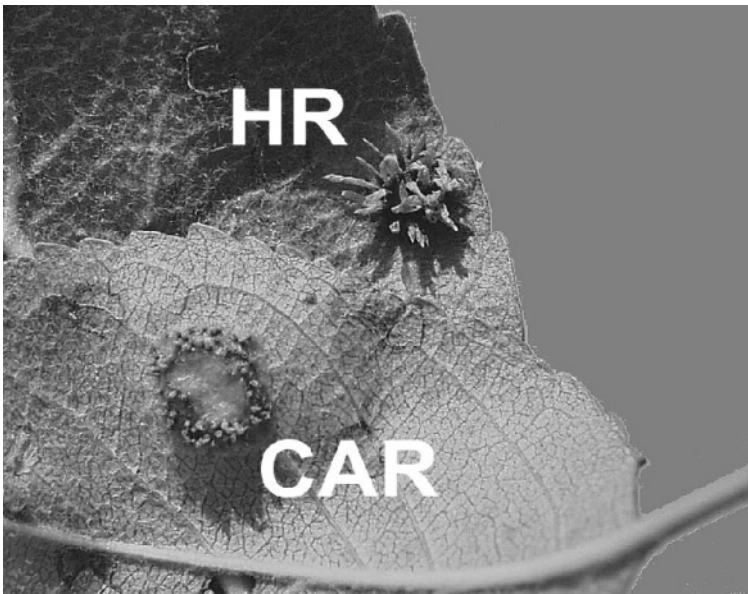
Figure 10: Aecia of hawthorn rust (HR) and cedar apple rust (CAR) (Courtesy of David Rosenberger).

Virginia, USA which called for the mass destruction of cedar trees. Although the law was very effective in reducing cedar apple rust, many home owners were apparently unhappy with having to destroy their cedar trees (Heald 1933).

2.4.1 Symptoms

Cedar apple rust attacks both the leaves and fruit of apple. On leaves, the disease first appears on the upper surface as small, faint, yellow spots approximately 10 days after the appearance of active cedar galls on neighboring cedar trees. As the spots enlarge,

they turn dark yellow to yellow-orange in color and the oldest lesions often have a reddish border. Tiny pustules or pycnia begin to form in the center of these lesions and eventually turn black. Blisters form on the underside of leaf lesions and small, tubular projections develop on the surface of these blisters. As the lesions reach maturity, they split and the walls curve back to form a cup revealing a powdery mass of orange to brown spores. The number and size of lesions varies depending upon the susceptibility of the variety. Heavily infected leaves may curve and/or turn yellow and drop prematurely. On twigs, the disease only affects current season's growth and appears as a small swelling in which the fruiting bodies develop.

On the fruit, lesions usually appear on the calyx end and appear similar to those on leaves, but only a bit larger. As lesions age, the apple tissue surrounding the lesion turns a darker green forming a border around the yellow to orange colored lesion.

Figure 11: Symptoms of quince rust on apple fruit (Courtesy of David Rosenberger).

Unlike leaf infections, lesions on the fruit often will not develop the tubular projections, but when they do occur, small black pycnia form around the periphery on a raised and roughened cushion of tissue. The tissue beneath the lesion is typically alive, although slightly corky. This is in contrast to quince rust in which the tissue beneath the lesion is necrotic.

On cedar, infections result in the formation of galls. Initially, the galls appear as a deep red to chocolate brown, globular swelling from 1 to 50 mm in diameter in the axils of leaves and may be present throughout the tree (Fig. 12). These young galls are first evident in June and reach their full size by fall. Mature galls are larger and may have slight depressions across their surface. In April or May of the following season, gelati-

nous yellow "telial horns" project from the gall. The horns are approximately 1.5 mm in diameter and range from 5-15 mm long when dry and may extend to over 50 mm long when wet. Under these conditions, the galls are quite obvious and have often been referred to as "cedar flowers" or "cedar apples". Small galls may produce only a few horns where the larger galls may produce up to 300 horns. As the galls age, they dry and become black. The galls may fall to the ground or may remain hanging in the tree (inactive) for several years.

2.4.2 Disease cycle (Cedar apple rust)

The fungus overwinters as mycelium in galls on cedar trees. In spring, the galls become active and during rainy periods they expand and produce the spore-bearing telial horns.

Figure 12: A cedar apple gall on red cedar (*Juniperus virginiana* L.) (Courtesy of David Rosenberger).

Wetted telial horns are typically 10-20 mm in length, appear orange-brown and are gelatinous. Upon drying, telial horns shrivel and turn black but will rejuvenate at the next wetting period. The telial horns can survive this period of wetting and drying for several weeks in the spring. The telial horns produce a type of spore, called a teliospore, whose sole function is to produce a second type of spore called a basidiospore. Basidiospores are formed within 4 hours of a wetting event at optimum temperatures and are the spores which infect apple (*see* table 4) (Aldwinckle *et al.,* 1980). Basidiospores are disseminated by wind and may be carried for several miles without losing their ability to infect apple. They can infect both the leaves and fruit of apple. In fact, leaf infections often serve to distinguish cedar-apple rust from quince rust, which infects only the

fruit. Apple leaves are most susceptible to infection when they are young, approximately 4-8 days old, and fruit are susceptible to infection from the tight cluster stage to just after petal fall. Pycnia form on the upper leaf surfaces or fruit about 1 to 2 weeks after infection.

Aecia form on the underside of leaf surfaces approximately 1-2 months after the formation of pycnia. Aecia produce another type of spore, called an aeciospore, and

Table 4: Temperature and moisture requirements for cedar apple rust infection periods on susceptible apple cultivars (Reproduced, with permission, from Compendium of Apple and Pear Diseases, 1990, The American Phytopathological Society, St. Paul, MN).

Temp (C)	Badsidiospore Formation	Wetting Period Light Infection	Severe Infection
2	NB	24	NSI
4	NB	12	24
6	NB	8	10
8	7	6	7
10	5	5	6
12	4	4	5
14	4	3	5
16	4	3	4
18	4	3	4
20	4	2	4
22	4	2	4
24	4	2	4
26-30	NB	NI	NI

NB = No basidiospore formation; NSI = No severe infection; NI = No infection

these are released under dry conditions late in the summer. The spores are wind disseminated and infect young twigs of eastern red cedar. Mature galls do not form until spring of the following season (18 months after infection of the cedar) and new galls are required each year in order to initiate disease on apple (Table 4).

2.4.3 Disease management

Cedar apple rust is not a problem where red cedar does not grow. It is possible then to reduce disease pressure by thinning or removing stands of wild red cedar, and indeed, this was done in the early 1900's in the United States. However, because basidiospores are able to travel on air currents and remain infectious for several miles, it is difficult to remove all sources of disease. If cedar trees are to remain in the locality of an apple planting, then it is suggested that the cedar apples be pruned from these trees.

Where disease is a problem, it is largely controlled through the use of fungicides. Apples are most susceptible to infection from tight cluster through petal fall. In areas, or when conditions are predicted to be favorable, a 7-10 day schedule starting

from pink until 2-3 weeks after petal is recommended. Mancozeb provides good protectant activity against both cedar-apple rust and quince rust. The SI fungicides provide eradicant activity against rust diseases and can be applied within 3 days of an infection event (*see* Table 4). The strobilurin fungicides provide only moderate activity against cedar-apple rust and poor activity against quince rust.

It is important to remember that secondary cycles of rust do not occur on apple. That is, rust infections on apple do not reinfect apple. All infections that occur on apple result from spores produced on cedar. Therefore, when cedar-galls exhaust their spore load, usually by mid-summer, inoculum will no longer be available to infect apple. Therefore, extending fungicide applications beyond, 1st or 2nd cover will provide no additional protection against these diseases.

Cultivars susceptible to cedar apple rust include 'Golden Delicious', 'Rome', 'Jonathan', 'Lodi', 'Idared', 'Mutsu' (= 'Crispin'), 'Fuji', 'Braeburn', 'Gala', 'Cameo', 'Ginger Gold', 'Gold Rush', and 'Alert'. 'Honeycrisp' is moderately susceptible and 'McIntosh' and 'Delicious' are considered resistant to the disease. All cultivars are considered susceptible to quince rust, but 'Cortland', 'Delicious', 'Golden Delicious', and 'Rome' are considered to be the most susceptible.

3. Root and crown disorders

3.1 *Phytophthora* root, crown, and collar rot

Root, crown, and collar rot are diseases of worldwide importance. Crown and collar rot are often and mistakenly used interchangeably (Jeffers and Wilcox, 1990). Collar rot affects the bark tissue of the scion portion of the tree at or just below the soil line, whereas crown rot affects the bark tissue of the rootstock portion of the tree. Collar rot is becoming less of an important disease due to the practice of raising graft unions well above the soil line. The use of highly-susceptible clonal rootstock, however, has led to an increase in importance of crown rot. Root rot often occurs together with crown rot and refers to rotting of the roots away from the crown region.

The disease occurs in nearly all apple growing regions of the world. The Commonwealth Mycological Institute prepared a map of the worldwide distribution of *Phytophthora cactorum* (Lebert & Cohn) Schröter, one of the primary pathogens implicated in causing the disease (Utkhede 1986). The countries known to harbor the pathogen according to this list and citations from more recent literature include Argentina, Australia, Brazil, Canada, Chile, China, Continental Europe, El Salvador, Japan, India, Kenya, Korea, Morocco, Mozambique, New Zealand, Peru, South Africa, Taiwan, United Kingdom, United States, and Uruguay.

The disease was first described by Baines in 1939 on cuttings of Cox Orange in England. It was of considerable importance in the United States in the late 1800's and early 1900's after widespread plantings of 'Grimes' were established throughout the north central states. In fact, the disease was also referred to as "Grimes collar rot" before the causal agent of the disease was known. "Grimes collar rot" was reported as early 1858 and was particularly problematic in Ohio and Indiana, USA where the reports from Selby and Burton originated. The disease was often confused with winter injury, since

it was more prevalent after hard winters, or from cankers caused by fire blight. Yet pathologists were having a difficult time consistently isolating the fire blight bacterium from lesions, and this eventually discounted fire blight as the cause. It was in 1939, though, when Baines (1939), conclusively showed that the Grimes collar rot was caused by *Phytophthora*.

3.1.1 Symptoms

The symptoms on apple usually develop over several seasons becoming progressively worse over time. The rate of disease development is dependent upon the inherent susceptibility of the variety/rootstock, environmental conditions, the degree of fungal infection, and the overall physiological and nutritional health of the tree. Disease symptoms may become noticeable in early spring as delayed bud break and possibly tip dieback. These symptoms are not a result of direct infection at these points, but are characteristic of a plant under stress. Often, these early symptoms may not appear or simply pass unnoticed. Foliar symptoms usually become evident in mid- to late summer and begin with chlorosis followed by a reddening or purpling of the leaves. Infected trees often have a normal bloom, giving a false impression of good health. However, developing fruits typically remain small, leaves begin to wilt and drop, and the tree shows a general decline. The decline generally progresses until the trunk is girdled and the tree dies.

Cankers are almost always found at the soil line. As the bark dies, an exudate may form on its surface and, if one peels the bark back from the wood, the inner bark will be slimy. Cankers are irregular in shape and expand in all directions. Depending upon the age of the tree and the environmental conditions, young cankers may encircle or girdle the tree in the first year. This, however, is not the norm and young cankers are usually confined to one side of the tree but these young cankers can often be hard to detect. With older cankers, the bark tends to dry out, giving the canker a definite outline. Callus tissue may form around the periphery of these cankers, but the callus formation does not completely arrest the development of the canker.

It should be noted that the general decline and wilting of trees associated with *Phytophthora* infection is characteristic of a number of other conditions other than those caused by *Phytophthora*. Rootstock blight (caused by fire blight), "wet feet" (root asphyxiation), borers in burr knots, winter injury, and graft union necrosis (tomato ringspot virus) are often misdiagnosed as Phytophthora crown rot. To distinguish Phytophthora crown rot from these other possibilities is not always so simple. *Phytophthora*-infected tissue often shows a characteristic reddish-brown discoloration of the inner bark several inches below the soil line (where the fungus first enters the tree). Also characteristic is a clear-cut margin of diseased from healthy tissue (Fig. 13). Aside from these diagnostic symptoms, the only other means to positively diagnose the disease is to isolate and culture the pathogen in the laboratory; this may take several weeks. Winter injury is most often confused with *Phytophthora*, almost exclusively affects the above ground portion of the tree and, even then, only the side of the tree facing the southwest. The bark from winter-injured trees will usually separate from the tree over time; this is not a typical symptom of crown rot (Utkhede 1986).

3.1.2 Disease cycle

Phytophthora root and crown rot is caused by a group of fungi in the genus
Phytophthora. *P. cactorum* is probably the most ubiquitous worldwide (Jeffers and
Aldwinckle 1988, Latorre *et al.,* 2001), although *P. cambivora* (Petri) Buisman, *P. citricola*
Sawada, *P. cryptogea* Pethybr. & Lafferty, *P. drechsleri* Tucker, *P. megasperma* Drechs.,
P. parasitica Dastur, *P. syringae* (Kleb.) Kleb., and several unidentified species of
Phytophthora have been isolated from orchard soil throughout the world and are pre-
sumed to cause root and crown rot as well (Latorre *et al.,* 2001). Determining the source
of primary inoculum is generally not straightforward. In a study conducted in New York,
Phytophthora was easily isolated from the rootstocks of trees collected from nurseries
in the United States, Canada, and Europe (Jeffers and Aldwinckle 1988). In the same

Figure 13: Typical red discoloration of apple roots infected with *Phytophthora*.

study, *P. cactorum* was isolated from nearly half of the soil samples collected in or-
chards showing symptomatic or asymptomatic trees. Moreover, *P. cactorum* was iso-
lated from 17 of 37 nonagricultural soils sampled. Together, this is evidence that this
fungus is a natural inhabitant of both agricultural and forest soils.

     Once established, the fungus can survive in living or decaying tissue as myce-
lium. In general, *Phytophthora* spp. are not considered good saprophytes, although *P.
cactorum* and *P. syringae* are capable of colonizing fallen fruit (Jeffers and Wilcox 1990).
All *Phytophthora* spp. can survive freely in the soil as oospores and these may survive
for several years in the soil. Sporangia and encysted zoospores can also survive for a
short period of time in soil. *P. cactorum* is also suspected to produce chlamydspores, a

resistant asexual spore. It should be noted that the study of this disease was greatly facilitated in the 1990's with development of better and repeatable isolation techniques, particularly SADAMCAP (Soil Air-Dried And Moistened Chilled And Plated)(Horner and Wilcox 1995).

Oospores germinate into either sporangium directly or vegetative mycelium after a 6-8 week period of dormancy. Zoospores are produced in sporangia and are the primary spore infecting apple. They are produced under a wide range of conditions, but maximum production occurs when soils are, or are very close to being, saturated with water and temperatures are around 16°C. When soils are saturated, zoospores are discharged and swim to their host. In a 2 year study, Horner and Wilcox (1996) found that dormant spore populations were highest in spring, declined steadily through summer and fall, and increased once again the following spring in New York apple orchards. In the same study, active zoospores were found after the month of March and typically coincided with soil temperatures exceeding 10°C. Chemotaxis, that is, chemical signals emitted by the host that the fungus utilizes for host detection, is used and acts over a few centimeters. However, fully saturated soils promote the passive movement of these spores as well. The role of directly germinating sporangia, oospores, or chlamydospores in infection is not well understood.

Apples are most susceptible to infection between the pink phonological stage and shoot elongation. *P. syringae*, *P. megasperma*, and *P. cryptogea* are capable of extensively infecting apple during the dormancy period. Furthermore, apple varieties grown on vigorous rootstock are more susceptible than those on less vigorous rootstock. Wilcox (1993) looked at the susceptibility of the rootstocks M.7, M.26, MM.111, and Ottawa 3 (O.3) to *P. cactorum, P. cambivora, P. cryptogea,* and *P. megasperma* after 0, 24, 48, or 72 h of flooding every 7 days for 4 months. Not unexpectedly, disease incidence increased with increasing flooding duration across all rootstocks and pathogens. When averaged across all rootstocks and flooding treatments mean crown rot incidences of *P. cryptogea, P. cactorum, P. cambivora,* and *P. megasperma* were 36%, 26%, 15%, and 9%, respectively. When averaged across all pathogens and flooding periods MM.111 was most susceptible followed by M.26, O.3, and M.7. There was significant interaction among the factors explored meaning that no one factor can explain nor predict the level of crown rot alone. This is not the only study to evaluate the susceptibility of rootstock, however, it is a comprehensive study in which many factors were investigated in their evaluation, and it demonstrated clearly that future screening efforts may need to take a more comprehensive approach.

3.1.3 Disease management

Successful control of *Phytophthora* can be accomplished through a combination of cultural and, when necessary, chemical practices. The most important factor in disease management is choosing and preparing your planting site. Sites that drain poorly, are slow to dry, and/or experience periodic flooding should be avoided. In many cases, marginal planting sites can be greatly improved with the installation of drain tiles and water-management ditches. The fungus needs standing water to infect. Planting trees on berms or ridges is recommended because it raises the crowns of the tree above the

portion of soil where pathogen activity is the greatest. For example, in a berm that stands 10 cm above a flooded orchard floor, fungal activity is reduce 90%; at 25 cm above the flooded floor the fungus is virtually inactive. In wet soils, the graft union should be at least 10 cm above the soil line.

Water and/or irrigation management is perhaps the next important practice in reducing the risk of crown and root rot. Special care should be taken to assure that adequate drainage is established, either in the form of drainage ditches or tiling. Standing water, for nearly any period of time, can result in infection as saturated soils promote the release of zoospores. Drainage appears to be more important than how water is delivered. Utkhede and Smith (1996) found no differences in infection between drip irrigated and sprinkler irrigated orchards.

The proper selection of rootstock and variety is perhaps as important as proper site-selection and preparation. Apple rootstocks vary tremendously in their suscepti-

Table 5: Volume of mefanoxem solution to apply relative to trunk diameter.

Trunk diameter (cm) at 30 cm above soil line	Solution (liter)
> 2.5	1
2.5-7.5	2
7.5-12.5	3
< 12.5	4

bility to *Phytophthora*. Among the apple rootstock, seedlings, M.9, M.2, M.4, M.111, MM.110, MM.114, and MM.115 are the most resistant; M.7, M.26, and MM.111 are moderately susceptible; and MM.106 and MM.104 are very susceptible. Preplant root treatments with mefanoxem (an isotope of metalaxyl) or copper hydroxide are effective in reducing the incidence of crown rot, even on highly susceptible rootstocks such as MM.106, but unfortunately label restriction prevent the use of these fungicides as a preplant dip (Jeffers, 1992).

The most effective fungicide for the management of Phytophthora crown and root rot is mefanoxem. Mefanoxem should be applied where crown rot has been a problem or in areas of the orchard where marginal drainage and rootstock susceptibility is likely to be a problem. For apples, make a solution containing 0.25 liters of mefanoxem (trade name Ridomil Gold 4EC) in 378 liters of water and apply the solution at the rate indicated in Table 5. Applications are made just as growth begins in the spring and immediately after harvest. On new apple plantings, delay first application until 2 weeks after planting (Table 5).

The effectiveness of chemical control is dependent on how far disease has advanced, the condition of the planting site, and the inherent susceptibility of the tree. Trees that show marked symptoms or are in a severe state of decline typically cannot be revived and should be removed. Trees that are planted in sub-optimal sites, *i.e.*, where disease pressure is likely to occur every year, may be good candidates for chemical

treatment, depending on the rootstock. Trees, however, which show mild symptoms or healthy trees that neighbor declining trees and are planted in a good site will most likely benefit from fungicide treatment. These trees may be saved or protected from infection when fungicide is applied according to label instructions.

Lastly, according to Utkhede (1986), there are a number of plants that can be used as a green manure to inhibit disease development. The plants include canola (*Brassica napus* L.), mustard (*Brassica juncea* L.), and turnip (*Brassica rapa* L.). These plants produce mustard or leek oil diallylsulfides that inhibit both mycelial growth and zoospore germination and are thus highly toxic to *P. cactorum*.

3.2 Southern blight

As the name implies, southern blight is a disease that plagues apple in warmer production regions. Although the disease can be problematic in orchards, most losses occur in the nursery because few attempts are made to establish orchards in regions where this disease is endemic. The disease is caused by the generalist pathogen *Sclerotium rolfsii* Sacc. which is known to attack over 200 species of plants. This is the notorious white rot pathogen of the southeastern United States and causes tremendous losses in vegetable crops each year.

The disease was first reported on 'Northern Spy' in South Africa in 1922. In the United States, both Turner in North Carolina and Cooley in Maryland reported the disease in 1936. Cooley reported a 5% loss in nursery stock over an 8 year period. The fungus is known to occur in many countries including Brazil, Peru, Chile, Japan, India, and Southern Europe to name a few.

3.2.1 Symptoms

Trees attacked by the fungus show a general decline, a characteristic symptom produced by many pathogens that affect the translocation of water and nutrients. Leaves of infected trees become discolored, turning red to brown and eventually die. Inspection of the crown will often reveal a white web of mycelium. The mycelium may surround the entire tree and may grow up the trunk as much as 17 cm. The mycelium eventually disappears and leaves behind masses of sclerotia (hardened, fungal bodies that serve as survival structures). The sclerotia are first white in color and turn tan to reddish brown to a dark brown. The sclerotia are small and vary in size from 0.5-2.0 mm in diameter. The bark tissue at the crown and upper portion of the roots will be completely rotted by the fungus and will girdle the tree causing death (Fig. 14). Young trees, particularly in nursery production, up to 3 years of age are most susceptible to attack.

3.2.2 Disease cycle

The fungus overwinters as sclerotia. Under favorable conditions the sclerotia germinate to produce mycelium. The fungus produces pectolytic and cellulytic enzymes that allow the fungus to penetrate the host directly. However, the fungus is quite capable of penetrating wounds and injuries. Incidence of the disease increases with increasing

temperatures and soil moisture, with the availability of organic matter, and also in sandy soils as opposed to heavier clay soils. In new apple orchards, disease may appear in locations where the fungus attacked a previous crop. The primary means of dissemination is through mycelial growth. Thus, plants grown closely together are more apt to suffer extensive losses to the disease than those grown farther apart.

3.2.3 Disease management

The best means to managing this disease in the orchard is through avoidance. That is, new orchards should be established on a site that does not have a history of this disease on any of the many host plants that it can attack. Prior to planting, trees should be inspected for signs of the fungus and, if possible, it should be determined whether
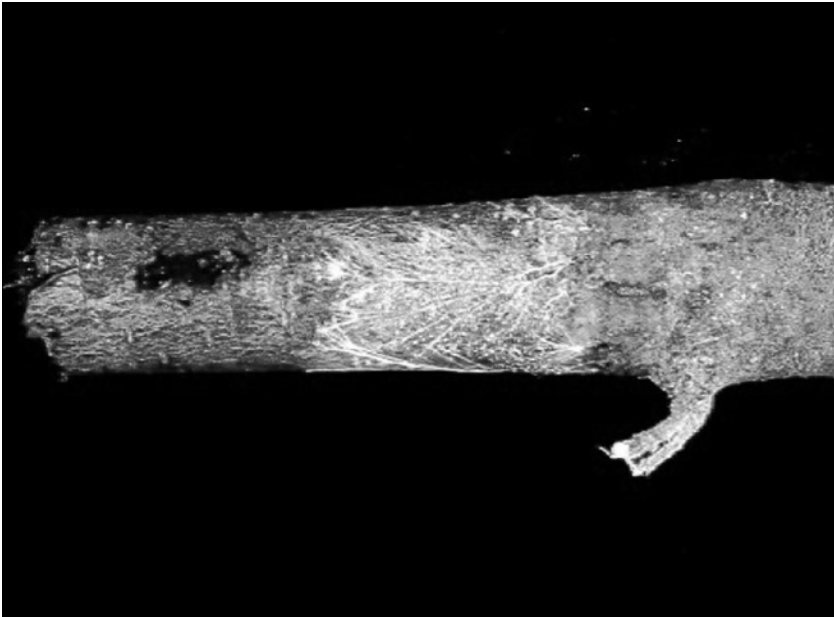
Figure 14: Characteristic white mycelial growth of southern blight infected root (Courtesy of Turner Sutton).

the trees were exposed to the fungus while in the nursery. If, however, one has to plant in to a site with a history of the disease then certain precautions should be taken before planting into the site. Deep plowing the field will bury sclerotia and infected crop debris and prevent the germination of sclerotia. Sclerotia degrade over time, so rotation with a non-host crop such as corn, wheat, or oats can significantly reduce the amount of inoculum in the soil. Other nonchemical methods include the use of soil-solarization, the application of biocontrol agents such as *Trichoderma harzianum*, and soil replacement. All have been used to some degree with varying levels of success.

It is important to realize that the mycelium and sclerotia of the fungus are moved effectively over great distances only with the aid humans (*i.e.*, movement of infected nursery stock) or on machinery contaminated with infected soil or debris (*e.g.*, mowers, cultivators, tractor tires). The use of machinery facilitates the spread through the orchard, eliminating their use through infected portions of the orchard can help to contain the disease in the establishment years.

The use of soil fumigants such methyl bromide can effectively reduce inoculum in an orchard (fumigants may not penetrate the soil deeply enough into subsoils to eliminate all inoculum). However, the use of the most effective fumigants is costly and on the verge of becoming illegal in many countries. High levels of nitrogen to produce ammonia apparently reduces germination of the sclerotia. This is probably not effective on its own, and should only be used in consideration of the demands of the tree.

3.3 Apple replant disease

Apple replant disease (ARD) is a disorder that is characterized by poor growth in young trees. Over a 10 year period, ARD cost growers in Washington state nearly $100,000 US per hectare through poor yields, tree death and replacement costs. To date, many factors have been cited to contribute to the disease but no single organism or abiotic condition has been cited as the explicit cause. Moreover, factors contributing to replant problems vary from region to region. However, it does appear that apple replant is caused largely by biological factors since chemical fumigation effectively manages the disease. Apple replant occurs wherever apples are grown. Two types of replant disease are recognized "specific replant disease" and "nonspecific replant disease" (Mai and Abawi 1981). Specific replant disease refers disorders in young plantings that were planted previously to apple or very closely related species. Nonspecific replant disease occurs when symptoms appear in plantings that were planted to crops other than apples. Nonspecific replant disease is often correlated with high populations of plant parasitic nematodes (Ogawa and English 1991).

3.3.1 Symptoms

The symptoms of this disease are varied and no truly diagnostic symptom distinguishes apple replant disease from other maladies that cause decline or poor growth. In general, trees suffering from replant disease show slow and uneven growth within the first three years of planting. This is characterized by reduced shoot growth, severe stunting, rosetted leaves, and reduced fruit production. Moreover, fruit production can be delayed 2-3 years and yields thereafter are diminished. The root systems of affected trees are fibrous, poorly developed and are often in a state of decay. In some orchards, trees can grow through the symptoms if replant pressure is not severe and the trees are well nourished. In severe cases, the trees decline rapidly and die.

3.3.2 Causal factors

A number of organisms have been implicated in replant disease including a number of

fungi and nematodes and the consensus is that replant is caused by a complex of organisms. In Washington State, *Cylindrocarpon destructans*, *Phyotophtora cactorum*, *Pythium* spp., and *Rhizoctonia solania* were consistently isolated from soils affected with ARD (Mazzola 1998). In Queensland, *Fusarium tricinctum*, *C. destructans*, and *Pythium* spp. were consistently isolated from ARD soils (Dullahide *et al.,* 1994). There have been mixed reports of the importance of nematodes in ARD. The lesion nematode, *Pratylenchus penetrans* (Cobb) Filipjev & Schuur.-Stek., when mixed with *Phytophthora parasitica* resulted in levels of ARD greater than the additive affect of the individual organisms (Utkhede *et al.,* 1992). More recent studies, however, have suggested little to no interaction between nematodes and fungi (Mazzola 1998, Dullahide *et al.,* 1994). Bacteria seem to play no role in disease development.

3.3.3 Disease management

Although tolerance to ARD exists in *Malus* germplasm (Isutsa and Merwin 2000), resistance is currently not a viable option for disease management. As a preventative measure, it is recommended that soils from sites be tested for known causal organisms (if possible) and for pH, since low pH is known to exasperate ARD (Tsc and Utkhede 1991). Cultural methods that have shown to reduce ARD include planting new trees in the drive alley or digging holes the autumn prior to planting to expose the causal organisms to harsh conditions.

Pre-plant soil fumigation, particularly with methyl bromide, metam sodium, and chloropicrin, is often (but not always) effective at minimizing losses due to ARD. However, methyl bromide will soon be unavailable for use in the United States, is currently not used in many European countries, and is often not recommended in Integrated Fruit Production (IFP) protocols. Other soil fumigants, such as 1,3-dichloropropene are only effective against nematodes and not effective against ARD. Therefore, alternative control strategies have been extensively investigated over the past 10 years.

A great deal of research looking at alternative strategies for managing ARD has been done by Mazzola at the USDA Tree Fruit Research Laboratory in Wenatchee, WA. Recent research has shown that biological control of *Rhizoctonia solani* AG-5, a component of ARD in Washington State, can be achieved with a root dip or drench of a preparation of *Pseudomaonas putida* strain C28 (Gu and Mazzola 2001). Because this preparation only targets *R. solani*, it will have to be combined with other treatments that suppress other components contributing to ARD.

Greenhouse studies investigating short-term rotation with certain varieties of wheat reduced populations of *Rhizoctonia* and *Phythium*, but stimulated the growth of *Cylindrocarpon* and *Fusarium* (Mazzola and Gu 2000). The suppression, however, was influenced by the selection of the wheat variety. Apple seedlings planted in soils cultivated with the variety 'Penewawa' grew better than those planted in soils cultivated with either 'Eltan' or 'Rely'. Furthermore, soils cultivated with wheat suppressed populations of fluorescent *Pseudomonads* but enhanced the growth of *P. putida*.

The use of *Brassica napus* (rapeseed) as biofumigant crop has been investigated (Mazzola *et al.,* 2001). Greenhouse experiments looked at cultivating rapeseed and then incorporating the residue in to the soil or amending soil with rapeseed meal, a

by-product of the oil extraction process. The use of seed meal appeared to produce better results than cultivating the crop. Amending the soil at the rate of 0.1% (vol/vol) significantly enhanced growth of apple seedlings while suppressing infections by *Rhizoctonia* and *Pratylenchus penetrans*. When applied at the rate of 2% (vol/vol) the seed meal was toxic to the apple trees, even when planting was delayed 12 weeks.

Apple replant disorder will undoubtedly represent a significant challenge to growers as chemical fumigants become outlawed. The combination of cultural and biological methods shows promise, but as these become standard in orchard management other factors, such as nutrition, will determine if these practices will be incorporated as part of sustainable system.

4. Canker diseases

Apples are susceptible to a number of canker diseases. Most cankers are caused by fungi (with the exception of fire blight, caused by the bacterium *E. amylovora*) and are of varying levels of importance, depending on the severity of losses they cause. The amount of damage caused by cankers in an orchard is not as readily determined perhaps as losses due to other types of disease, such as apple scab, where fruit infections render fruit unmarketable or of reduced value. Canker diseases have a more indirect, yet still significant impact on orchard production, reducing tree growth and productivity, and providing inoculum sources for other related diseases such as leaf spots, fruit rots or shoot blights that these same pathogens may also cause. What starts as a small number of cankers, left uncontrolled under favorable weather conditions, may result in infection and weakening or death of entire orchard blocks.

In general, cankers begin as small spots, or lesions, which appear as dead, diseased, often sunken areas on stems, twigs, or branches. Lesions enlarge over time, through repeated callusing on the part of the plant host, into usually well defined cankers with dead bark above and necrotic, darkened tissue below. Cankers may be superficial in nature or extend deeply into the wood. Large single cankers, or fusion of 2 or more smaller cankers, may lead to girdling of twigs, stems or branches, causing death of those plant parts. Cankers often form at sites of pruning, insect infestation, diseased tissue, or wounds from horticultural practices or adverse weather conditions. Factors impacting canker development include the age of a wound, environmental conditions, cultivar, tree age, and other orchard characteristics such as altitude (Sharma and Bhardwaj 1999, Xu and Butt, 1996).

Cultivars vary in their degree of susceptibility to infection by the various canker fungi. With the exception perhaps of fire blight, few cultivars have been shown to have complete resistance to canker diseases. Much of the current apple breeding work in Europe and Japan is focusing on incorporation of canker disease resistance, especially to *Nectria galligena,* along with multiple resistance to other major apple diseases such as scab and powdery mildew (Kemp and Dieren 2001, Fischer 2000, Kozlovskaya *et al.,* 2000). Use of resistant cultivars may become an integral part of canker disease management in the future as new resistant varieties are developed. However, at this point, the use of resistant varieties is not typically considered in canker disease management.

While pesticide applications reduce the spread of cankers and their associated

diseases they are not usually effective in eradicating existing cankers (Xu and Butt 1996). Thus it follows that the best management practices for canker diseases are pro-active, rather than reactive. It is better to prevent canker establishment by following good horticultural practices, than to try to eradicate existing cankers.

These practices include:

i. Keeping pruning tools sharp to avoid torn bark or jagged cuts.
ii. Removing cankers immediately, making the cut in healthy tissue several inches below the canker.
iii. When removing whole branches, prune back to branch collars to avoid stubs, leaving collars intact to promote wound healing.
iv. If main trunks are involved, trees may sometimes be saved by cutting away diseased tissue and encouraging bark to heal around wounds.
v. Pruning when trees are dormant to minimize disease spread by pruning tools.
vi. Removing and destroying all dead wood, fruit mummies and prunings from the orchard. If this is not possible, chop debris with a flail mower or similar equip ment to hasten breakdown.
vii. Removing other hardwood trees with cankers from surrounding hedgerows.
viii. Buying nursery stock only from reputable nurseries. Inspect each tree prior to planting, discarding any with existing cankers.
ix. Maintaining tree health and vigor with a well-balanced fertilizer program.

A more detailed discussion of the more ubiquitous apple canker diseases follows. White rot and black rot, although capable of producing damaging cankers, have a significant fruit rot phase and are addressed under summer fruit rots. Other apple cankers of note not addressed here include Valsa canker, caused by *Valsa ceratospermae* (Tode ex. Fries) Maire, is found predominantly in China, Korea and Japan; Phomopsis canker, caused by *Phomopsis mali* Roberts, has been reported in Europe, Japan and North America; silver leaf canker, caused by *Chondrostereum purpureum* (Pers. ex Fr.) Pouzar, is a canker disease of minor importance occurring in 38 countries world-wide; nail head canker, caused by *Nummularia discreta* (Schwein.) Tul. & C. Tul., was of serious importance in the US in the early 1900's but found only infrequently today; and Monochaetia twig canker, a minor disease occurring in the North central US, is caused by *Monochaetia mali* (Ell. & Ev.) Sacc. Increasingly, *Leucostoma* spp. have been identified as causing serious canker problems on pome fruits (Proffer and Jones 1989, Brown-Rytlewski and McManus 2000a).

4.1 Perennial canker and apple anthracnose

Apple anthracnose, also referred to as "Northwestern anthracnose", "black spot canker", "Pacific coast canker", and "apple-tree anthracnose", is caused by the fungus *Pezicula malicorticis* (Jacks.) Nannf. The disease was first reported in the Pacific northwest by the USDA scientist Pierce in the 1890's. Although his work with the disease was largely unpublished, he determined the parasitic origin of the disease and reported

that it was confined to the states of Washington and Oregon. The disease was studied extensively by Cordley in the early 1900's (Cordley 1900 a,b,c) and then later by Jackson (Jackson 1911, Jackson 1912, Jackson 1913). Jackson (1913) was first to discover and describe the sexual stage of the fungus and proposed the generic name *Neofabraea* for the newly discovered teleomorph. Nannfeldt (1932), however, recombined *Neofabraea* with *Pezicula* and most taxonomists follow this classification considering *Neofabraea* as a synonym of *Pezicula* (Abeln *et al.,* 2000).

In the 1920's, a similar canker disease was described by Zeller and Childs (1925). Originally named "false anthracnose" or "target canker" because of its similarity to anthracnose, it is now known as perennial canker. The disease is caused by the fungus *Pezicula perennans* (Kienholz) Nannf. (anamorph *Cryptosporiopsis perennans* (Zeller and Childs) Woolenweb., syn. *Gloeosporium perennans* (Zeller and Childs)). Anthracnose and perennial canker are etiologically similar diseases. Consequently, there is much debate amongst mycologists about the classification of these two fungi (Dugan *et al.,* 1993, Abeln *et al.,* 2000). Kienholz (1939) was unable to distinguish differences in morphological, physiological, or mycological characteristics of either the sexual or the asexual stage of the fungi causing these diseases but, because of pathological differences, recommended that the organisms remain as separate species.

The primary difference between the two diseases is in their geographical distribution. Whereas anthracnose prefers the more humid regions of the Pacific Northwest, mainly occurring in the coastal fruit-producing regions west of the Cascade Mountains, perennial canker prefers the more arid regions of the Pacific Northwest, residing east of the Cascade Mountains such as in the Hood River Valley. Grove (1990a) reports two field-level characteristics that serve to distinguish the two diseases. First, he reports that the fungicides which control anthracnose are ineffective against perennial canker and, second, the anthracnose organism penetrates the bark directly, whereas the canker organism enters only through wounds. The latter may be more difficult to assess directly in the field.

Both anthracnose and perennial canker have been reported to occur beyond the Pacific northwest. Anthracnose has been found throughout North America including British Columbia (Canada), California, Idaho, Illinois, Maine, Massachusetts, Michigan, and Nebraska, as well as in the countries Denmark, Great Britain, Holland, and New Zealand. Perennial canker has been reported in California, Idaho, Montana, British Columbia, Great Britain, and continental Europe.

4.1.1 Symptoms

Anthracnose lesions first appear as small, circular spots that are purple or red when wet. Younger branches are usually attacked, but it is not uncommon for scaffold limbs or the trunk to be attacked. As lesions enlarge, they become elliptical, sunken and turn orange to brown. A distinct margin develops between healthy and diseased tissue which eventually causes the bark to crack around the infected area. The infected bark tissue over the canker separates into small pieces and curls upwards from the lesion. This exposes the acervuli which appear as small cream-colored pustules when the spores ooze from them. As they age, however, the acervuli become blackened. On older

cankers, the bark sloughs off leaving only the bast fibers behind. These fibers run lengthwise across the lesion and their appearance as such has often been referred to as "fiddle strings". Anthracnose cankers typically do not enlarge during their first year of growth (Fig. 15).

Perennial canker lesions are elliptical, sunken, and orange, purple, or brown in color. The apple produces a raised layer of callus tissue around the infected tissue to isolate the diseased tissue. This occurs year after year as the fungus continues to invade healthy tissue resulting in a series of concentric callus rings. Acervuli are produced in the most recently colonized tissue and appear as small, raised black bodies. When sporulating, an opaque gelatinous ooze appears over the acervuli. In regions where they both exist, the wooly apple aphid (*Eriosoma lanigerum*) can be found invading these cankers (Fig. 16). In 1904, Lawrence (1904) made the connection be-

Figure 15: Apple anthracnose canker (Courtesy of Gary Grove).

tween the canker phase of anthracnose and bull's eye rot, a common storage rot (*see* post harvest diseases). Since then, it has been well established that perennial canker fungus also causes bull's eye rot.

4.1.2 Disease cycle

Both fungi survive the winter in cankered limbs as mycelium or in fruit left lying on the orchard floor. The perennial canker fungus sporulates throughout the year. Peak spore production is dependent upon location. In the Pacific Northwest, sporulation peaks during late autumn and winter (Grove *et al.,* 1992) where in Great Britain the peak occurs

in autumn. Conidia are disseminated via splashing rain to wound sites. Thus, cracking due to frost injury, wooly apple aphid damage, and certainly pruning damage are primary sites of infection for the fungus. The wooly apple aphid is not a vector of the disease (Grove *et al.,* 1992). The cankers become active and enlarge in late winter and early spring as a result of new conidial infections. In laboratory studies, symptoms of the disease are evident 2-3 months after inoculation and begin to produce conidia 5-6 months after infection (Grove *et al.,* 1992). The wooly apple aphid is attracted to the new callus tissue, feeds on it, and results in the formation of galls. The galls are more sensitive to winter injury than the surrounding tissue and once ruptured provide additional wound sites for further infection. The sexual stage of the fungus is not commonly encountered in the field thus its role in the disease cycle is not well understood. However, it may play a role in the long distance dispersal of the fungus.
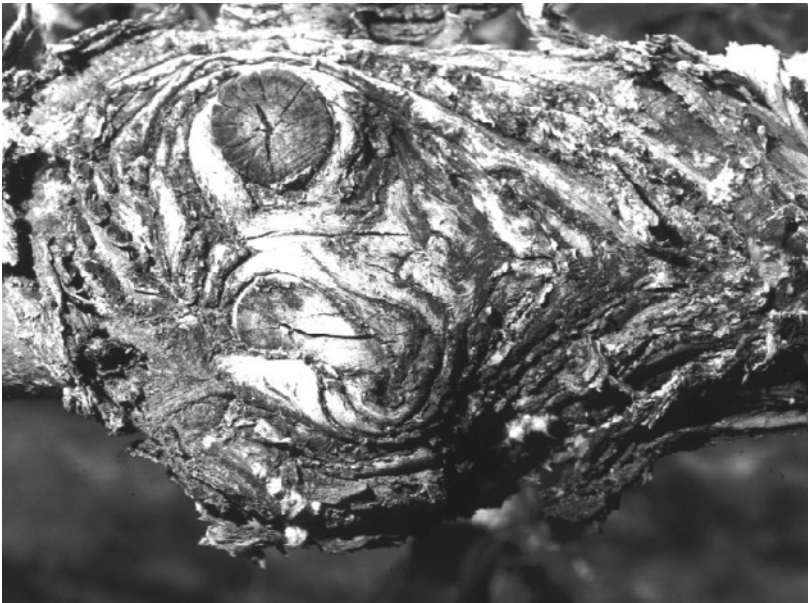
Figure 16: Perennial canker (Courtesy of Gary Grove).

The epidemiology of anthracnose is less understood. New cankers appear, typically on uninjured bark, in autumn and enlarge the following spring. It is assumed that most infections occur through the lenticels (Kienholz 1939). Cankers reach their full size by late spring early summer. Acervuli are present in cankered tissue in late summer and fall and the spores are disseminated by splashing rain.

4.1.3 Disease management

Control of anthracnose can be accomplished with 1 to 3 well-timed fungicide applica-

tions. Applications are typically applied anywhere from early autumn through winter when the new growth is most susceptible to infection. Because rain is necessary for infection to occur, fungicides should be applied prior to significant rain events in order to ensure adequate protection. In the Pacific northwest, the first application is usually made prior to harvest. This preharvest application offers protection against bull's eye rot. The most effective fungicides that are labeled for use preharvest are captan, ziram, and mancozeb. Bordeaux mixture or other fixed copper products can be used after harvest as preharvest usage may lead to fruit russet.

Control of perennial canker focuses on sanitation practices and management of the wooly apple aphid. Cankers should be pruned out in a timely manner to remove inoculum sources. Smaller infected branches should be removed entirely. Cankers on larger limbs or on the trunk can be excised by scraping and cutting the dead tissue from the infected wood. Cutting or large pruning cuts should be done well below or away from the margin of the canker. Care should be taken to leave clean cuts, as ragged cuts leaves areas in which the wooly apple aphid can establish itself. In severely infected orchards the removal of infected trees may prove to more beneficial than a severe pruning.

In eastern Washington, trees are most susceptible to infection from October to March. This coincides with peak spore production of the fungus and, unfortunately, with dormant pruning. Thus, it seems reasonable that pruning schedules should be adjusted around rain events during the period of peak spore production to reduce the spread of the disease, since pruning wounds are susceptible to infection. However, Grove *et al.,* (1992) points out that more information is needed on the duration of wound susceptibility in order to effectively adjust pruning schedules. Preliminary studies suggest that pruning wounds are susceptible for approximately 1 month (Grove *et al.,* 1992).

4.2 Nectria canker

Nectria canker, also called "European canker", "apple canker" or "crotch canker", is caused by the fungus *Nectria galligena* Bres. (anamorph *Cylindrocarpon heteronemum* (Berk. and Broome). This economically important disease kills young trees; reports on epidemics in some growing regions document removal of entire orchards as a direct result of *N. galligena* infection. Infection in older trees may result in the loss of whole limbs. Fruit infections occur commonly in the British Isles, causing "eye rot"; this problem also appeared briefly in California in 1965.

N. galligena infects not only apple and pear, but also quince, aspen, beech, birch, maple and hickory. The disease is widespread in fruit production areas throughout the world including North America, Northern Europe and the British Isles, Australia, New Zealand, South Africa, Japan, and Chile. Nectria canker is of particular importance in the Pacific costal regions of California, where disease development is favored by moderate temperatures, fog and annual rain fall often in excess of 100 cm. The same is true for portions of Oregon, Washington and British Columbia where similar conditions exist. Disease development is quite problematic in the British Isles where disease development is favored by frequent summer rains (Grove 1990b, Xu and Butt 1994,

Sharma and Bhardwaj 1999).

4.2.1 Symptoms

4.2.1.1. Fruit rot phase (eye rot)

Eye rot begins as brown, necrotic, slightly depressed lesions on the fruit surface. Infection typically occurs through wounds, calyxes, and fruit lenticels. Lesions around lenticels appear as circular, brown, necrotic areas with pale brown centers. A characteristic sign observed on fruit in later stages of eye rot is the appearance of white mycelial mats near the calyx.

4.2.1.2. Canker phase

N. galligena cankers are often associated with nodes, appearing as elliptical sunken areas, which later become dark and water-soaked. Sometimes callus production stops fungal invasion and cankers die by seasons end. Other times, the fungus, walled off by callus formation during the current growing season, re-invades callus tissue when active growth resumes the following season, giving older cankers a zonate appearance. These zones are absent in regions where the cankers are able to enlarge continuously. Enlarging cankers girdle infected twigs and branches, killing tissue above cankers. During damp weather, gelatinous spore masses (sporodochia) of the fungus ooze from cankers; bright red to orange fruiting bodies (perithecia) may appear on older cankers during the winter (Fig.17).

4.2.2 Disease cycle

N. galligena persists through the winter and under adverse environmental conditions as mycelium in both twig and branch cankers. Young cankers usually produce only sporodochia the first year after they develop. Perithecia develop the year after canker formation, and cankers may continue to produce both conidia and ascospores as conditions are favorable throughout the year (Xu and Butt 1994). Both ascospores and conidia are capable of causing infection, which commonly occurs through wound sites such as leaf scars, fruit scars due to chemical thinning or natural abscission, pruning wounds, and even apple scab lesions. Leaf scars are most susceptible to infection 1 hour after leaves abscise, and remain susceptible for up to 30 days after leaf drop. Six hours of wetness are required for the fungus to be able to initiate leaf scar infections.

Production of conidia occurs in gelatinous spore masses or sporodochia and is favored by cool, wet weather. Conidia are dispersed by rain splash from these to infection sites. The timing of peak ascospore release varies from region to region, but coincides with duration of rainfall in all cases. Ascospores are forcibly ejected from perithecia during rainy periods and wind dispersed or may also be exuded in a gelatinous mass and splash-dispersed. Peak spore production for both spore types occurs when temperatures fall between 10 and 16°C. The most important factors contributing to canker formation have been identified as inoculum dose, cultivar and wound age (Xu *et*

al., 1998). Where greater numbers of spores landed on younger wound sites, incubation periods were shorter, and canker incidence higher, following the pattern of most canker pathogens. These types of infection also resulted in greater fungal biomass for the invading pathogen, rapid tissue colonization and symptom expression.

4.2.3 Disease management

One report from Chile suggests that infection by *N. galligena* occurs mainly through leaf scars in autumn. Fall applications of copper fungicides, benzimidazole compounds, captafol and strobilurins were found to reduce leaf scar infections from 62 to 92 percent (Lolas and Latorre 1997). Other reports confirm these findings and add that spring-summer applications of benzimidazoles with dithianon, generally targeted to control

Figure 17: Orange sporodochia of *Necrtria cinnabarina* (Courtesy of David Rosenberger).

apple scab, provide substantial reductions in *N. galligena* canker (65-76%), as well as reducing fruit rot in storage. No significant differences in incidence of new cankers or storage rot were found between this program and a similar program with spring-summer applications of myclobutanil and mancozeb (Cooke 1999).

An infection warning system for *Nectria galligena* similar to that previously developed for apple scab (Ventem™) has been incorporated in the ADEM system (Butt and Xu 1996). The warning system identifies when weather conditions favor infection and predict canker and fruit rot incidence centered around sporulating cankers, taking in to account the age of pruning cuts and leaf scars (Xu and Butt, 1994).

5. Summer fruit rots

5.1 White rot

White rot of apple also known as "*Dothiorella* rot", "*Botryosphaeria* rot", "Bot rot", "bark canker", "*Dothiorella* canker", "*Botryosphaeria* canker", and "stem brown disease" is caused by the fungus *Botryosphaeria dothidea* (Moug.) Ces. & De Not. (anamorph *Fusicoccum aesculi*). The disease is worldwide in distribution, with serious occurrences on apple being reported in the United States, Australia, Korea, Argentina, India and Brazil (Sharma and Bhardwaj 1999, Sutton 1990b).

The fungus is ubiquitous in nature, causing disease on a wide variety of other woody hosts such as birch, chestnut, willow, mountain ash, quince, pear, sweet gum, Rhododendron, avocado, grape, roses, stone fruit, blueberry, blackberry, currant and gooseberry (Sharma and Bhardwaj 1999, Sutton 1990b). Losses on apple result from shoot, limb and trunk death, as well as fruit rot (Travis *et al.,* 1995b). The *Fusicoccum* stage of the disease is most commonly found in nature, with pycnidia being produced on fruit, cankers, and dead bark. The disease on apple occurs in two phases: the fruit rot phase and the canker phase. The fruit rot phase was first described in the United States in 1925 by E. A. Fenner. The canker phase, while described several years earlier (1919) in South Africa, was not considered important in the US until an epidemic in Indiana in 1952. Fruit losses of up to 50% have occurred in the southeastern US where the disease is prevalent. The canker phase causes considerable losses in southern, midwestern and northeastern portions of the US, from extensive loss of large scaffold limbs to death of whole trees.

5.1.1 Symptoms:

5.1.1.1 Fruit rot phase

Fruit lesions become visible 4-6 weeks before harvest, and appear as small, circular, slightly sunken tan to brown spots, sometimes surrounded by a red halo on yellow skinned fruit. On red pigmented fruit, the halo appears dark purple to black (Fig. 18). Latent infections result in formation of corky areas beneath the fruit epidermis, walling off the pathogen at the point of entry. These latent infections may occur on immature fruit up to 7 weeks after petal fall (Parker and Sutton 1992, Kim *et al.,* 2001). Expanding lesions develop in cylindrical fashion to the fruit core, unlike bitter rot lesions caused by *Glomerella cingulata*, which tend to be V-shaped.

Most rotted fruit drop, but some may shrivel and remain attached to the tree, serving as a source of secondary inoculum. Scattered clumps of black fruiting structures (pycnidia) develop on surfaces of fruit with advanced stages of white rot. Rotted fruit appear clear tan to light brown, soft, and watery under warm conditions. This "bleaching" of red-skinned apple cultivars during the decay process has led to the name "white rot". Fruit rot developing under cooler conditions is firmer and deeper tan in color, similar to black rot caused by *B. obtusa*.

5.1.1.2 Canker phase

The canker phase starts in sunburnt or wounded areas of stems and twigs or around lenticels. Cankers begin as small, sunken, reddish brown lesions, bordered by purple margins, often with depressed bark and blisters which exude watery liquid on the lesion surface. Pimple-like pycnidia develop on canker surfaces in the spring, 4-8 weeks after infection. Cankers stop enlarging by fall and are indistinguishable in the field from black rot cankers at this point.

Shoot dieback may appear above the canker as wrinkled, loosened, tan to burnt orange-brown bark, which sometimes peels back. Large limbs may be girdled where several cankers fuse. The appearance of yellow foliage in late May to early June on infected limbs is one of the more striking symptoms of the disease, and is associated

Figure 18: Symptoms of white rot on fruit (Courtesy of David Rosenberger).

with limb girdling by the fungus. Infected wood appears necrotic and darkened below lesions, becoming slimy and fissured. Pseudothecia develop in older cankers (Rytter and Travis 1994).

5.1.2 Disease cycle

The fungus survives from season to season as mycelia, pycnidia or pseudothecia in cankers, mummified fruit, and dead bark. *B. dothidea* readily colonizes current season fire blight strikes and mummified fruit. These infections serve as important sources of

secondary inoculum. While *B. dothidea* is also capable of infecting forest hosts, the importance of this source of inoculum has not yet been established. Ascospores and conidia are produced throughout the growing season in southeastern United States. Conidia are more abundant than ascospores; both are released during wet periods, when they ooze out of fruiting structures, and are dispersed by rain or wind, respectively.

The optimum temperature for germination of both spore types is between 28-32ºC. Germination occurs in less than 90 minutes at 28ºC in free water, and may occur in the absence of free water when the relative humidity is greater than 95%. Ascospores have been shown to germinate over a wider range of relative humidities than conidia (92-100%). Germination of both spore types occurs rapidly under favorable conditions, with germination reaching almost 100% in as little as 4 hours (Parker and Sutton 1993) Wounding is not necessary for infection but is probably the most important entry point for the fungus. Infections through wounds on the apple cuticle may develop rapidly under favorable conditions; this argues for the use of a pre-infection control strategy rather than a post-infection strategy (Sutton and Arauz 1991). Drought stress and winter injury have been observed to promote canker development, especially on older limbs (Travis *et al.,* 1995a).

5.1.3 Disease management

As wounds provide a primary means of entry for the pathogen, care should be taken to avoid wounding or pruning during periods when trees may be subject to drought stress. Summer pruning in particular may increase the incidence of infection (Brown-Rytlewski and McManus 2000b). Sanitation is also key in controlling the disease. Removal and destruction of infected branches, cankers and other sources of inoculum, such as mummified fruit, is recommended. Applying fungicide to pruning wounds or tree canopies after pruning may provide an additional level of protection for growers. Brown-Rytlewski and McManus (2000b) report that routine applications of benomyl, kresoxim-methyl, or trifloxystrobin made for control of summer diseases such as apple scab, sooty blotch and flyspeck, decrease canker symptoms. However, the ability of the pathogen to move systemically in the xylem may limit the value of these fungicides in controlling the canker phase of this disease. They add that actively growing trees inoculated with the pathogen remained free of disease as long as they were not stressed, for example by transplanting or drought.

5.2 Black rot

Black rot of apple, also referred to as "black rot canker", "black canker", "smoky blight canker", "NY apple tree canker", "dieback", or "twig blight", is caused by the fungus *Botryosphaeria obtusa* (Schwein.) Shoemaker (syn. *Physalospora obtusa* (Schwein.) Cooke; anamorph *Sphaeropsis malorum* Berk.). *B. obtusa* has been reported to infect plants from at least 55 families, including other members of the rose family such as quince and pear (Sharma and Bhardwaj 1999, Sutton 1990c). *B. obtusa* can be found over a wide geographic range including Australia, New Zealand, Europe, India, North

and South America, and Zimbabawe. In the United States black rot is most severe in the southeastern US, although it also occurs in eastern growing regions.

     B. obtusa infection on apple occurs in three phases, a leaf spot phase, a fruit rot phase, and a canker phase (Travis *et al.,* 1995a). The disease was first reported in 1879 on apple fruit in New York State by C. H. Peck. The leaf spot phase, designated frogeye leaf spot, was later described by W. B. Alwood in 1892. Paddock reported in 1899 that *Sphaeropsis malorum* (*i.e.*, *B. obtusa*), previously associated with fruit rot and frogeye leaf spot, was also associated with canker symptoms. The causal agent was not positively identified until 1908, when W. M. Scott and J.B. Rorer demonstrated *S. malorum* as the pathogen (Sharma and Bhardwaj 1999, Sutton 1990c).

5.2.1 Symptoms:

Figure 19: Frog eye leaf spot lesions caused by the black rot pathogen.

5.2.1.1 Frogeye leaf spot

Leaf infections occur in early spring as leaves unfold, often through stomata. Spots or lesions appear as small purple specks on leaf upper surfaces within the first 1-3 weeks after petal fall. These circular lesions enlarge to 3-6 mm in diameter. Lesion margins remain purple while the centers turn tan to brown. Lesions continue to enlarge and take on a "frog eye" appearance over the next several weeks (Fig. 19). As lesions age, a series of concentric rings form around the infection point. Small black spore producing

structures (pycnidia) sometimes form in the center of older lesions. Heavily infected leaves become chlorotic and fall off. Complete defoliation may occur in cases of severe infection.

5.2.1.2 Fruit rot phase

Fruit infection most commonly occurs through stomata of sepals early in the season as soon as bud scales start to loosen; studies in Georgia, USA indicate black rot infection may occur as early as silvertip (Beisel and Hendrix 1982, Beisel *et al.,* 1984). These infections begin as red spots bordered by purple rings, with the entire sepal later turning dark brown. Sepal infection leads to blossom end rot development later in the season.

Figure 20: Symptoms of black rot on fruit (Courtesy of David Rosenberger).

Late fruit infections occur through cracks in the cuticle, wounds and possibly lenticels. Harvest injuries are also subject to infection, causing fruit to decay during or after storage. Lesions on mature fruit enlarge rapidly becoming black and irregular in shape, occasionally bordered by a red ring; these lesions are often found to be infection courts for secondary pathogens (Fig. 20). A series of concentric bands forms as the rotted fruit area enlarges, which alternates in color from brown to black. The flesh beneath the rot remains firm and leathery. Rot may develop in the core or seed cavity, especially in cultivars with 'Delicious' parentage. Infected fruit color early and ripen 3-6 weeks in advance of healthy fruit. Pycnidia often form on rotted fruit surfaces. Even-

tually infected fruit dry down to mummies which remain attached to the tree, serving as inoculum sources in the spring.

5.2.1.3 Canker phase

Bark wounds are often the point of entry for black rot infection. The fungus also colonizes wood previously infected by the fire blight bacterium, tissues having cold injury or other wound sites. Cankers first appear as slightly sunken reddish brown lesions on bark or a superficial roughening of bark tissue. These cankers turn smoky and develop a series of alternating rings, enlarging lengthwise, rapidly becoming elliptical in shape, and extending up to 1 meter in length. More severe cankers invade bark to the wood, causing cracking of the infected area. Some cankers remain small, dying out by the end of the first year, while others continue to enlarge from year to year. Fruiting bodies form in cankered areas by the end of the second year after infection; limbs may be completely girdled at this point. Cankers infections occur throughout the growing season on bark or dead wood (Rytter and Travis 1994).

5.2.2 Disease cycle

B. obtusa overwinters in cankers or as mummified fruit on trees. There is no indication at this point that other hosts play an important role in apple infection. Fire blight strikes and mummified fruit from chemical thinning are rapidly colonized by the black rot fungus, and serve as important sources of secondary inoculum. Both ascospores and conidia are released from cankers or mummified fruit during periods of rainfall. Conidia are released throughout the growing season in southern regions of the US, but are primarily released during the period after bud break in northern growing regions. During rainy periods, conidia ooze by the thousands from fruiting structures, and are carried by splashing wind, rain and insects to infection courts. Airborne ascospores are released in the 4-6 week period following petal fall. Both spore types germinate in free water after 4 hours at 16-32°C; longer wetting periods are required for germination at lower temperatures. Spores also germinate at relative humidities between 96-100% in the absence of free water, but at lesser rates; the germination threshold falls at 92% RH, with no germination occurring at lower RH. Environmental conditions required for foliage and fruit infection differ. Optimal conditions for infection of the foliage occurs at 26.6°C with a minimum of 4.5 hours of wetting; no infection will occur at 8°C with wetness periods shorter than 48 hours. Optimal conditions for fruit infection occur between 20-24°C with a minimum of 9 hours of wetting (Arauz and Sutton 1989a, Arauz and Sutton 1989b).

5.2.3 Disease management

As in the case of *B. dothidea*, an integrated approach to disease management is needed. Prunings and other inoculum sources such as dead wood and mummified fruit should be removed and destroyed. Removal of current season fire blight strikes is also important as they provide infection courts and a source of secondary inoculum.

In addition to these cultural methods, a fungicide program beginning at silver tip and continuing on a 10-14 day schedule is advisable for control of the frogeye leaf spot and fruit rot phases of the disease. Captan, folpet, and the benzimidazoles are fairly effective against the leaf spot and fruit rot phase. A study by Arauz and Sutton (1990) indicated that the SI fungicides flusilazole, penconazole and tebuconazole reduced the severity of frog eye leaf spot 50% when applied up to 48 hours after infection (inoculation). However, tebuconazole was the only SI fungicide in the course of the study that provided adequate levels of both protectant and eradicant activity against *B. obtusa*, making it an excellent candidate for use in an integrated management program for scab, mildew, cedar apple rust, and black rot.

An empirical model for predicting black rot fruit and leaf infections based on temperature and the duration of wetting was developed by Arauz and Sutton (1989a). Unlike apple scab, though, periods of dryness lasting 1 hour or more irreversibly interrupted *B. obtusa* infection events. Thus, split wetting periods are not added together as would be the case when forecasting apple scab. Moreover, because the times required for infection become increasingly shorter as temperatures rise above 20ºC, successful use of this model relies heavily on the availability of fungicides with eradicant activity against *B. obtusa*; such as tebuconazole.

5.3 Brown rot

Brown rot is a common fruit rot in Europe. The disease is caused primarily by the fungus *Monilinia fructigena* Honey, and less often by *M. laxa* (Aderh. &Ruhl.) Honey and *M. fruticola* (Wint.) Honey, the latter being a serious pathogen of peaches, cherries, plums, prunes, nectarines, and apricots. *M. fructigena* can infect blossoms, immature and mature fruit, spurs, and small branches. Losses of up to 40% can occur if weather conditions favor disease development and fungicide protection is lacking during bloom or just before ripening. Additional losses are possible in storage if fruit are not handled properly during harvest.

5.3.1 Symptoms on blossoms, twigs, and fruit

Infected flowers turn brown, wither, and collapse. If infected blossoms do not drop off, the fungus may grow through the pedicel (flower stem) into the twig below, causing twig infections (Fig. 21). Twigs develop elliptical to fusoid cankers and active lesions may develop tufts of gray mycelium and spores.

On the fruit, brown rot infections first appear as soft brown spots. These rapidly expand and become covered with powdery masses of tan spores. The spore may form in concentric circles on the surface of the fruit. Infections may spread rapidly from fruit to fruit, particularly if environmental conditions are favorable and the fruit are touching one another. Under optimum conditions, an entire fruit may be rotted in 48 hrs.

5.3.2 Disease cycle

M. fructigena overwinters in cankers on twigs and branches. The cankers produce

spores in the spring that are spread by wind, rain, and insects to blossoms and young fruit. Infection occurs under warm and wet weather. Mature fruit are infected through wounds or through latent infections (*i.e.*, early infection of the blossoms). Severe infection is usually associated with high levels of disease in neighboring stone fruit (Jones 1990)

5.3.3 Disease management

Orchard sanitation is usually all that is needed for reducing disease pressure and effectively managing disease. Infected fruit and cankers should be pruned out during the dormant season and either burned or buried deep in the soil. Wild or neglected stone fruit trees should be removed from the area because they may serve as reservoirs for

Figure 21: Symptoms of European brown rot (Courtesy of David Rosenberger).

disease.

Any type of injury will provide a point of entry for the fungus: hail damage, insect feeding wounds, bird pecks, fruit cracking, limb rubs, twig punctures, picking injury and damage sustained during packing. It is essential to control insect fruit feeding and take special care during harvest and packing not to puncture or bruise fruit. Cool fruit to as close to 0°C as possible after harvest.

Fungicides are often not needed to manage disease. However, if disease is a problem sprays should be applied during bloom to control blossom blight and 2-3 weeks before harvest to control fruit rot. A number of fungicides labeled to control

apple scab are also effective against brown rot, such as the SI's (*e.g.* myclobutanil, bitertanol, and triflumizol) and where effective the benzimidazoles (*e.g.* benomyl, thiophanate-methyl, and carbendazim). The dicarboximides (*e.g.* iprodione and vinclozolin) are also very effective against brown rot.

5.4 Bitter Rot

Bitter rot is a disease of nearly worldwide distribution. It is an important fruit rotting disease in warmer apple growing regions, for example the mid-Atlantic or southern regions of the United States. The disease is caused by the fungus *Colletotrichum gloeosporioides* (Penz.) Penz. &Sacc. (teleomorph *Glomerella cingulata* (Stonem.) Spauld. & Schrenk). A number of hosts, including peaches, nectarines, grapes, straw-

Figure 22: Characteristic sunken lesions of bitter rot (Courtesy of David Rosenberger).

berries, and blueberries are attacked by this pathogen. The fungus is also known to cause a canker, but this is not common. The disease was first described on apple by Berkley in 1856 and shortly afterwards it was described in the United States.

5.4.1 Symptoms

The disease begins as small, brown lesions as the fruit begin to develop, typically in early summer. The number of lesions per fruit is variable but can be as few as one. Lesions expands rapidly, depending upon environmental conditions, are circular, and

turn a darker brown and become sunken as they age (Fig. 22). Lesions begin to produce fruiting bodies when reach approximately 3 cm in diameter. Spores are produced in a creamy white to pink matrix, often in concentric circles. The rotted flesh is typically watery and appears V-shaped in cross section. The fruit eventually dries and mummifies where it may fall to the ground or remain hanging from the tree throughout the duration of the winter.

5.4.2 Disease cycle

The fungus overwinters in mummified fruit, or at the margins of rotting canker wood. The fungus infects fruit through wounds or it is capable of infecting the tissue directly. Spores of the fungus are disseminated by splashing water (conidia) or wind or wind-driven rain (ascospores). Infection is favored under warm and wet conditions, and mature or wounded fruit are most vulnerable to infection, although fruit of any age, starting from just after bloom, are capable of becoming infected. The optimum temperature for infection is $26^{\circ}C$ and can occur in as little 5 hours; the optimum temperature for lesion expansion is $30^{\circ}C$ (Sutton 1990a).

5.4.3 Disease management

Sanitation is a key element to managing this disease. Mummified fruit and cankered wood should be removed to reduce inoculum sources. Wood cankered from other diseases and other dead branches should be removed as well because they serve as sites of entry for the pathogen. Regular fungicide applications from 1st cover through harvest on a 10-14 day schedule are usually necessary to effectively manage disease. The EBDC fungicides, captan, and the strobilurins fungicides are the most effective, whereas the benzimidazoles and SI fungicides are relatively ineffective. Unfortunately, the EBDC fungicides cannot be applied when fruit are at greatest risk of infection during late July and August in the United States because of label restrictions. No apple variety is completely immune to disease; however, some varieties like 'Fuji', 'Golden Delicious', and 'Empire' are more susceptible.

5.5 Bull's-eye rot

Bull's-eye rot is caused by the pathogen *Pezicula malicorticis* (Jacks.) Nannf. (anamorph *Cryptosporiopsis curvispora* (Peck) Gremmen). The disease is known to cause serious losses in Washington, Oregon, and British Columbia, is sporadic in California, and is of minor importance in the eastern United States (Spotts 1990b). Although all varieties are considered susceptible to some degree, the most susceptible cultivars appear to be 'Yellow Newtown', 'Winesap', 'Delicious', and 'Golden Delicious' (Pierson *et al.,* 1971).

5.5.1 Symptoms

Bull's eye rot lesions begin most often around fruit lenticels and wounds, developing rather slowly in cold storage. Lesions are typically light-tan to brown with a lighter pale-

yellow to tan center which gives these lesions a bull's eye appearance (Fig. 23). The spots are generally circular, flat to slightly sunken, and the affected tissue remains relatively firm. As lesions age, the fungus produces tufts of cream-colored spores in the center of the lesion; they are not always produced in cold storage.

5.5.2 Disease cycle

The fungus overwinters in cankers on branches or in infected fruit left lying on the orchard floor. Fruit infection can occur anywhere between petal fall and harvest and the fruit become more susceptible to infection as they age. The spores are splash dispersed and, consequently, the disease is more severe in seasons when heavy rains occur after bloom. The fungus does not produce symptoms in the field. Symptoms develop in cold

Figure 23: Symptoms of bull's eye rot (Courtesy of Gary Grove).

storage about 5 months after harvest. The fungus does not spread from fruit-to-fruit in cold storage.

5.5.3 Disease management

Managing the disease begins by pruning out infected branches and cleaning the orchard floor of infected fruit. A fungicide application applied shortly after petal fall can reduce the incidence of disease, especially under favorable conditions. Postharvest fungicide applications are effective only if the fungicide can reach the fungus. Estab-

lished infections, *i.e.*, those which occurred shortly after bloom, will not be controlled with postharvest fungicides.

To forecast the level of infection that has occurred, it is possible to hold a sample of fruit at 18-21°C under high humidity for 30 days and determine incidence. Fruit from lots with high disease incidence can be marketed early, prior to the development of symptoms, to minimize losses attributed to the disease (Kienholz 1951, Kienholz 1956, Pierson 1958). CA storage with low oxygen reduces the incidence and severity of bull's eye rot.

6. Minor fungal diseases

There are a number of minor diseases caused by fungi whose importance varies from

Figure 24: Discrete lesions of *Alternaria* blotch on leaves (Courtesy of Turner Sutton).

region to region as well as within specific regions. Many of the so-called minor diseases are unimportant simply because they are well controlled by disease management practices targeted to control other diseases such as apple scab, powdery mildew, and the summer fruit rots. Others are less important because the pathogen has not been introduced or has not acclimated or established in a specific region.

6.1 *Alternaria* blotch

Alternaria blotch is caused by *Alternaria mali* Roberts. The disease is a serious disease in Japan, Korea, and China and is becoming more problematic in the eastern United

States (Filajdic and Sutton 1991). The disease primarily affects the foliage, causing circular lesions that are necrotic with a light-brown interior surrounded by a darker purplish halo (Fig. 24). The pathogen can also attack green woody tissue, but rarely attacks the fruit. Mid-summer defoliation can occur when infection is severe.

6.2 Brooks fruit spot

Brooks fruit spot is caused by *Mycosphaerella pomi* (Pass) Lindau (anamorph *Cylindrosporium pomi* C. Brooks). Symptoms on fruit begin as irregular, slightly sunken dark green lesions on immature fruit (Fig. 25). These symptoms are often confused with physiological disorders such as cork spot, bitter pit, and Jonathan spot. However, these disorders are often not evident until later in the season and typically cause

Figure 25: Symptoms of Brooks fruit spot (Courtesy of Turner Sutton).

browning of the flesh under the lesion, whereas Brooks spot does not. The disease is particularly common in eastern North America (Yoder 1990).

6.3 Dry eye rot

Dry eye rot (blossom end rot) (Rosenberger 1990a) and calyx end rot (Hickey 1990) are diseases that have been reported on apple throughout North America, Europe, and New Zealand. Dry eye rot is caused by *Botrytis cinerea* Pers., the "gray mold" fungus. Calyx end rot is caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. The two diseases

are often confused with each other because symptoms of both begin at the calyx end of the fruit and both cause a reddish discoloring at the site of infection (Fig. 26). Usually isolation of the pathogen is necessary for positive identification. Fruit infected with either of the pathogens have a tendency to drop prematurely. If harvested, though, fruit infected with dry eye rot will develop gray mold in storage.

6.4 Moldy core and core rot

Moldy core and core rot (Spotts 1990a) have been reported in many apple growing regions of the world. The disease is associated with many fungi including *Alternaria* spp., *Stemphylium* spp., *Cladosporium* spp., *Ulocladium* spp., *Epicoccum* spp., *Coniothyrium* spp., and *Pleospora herbarum* (Pers.) Rabenh. and most apple varieties
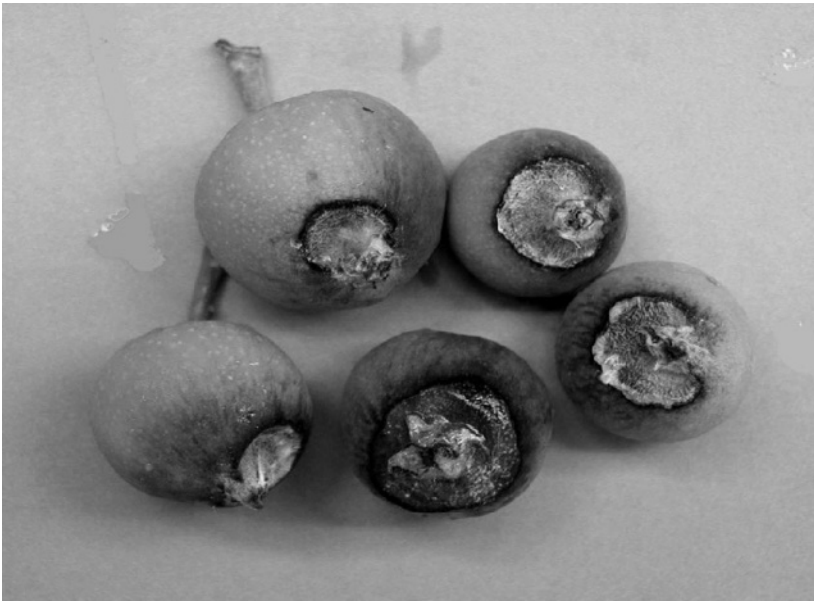
Figure 26: Dried lesions of dry eye rot.

are susceptible to the disease. External symptoms are rare. The rot is associated with the core of the apple which tends to be dry and localized around the core, hence the name. Wet core rot is typically found after harvest when the apples are in storage. The disease develops rapidly and can partially or completely rot the fruit. Wet core rot is typically associated with *Penicillium* spp.

7. Bacterial diseases

Diseases caused by bacteria are some of the most devastating and difficult to manage. This is partly true because we have few effective bactericides labeled for use in com-

mercial agriculture and, for those that are registered, resistance is a serious issue. Because most of the fungicides available for control of apple diseases are ineffective against bacterial diseases, growers must take special care in monitoring disease caused by bacteria so that appropriate action can be taken.

7.1 Fire blight

Fire blight, caused by the bacteria *Erwinia amylovora* (Burrill) Winslow *et al.*,, is one of the most devastating diseases of apple. The pathogen attacks many plants in the family Rosaceae including most notably apples, pears, and quince. Other susceptible genera include Cotoneaster (cotoneaster), Crataegus (hawthorn), Pyracantha (firethorn), Sorbus (mountain ash), and Stranvaesia (stranvaesia).

The disease was first reported by William Denning in 1780 in the Hudson Valley of New York (the disease wasn't officially described until 1794). At the time of Denning's description, it was believed that the symptoms fire blight were caused by various other natural phenomenon or disorders, such as lightning, heat scald, and insects, as opposed to being of parasitic origin. It wasn't until 1880, nearly 100 years later, when Thomas J. Burrill at the University of Illinois discovered that the disease was caused by a bacterium. In 1884, Joseph C. Arthur at Cornell University did the first inoculation experiments confirming Burrill's results (van der Zwet and Kiel 1979). In 1895, Waite was able to demonstrate insect transmission of the disease and the importance of puncture wounds in plant parts other than blossoms. Several years later, Whetzel (1906) reported his findings on the canker blight phase and speculated on its importance as an overwintering site for the bacteria. Research continued on bacterial dissemination and, in 1918, Stevens *et al.,* (1918) published results demonstrating the importance of wind and rain dispersal of the organism. This was verified by a number of researchers in the following years, changing the hypothesis of the day that the bacteria were largely disseminated by insects (van der Zwet and Kiel 1979).

Fire blight is indigenous to the eastern United States, moving westward across the United States around the middle of the 19th century. The disease was reported in the mid-western United States in 1880 and in the early 1900's it was discovered in California, although fire blight-like symptoms were reported earlier. Over the next 10 years, fire blight caused devastating losses to California's pear industry. The spread continued up the Pacific coast and, in 1908, the disease was discovered in Rogue River Valley of Oregon. By 1914-1915 it had spread to the Yakima Valley of Washington and, essentially invading all major apple producing states in the USA (Bonn and van der Zwet 2000, van der Zwet and Kiel 1979).

Currently fire blight is present throughout continental North America from Canada to Mexico. Outside of North America the disease was first observed in England in 1957 and has since spread throughout Western Europe, including South Tyrol and the Po Valley of Italy. In the 1960's the disease was discovered on pears in the Nile Delta of Egypt and over the course of the following 10 years it spread to Cyprus, Israel, Iran, Jordan, Lebanon, Turkey, Greece, Bulgaria, Romania, and then to then former Yugoslavia. In the southern hemisphere, reports of the disease are not as widespread. The disease is not known to occur in South America, or Australia, but it has been found in

New Zealand and Japan (Bonn and van der Zwet, 2000).

Over the last decade, consumer and market demands have forced major changes in horticultural practices throughout much of the world. For example, high density orchards of 1700-3000 trees/hectare, grafted on to fire blight susceptible dwarfing rootstocks such as M.9 and M.26, are replacing older orchards with 400 to 1000 trees/hectare (Westwood 1993).

Fire blight susceptible varieties such as 'Gala', 'Fuji', 'Honeycrisp', 'Jonagold', and 'Braeburn' are replacing older, less susceptible varieties. These changes were implemented to encourage bearing by second or third leaf and, of course, to increase yield. Collectively, these changes have not only increased chances for fire blight infection but the level of damage likely to occur (van der Zwet and Beer 1995). For example, it is now known that systemic invasion of rootstocks resulting from blossom infection

Figure 27: Blossom blight symptoms caused by fire blight (Courtesy of Megan Dewdney).

is common in some areas such as Michigan and New York states. When this occurs often the tree is girdled, killing it within one season (Norelli *et al.,* 2000, van der Zwet and Beer 1995).

7.1.1 Symptoms

Fire blight symptoms are often very characteristic and not easily confused with other diseases or conditions during the growing season. There are five different stages of the disease recognized which are described separately below.

7.1.1.1 Blossom blight

Blossom blight occurs in the spring when blossoms become infected. The first sign of infection is water soaking followed by shriveling, wilting and their eventually turning brown to black. In a given cluster, individual flowers or the entire cluster may be affected. Typically, infected blossoms do not fall and bacteria progress into the tender shoot growth. In the shoot, the bacteria travel along the midvein of the leaves and they soon wilt, shrivel, and turn brownish-black, killing the entire shoot (Fig. 27). Flowers will cling to the infected stem and often remain attached throughout the season and even well past petal fall. Infected fruit appear black and shriveled and usually remain attached to the tree (Fig. 28).

7.1.1.2 Shoot blight

Shoot blight is caused by secondary infections that originate on young terminal shoots,

Figure 28: Bacterial ooze present on infected fruit (Courtesy of Megan Dewdney).

including suckers and water sprouts. It usually develops in late spring or early summer when the most actively growing tissue is present. Infected shoots may at first have an oily appearance and turn a dark green. In apples, the infected shoot becomes light to dark brown in contrast to pear which becomes black. Under favorable conditions shoot blight can progress very rapidly, moving 15-30 cm over the course of a few days (van de Zwet and Beer 1995). Blighted shoots will often form the characteristic "shepard's

crook" at their tip and the dead leaves remain clinging to the affected twigs (Fig. 29). From there the disease can progress to whole limbs and when infection is severe, the whole tree appears to be scorched by fire, hence the name fire blight.

7.1.1.3 Canker blight

Canker blight is also referred to as 'limb', 'trunk', or 'body' blight depending on where the infection occurs. Cankers form as result of the bacteria traveling systemically into the woody tissue of the tree (Fig. 30). The canker itself is dead bark, phloem and cortex cells. There are two types of cankers; determinate and indeterminate. The determinate cankers have a distinct margin with cracks that circle the infected area where the tree has walled off the infection with periderm formation. The indeterminate cankers have

Figure 29: Characteristic "shepherd's crook" caused by fire blight.

indistinct margins that can be raised or blistered but a canker could simply be a water soaked depression. If the bark is peeled back from the margin area of both types of cankers, a distinct demarcation between healthy and diseased tissue is visible where the diseased tissue is a red-brown color. In spring, red-brown streaks will spread from the cankers down the tree limb in the sapwood as the bacteria become active again.

7.1.1.4 Trauma blight

Trauma blight is a term used to describe infections that occur when blight is initiated at leaf, fruit, stem or bark injuries resulting from hail or severe windstorms. When this

occurs on branches or limbs, trauma blight is easily confused with shoot blight because of the similarity of the symptoms. Often, the only defining feature that helps to determine whether a shoot is suffering from shoot blight or trauma blight is the origin of the infection. If the infection origin appears to be the shoot tip, it is often assumed to be shoot blight. Of course, rampant infections following, for example, a hail storm can be largely attributed to trauma infections.

7.1.1.5 Rootstock blight

Rootstock blight is not the most common phase of the disease, but it is the most destructive phase because it often kills the entire tree (Fig. 31). Trees on M.26 and M.9, the most commonly used dwarfing rootstocks, are the most susceptible. Infected root-

Figure 30: A young fire blight canker (Courtesy of Megan Dewdney).

stock turns dark brown to black depending on the severity of the infection and susceptibility of the rootstock and a stark contrast between the scion and rootstock at the graft union is often noticeable. Trees affected by rootstock blight generally show symptoms of decline and early death by mid to late season.

Sometimes symptoms may not be apparent until the following spring when the tree does poorly or does not break bud. Curiously, rootstock blight is not a problem in all areas of the world. It is most common in the northeastern United States for reasons that are unclear.

7.1.2 Disease cycle

The overwintering site of *E. amylovora* is thought to be the small numbers of cankers found on infected twigs and limbs. Only a small percentage of cankers become active in the early spring as temperatures warm and buds begin to develop. In general, cankers with indistinct margins on larger limbs become active. From these cankers, a yellowish to white ooze appears several weeks prior to bloom as the bacteria multiply in the healthy bark bordering the margin. During this period, insects (mainly flies) disseminate the bacteria throughout the orchard where they live on the surface of trees. During bloom, pollinating insects rapidly move the pathogen from flower to flower. Infections initiating the blossom blight phase of the disease (normally the primary phase) can occur within minutes after a rain or heavy dew when the average daily temperatures are

Figure 31: Rootstock infected with fireblight (note bacterial ooze).

equal to or greater than 16°C. Such rapid infection is favored by warm, humid weather before and during bloom. Blossoms do not have to be damaged for infection. The bacteria can enter through natural openings in the flower nectaries, the stigma, undehisced anthers and stomates on the sepals. Although symptoms may not be visible, the threat of blossom blight ends when the flower receptacles and young fruit become resistant after petal fall. Symptoms can be expected 5 to 30 days after infection depending upon daily average temperatures.

Bacteria produced from ooze on infected blossoms, the source of the secondary inoculum, is further spread by wind, rain, insects and farm practices such as spraying.
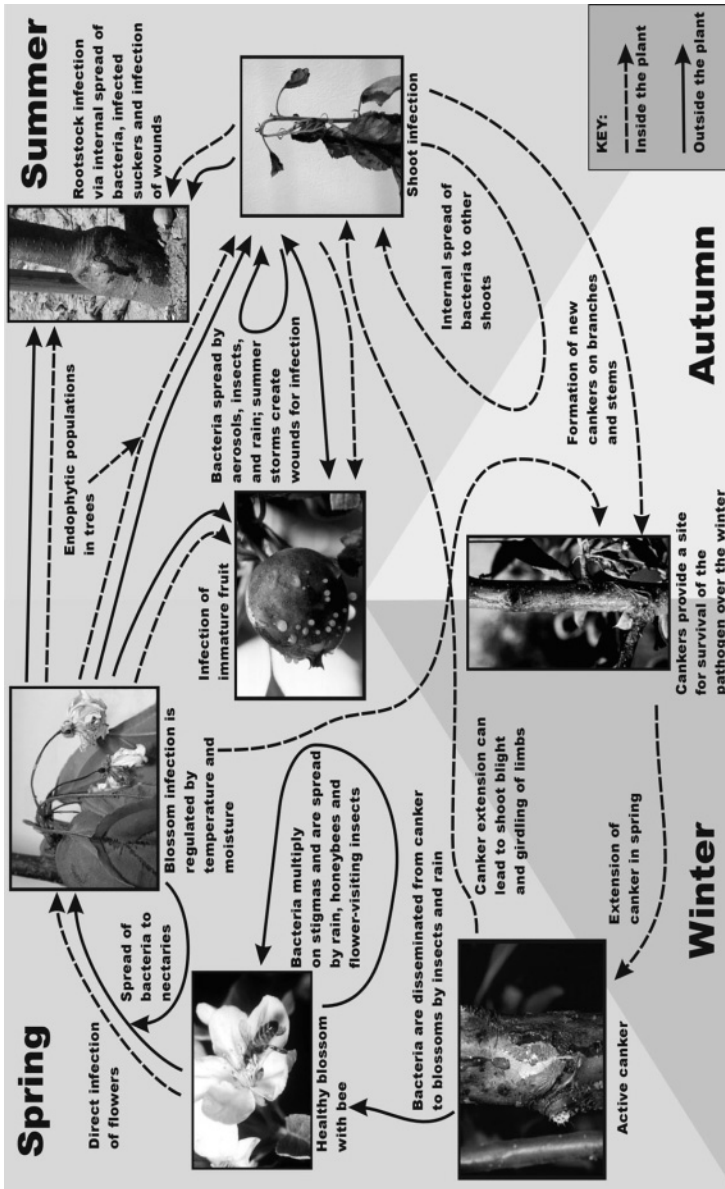
Figure 32: Fire blight disease cycle (Reprinted from Norelli, J.L., Jones, A.L., and Aldwinkle, H.S. 2003. Fire blight management in the twenty-first century: Using new technologies that enhance host resistance in apple. Plant Disease 87:756-765.)

Shoot tip infections are likely to occur when shoots are actively growing and daily temperatures average 16°C or more. In years with light blossom infections, the primary source of inoculum for shoot blight is the overwintering cankers. Young water sprouts near cankers are particularly susceptible because the bacteria can move to them systemically from canker margins. When blossom infections are absent, the development of shoot blight infections is often localized around areas with overwintering cankers.

Although mature shoots and limbs are generally resistant to infection by *E. amylovora*, injuries caused by hail, late frosts, or high winds that damage the foliage, breach the normal defense mechanisms in mature tissues. These infections at sites of injury are called trauma blight and may affect even normally resistant cultivars like 'Delicious'.

Rootstock blight occurs when bacteria from infected blossoms or shoots move internally through symptomless trunks and infects the rootstock. On these trees, just a few blossom or shoot infections on the scion cultivar can supply bacteria that move systemically into the rootstock where a canker may develop and girdle the tree. Although blossoms and shoots are a common source of inoculum for rootstock blight, it can also occur on symptomless trees. Other entry points for inoculum include burr knots, insect borer holes and root suckers.

Towards the end of the season, cankers begin to form with the slowing of bacterial multiplication. As the bark tissue dies in the center of infection, the bacteria in that area also begin to die. Live bacteria are only found at the margin of the infection where they can overwinter. Cankers can start with a raised or blistered area which may progress to a large area with a distinct edge and cracking. The cankers that are most likely to be the overwintering site of the bacteria are those that are found at the base of spurs, suckers, or limbs (Fig. 32).

7.1.3 Disease Management

Effectively managing fire blight requires a combination of disease management practices. For example, bactericides aimed at controlling blossom blight will be less effective in orchards where fire blight cankers have not been pruned out. Although fire blight can infect essentially all tissues of the tree and infection can occur at nearly any time during the growing season, management typically focuses on controlling the blossom blight phase of the disease. Most bactericides are applied to prevent blossom blight. Over the last 10-15 years, managing blossom blight has relied heavily on the use of forecasting models to time the application of bactericides. Interestingly, fire blight is one of the few diseases where forecasting has worked successfully on a commercial scale.

A variety of disease management techniques will be addressed in what follows. Although management of all phases of the disease is addressed, most of what is discussed focuses directly on managing blossom blight because it is the most economically important phase of the disease. Nonetheless, it should not be forgotten that managing fire blight requires season-long attention, but even so, the most prudent grower can suffer extensive losses because fire blight can strike very rapidly and unexpectedly.

7.1.3.1 Resistant varieties

Apple cultivars differ considerably in their susceptibility to fire blight (Thomas and Jones 1992). Highly susceptible cultivars should not be planted in orchards where severe epidemics occur frequently (van der Zwet and Beer 1995). In Table 6, the field susceptibility of several commonly grown cultivars to fire blight is given. Note, because a cultivars ranking is dependent on factors such as where and how the cultivar is grown and how susceptibility is evaluated, the ranking presented here may be inconsistent with other rankings. Although the genetics of a particular cultivar largely dictate whether a variety will be susceptible or not, other factors, particularly nutrition, contribute to a tree's susceptibility as well. Generally, vigorously growing trees are more sus-

Table 6: Relative susceptibility of commonly grown cultivars to fire blight.[1]

Very susceptible		Moderately susceptible		Slightly susceptible
Braeburn	Jonathan	Baldwin	Gravenstein	Delicious
Fuji	Mutsu	Cameo	Macoun	Liberty
Fuji 2	Northern Spy	Cortland	McIntosh	Prima
Gala	Paulared	Empire	Monroe	Priscilla
Ginger Gold	R.I. Greening	Enterprise	Mutsu	Stayman
Honeycrisp	Rome Beauty	Fortune	Jonafree	Winesap
Idared	Spigold	Gold Rush	Pioneer Mac	
Jerseymac	Twenty Ounce	Golden Delicious	Spartan	
Jonagold	Tydeman	Golden Supreme	Starkspur	
Jonamac	York Imperial	Granny Smith	Wealthy	

[1] Modified from Breth *et al.,* 2000.

ceptible than slow-growing trees. Orchards with high nitrogen levels, especially where young trees are being pushed to fill their allotted space, are at a high risk for fire blight (Table 6).

7.1.3.2 Non-chemical control

Non-chemical control is a broad topic that refers to practices such as orchard site selection, tree nutrition, and pruning. Orchard site selection is important for general tree health, which can directly affect a trees ability to withstand infection by *E. amylovora*. Conditions such as poorly drained, nutritionally deficient, and/or low soil pH can all contribute to greater disease severity. Soil analysis before planting can pinpoint some of these problems in advance and allow for soil amendments, such as the addition of lime to raise the pH. For increased drainage it is advised to install drainage tiles or plastic drainage tubes.

Nitrogen fertilization is a major concern for management of fire blight because it stimulates succulent and highly susceptible growth (Fisher *et al.,* 1959). In some re-

gions fire blight is a perpetual threat. Split applications of nitrogen are recommended in areas where fire blight is a perennial problem. The first application is made in the spring before blossoming and, if blossom blight was mild, the remaining nitrogen is applied. On light soils, avoid leguminous cover crops as they make the management of soil nitrogen more difficult. It was reported from Missouri, USA that orchards planted with such cover crops had more severe disease than when they were planted with grasses. Other sources of organic nitrogen, such as manure, should be avoided because the release of available nitrogen is later in the season stimulating the production of susceptible tissue (van de Zwet and Beer 1995). The interaction among potassium, calcium and magnesium should not be ignored. High levels of potassium reduce the amount of both calcium and magnesium which have been implicated in improving the resistance of trees to fire blight.

Dormant and seasonal pruning play an integral role in reducing the amount of disease pressure in an orchard. Dormant pruning is a good time to find cankers that were missed during the growing season. However, large cuts and biennial pruning stimulate succulent growth that is susceptible to infection; these practices should be avoided in orchard blocks planted with susceptible varieties. Seasonal pruning is the practice of removing infected limbs as soon as symptoms are detected. This is done throughout the year to prevent extensive damage from developing, and is an essential practice in orchards planted with highly susceptible varieties. When removing active cankers, cuts should be made at least 30 cm below symptoms. The effectiveness of sterilizing pruning-shears between cuts is debatable, and is often not done due to the impracticality. The risk of spreading fire blight is reduced substantially if pruning is done during dry weather.

Summer pruning is commonly done to encourage the production of new fruiting wood, particularly in newer high-density plantings. This practice extends the period of susceptibility to shoot blight by encouraging new growth and should not be done in years with high disease incidence. In large trees, semi-dwarf to standard size, the spurs that occur on scaffold branches should be removed because of the risk they pose of being infected by blossom blight which could girdle a major branch with a canker. The loss of these fruit is not an economic hardship because they tend to be small and have poor color.

In younger orchards, removing blossoms by hand will reduce the risk of blossom infection. This practice can be especially effective in minimizing losses due to rootstock blight, particularly when highly susceptible varieties such as 'Gala' or 'Gingergold' are grafted on to M.9 or M.26 rootstock. Although somewhat time consuming, blossom removal is a much less expensive alternative than replanting an entire block. A final non-chemical control measure that can be taken is to remove wild sources of inoculum around the orchard such as wild apples, pears and hawthorns. Cotoneaster, a common ornamental is also a host and is a potential inoculum reservoir.

7.1.3.3 Chemical control

Chemical control is almost always needed to manage fire blight on susceptible varieties when conditions are ideal for infection. Chemical control is largely targeted at managing

blossom blight, however, certain bactericides, particularly copper, are applied both early and later in the season to reduce inoculum pressure or to minimize shoot blight.

The application of copper hydroxide is often done to reduce epiphytic and overwintering bacterial populations on susceptible varieties in the early spring. Copper applied between bud-break and 1 cm green tip will reduce the amount of inoculum on the surface of infected trees. To be effective, thorough coverage is essential so dilute sprays are recommended. After 1 cm green tip there is a risk of phytotoxicity or fruit russetting, so the application of copper is best avoided after this stage (Breth *et al.,* 2000, Angello *et al.,* 2002). There are many different formulations so consult your local recommendations and registration.

Properly timed applications of an antibiotic during bloom are the primary defense against fire blight (Agnello *et al.,* 2002). There are two main antibiotics used; streptomycin and oxytetracycline. Oxytetracycline is not as effective as streptomycin but when streptomycin resistance is prevalent, it is one of a very limited number of alternatives. When applied at the correct timings, antibiotic applications during bloom are highly effective against the blossom blight phase of the disease. These sprays are critical because effective early season control often prevents the disease from becoming established in an orchard. Antibiotic applications are best used in a preventive mode; that is, applied just prior to an infection event. Using forecasting models (*see* below) it is possible to predict whether an infection event is likely to occur in the next day or two using local weather forecasts. Antibiotic sprays should not be used after bloom (van der Zwet and Beer 1995). They are generally ineffective against the shoot blight phase and unnecessary sprays can help select for resistant bacterial strains of both *E. amylovora* and, potentially, untargeted populations of bacteria (Lieberman and Wootan 1998, McManus and Stockwell, 2000).

Oxolinic acid is a newer bactericide that has been tested outside of North America for its ability to control fire blight. It has been tested for efficacy against *E. amylovora* on mainly pear in several European countries such as England, Belgium, Cyprus and Poland. Oxolinic acid has high antibacterial action against certain gram-negative bacteria by inhibiting DNA-gyrase and, subsequently, DNA replication, thus halting the ability of the bacteria to divide (Shtienberg *et al.,* 2001). The most comprehensive study conducted to evaluate the efficacy of was done in Israel. Shtienberg *et al.,* (2001) found that 300 µg ai/l was highly effective under both natural and artificial inoculations on pears and was more effective than streptomycin applied under the same conditions. Oxolinic acid at 200 µg ai/l was tested but did not consistently give adequate control. Statistically equivalent levels of control were achieved when applications were made 1 to 4 days prior to inoculation. Oxolinic acid was also effective when applied up to two days after inoculation but after this point had nearly no effect. Like other compounds there is some concern about the selection for resistant strains of bacteria, therefore it is advisable to treat this compound as conventional antibiotics (Shtienberg *et al.,* 2001).

BlightBan™ is a biological control agent used primarily on the west coast of the United States for blossom blight management. BlightBan contains beneficial bacteria, either *Erwinia herbicola* strain C9-1 or *Pseudomonas flourescens* strain A506. When these bacteria area applied to the blossoms, they colonize them quickly to produce a protective barrier that inhibits infection by the fire blight bacterium. Results have been

variable in field tests. In all cases they were found to have an inhibitory effect against *E. amylovora* but the range of efficacy was from minor to not significantly different from streptomycin (Hickey and Travis 1995, Reddy *et al.,* 2000, Sholberg *et al.,* 2001, Sutton and Anas 2002).

Messenger™ (Harpin) is a unique pesticide that labeled for blossom and shoot blight control. The active ingredient in Messenger is a protein derived from *E. amylovora* called harpin. Messenger does not have a direct effect on pathogen viability. Instead, Messenger activates natural defenses within plants to make them more resistant to diseases and physiological stresses. Plants apparently require 5-7 days for full induction of resistance, so Messenger must be applied several days prior to predicted fire blight infection periods. This is problematic because of the unreliability of available long-term weather forecasts. In experimental orchards, blight suppression provided by Messenger has been variable at best. There is not yet enough information to justify recommending routine use of Messenger.

Prohexadione calcium (Apogee™) is a growth regulator that has demonstrated potential for managing shoot blight infection in experimental trials conducted in New York, Michigan, and Virginia. Apogee is ineffective for control of the blossom blight phase of the disease and in the USA is only registered for apples, not pears. Apogee works by slowing or stopping tree growth and, therefore, is used primarily to control tree vigor and reduce the need for seasonal pruning. Apogee inhibits the biosynthesis of growth active gibberellins thereby stopping tree growth, rendering the trees relatively resistant to new infections and slowing the expansion of established infections (Evans *et al.,* 1999, Jones *et al.,* 1999). Thus, Apogee can significantly reduce shoot blight infections in orchards where antibiotic sprays failed to provide 100% control of blossom blight or there are a large number of active cankers (Yoder *et al.,* 1999). In trials conducted in New York, the best control of shoot blight was obtained when Apogee was applied during late bloom or early petal fall (when shoots were 2.5 cm long) at 90g/100L, with a second application 3 weeks later.

The problem with using Apogee to control shoot blight is that the first application of Apogee must be made before the effectiveness of blossom sprays can be evaluated (Yoder *et al.,* 1999). Research trials in New York have shown that if the first Apogee application is delayed until blossom blight symptoms appear, then Apogee will have almost no benefit for controlling shoot blight. Apogee has no effect on shoot growth or fire blight for at least 10 days after application, so it acts too slowly to be of value as a rescue treatment for orchards with blight symptoms.

In mature orchards where trees have already filled their spaces, the decision of whether to use Apogee or not can be based on a combination of its potential value as a vegetative growth inhibitor and as a supplement to fire blight control. In young orchards where trees have not yet filled their spaces, the decision is much more complex. Using Apogee for fire blight control in young orchards will cause reduced vegetative growth. This in turn will decrease profitability of the orchard in succeeding years because it will increase the number of years required for trees to fill their spaces and for the orchard to reach the break-even point. It is a difficult decision at petal fall that requires serious consideration as to whether the delay in reaching full production and/or the reduction in fruiting capacity outweighs potential losses due to fire blight and

the cost of application.

7.1.3.4 Forecasting

Successful blossom blight management can be achieved only with well-timed chemical sprays, typically of streptomycin. Normally, the number of applications is far less important than when sprays are applied. The ability to predict the onset of fire blight epidemics accurately and reliably has been the most limiting factor in improving the overall management of the disease. In this section, forecasting models used to precisely time antibiotic sprays are described.

MARYBLYT™ (Lightner and Steiner 1990, Steiner and Lightner 1992) is a forecasting program for fire blight of apples and pears that predicts blossom, shoot, canker, and trauma blight as well as the appearance of symptoms (Steiner 1990 a,b). It was first introduced in 1989 and released for commercial distribution in 1992 (Lightner and Steiner 1990, Steiner and Lightner 1992). Fruit growers and various research, teaching and extension programs in 30 US states and over 20 countries worldwide now use *MARYBLYT*. The program's popularity and widespread use are attributed to: 1) Its ability to predict specific infection events far enough in advance that protective treatments can be made and eradication measures can be timed for maximum effectiveness; 2) Data inputs, temperature and rain, are relatively simple and easy to acquire; 3) The programs (claimed) insensitivity to geographical climate differences; 4) It can be used with either U.S. or metric units; and 5) Predictions are quickly obtained and are accompanied by a variety of visual and audio prompts.

Blossom blight is the most threatening and destructive phase of the disease; providing the inoculum for the shoot, root, and trauma blight phases (Aldwinckle and Beer 1979, Breth *et al.,* 2000, Steiner 1990a). In the blossom blight submodel of *MARYBLYT* four risk factors are monitored to identify possible infection events. The risk factors and the associated minimum conditions necessary for blossom infection are as follows: (i). flowers open with intact stigmas and petals; (ii). accumulation of at least 110 cumulative degree hours (CDH) greater than 18.3 °C (198 DH > 65°F) from the start of bloom; (iii). heavy dew, = 0.25 mm rain in the last 24 hours, or = 2.5 mm the previous day; and (iv). an average daily temperature of 15.6°C (= 60 F) (Steiner 1990a). *MARYBLYT* characterizes risk as either low, moderate, high, or "infection" depending on whether one, two, three, or all four of the risk factors have exceeded their minimum values. Observation has shown that when all 4 of these parameters were met, early symptoms of blossom infection could be predicted and observed with the accumulation of 57 degree days >12.8°C (103 DD > 55° F) after an identified infection event. It was also found that the first symptoms of shoot blight occurred approximately 57 degree days >12.8°C (103 DD > 55°F) after the appearance of either blossom blight symptoms (except in years when the appearance of the winged adults of the white apple leafhopper was delayed (Steiner 1990a)). Table 7 provides a description of symptoms and when they are expected to appear based the criteria used to develop *MARYBLYT*.(Table 7).

Despite its appeal, *MARYBLYT* is not a perfect forecaster. The model tends to predict infections when none occur, especially in less susceptible varieties and in areas with no history of fire blight, resulting in unnecessary applications of antibiotics (Jones

Table 7: Appearance and timing of types of fire blight symptoms.

Blight Type	Early symptoms		Intermediate Symptoms	Late symptoms
	What to look for	When to look[b]		
Blossom blight	Dark spots to streaks and\or ooze droplets on flower bud petioles.	57 CDD>18.3°C (103 CDD>55°F) after a predicted BB infection event (can be 5-30 days).	Wilt and dis coloration of flower cluster.	Flower cluster and cluster leaves discolored or necrotic; spur canker may extend into supporting branch.
Canker blight	Narrow (1-2mm), water soaked or diffuse brown zone in green bark around margin of overwintering canker. Must cut through outer bark at canker margin to be seen. Brown streaks in inner bark extending several cm from canker margins.	109 CDD>12.7°C (196 CDD>55°F) after green tip. Most often appears about 1-2 weeks after primary petal fall, but can appear during bloom in seasons that are earlier\warmer than usual. Vegetative shoots near active canker sites show orange discoloration and wilt at tips; some basal leaves will show dark streaks and discolo -ration of mid-vein.	Dieback of vegetative shoots near canker sites; droplets of ooze often seen on shoots with symptoms.	Infected shoots near overwintering canker sites are necrotic: Infec tions often extend into the support- ing branch or may girdle that supporting limb.
Shoot blight	Vegetative shoot tips wilt; unlike early canker blight, these shoot tips remain green (no orange discolora -tion). Droplets of ooze sometimes appear on shoot axis.	57 CDD>12.7°C (103 CDD>55°F) after the appeara- nce of either BB or CB symptoms; most terminal shoots at this time show 10-12 leaves.	Varying amounts of necrosis on shoots.	Symptoms may progress from infected shoots down into supporting limb. Severity of symptoms is generally greater on trees with excessi- vely succulent growth or with marginal carbohy- drate reserves (weak?).

table 7 contd....

Trauma blight	Discoloration and wilt of shoot and spur leaves. Note: it trauma occurs during bloom (*i.e.*, severe wind), symptoms may appear as blossom blight, but with more damage to spur leaves.	57 CDD>12.7°C (103 CDD>55°F) after late frost, wind or hail event.	Many vegetative shoot tips showing yellow to orange discoloration along with wilt and leaf discoloration.	Blight symptoms usually throughout the tree canopy and may involve shoots, limbs, spurs, and fruit.
Rootstock blight	Ooze droplets evident on bark surface of rootstock just below graft union. Internal bark tissues of rootstock showing necrosis.	Usually evident 2-4 weeks after blight symptoms develop on scion shoots or spurs. Not all trees showing blight symptoms on scion tissues will show symptoms of rootstock blight.	Sudden decline and death of some infected trees by midsummer after infection of scions. Rootstock canker completely girdles tree.	Limb or tree showing early red foliage in late summer to early fall (canker symptoms on rootstock). Also look for early decline of trees in the spring following blight outbreaks and for the upward development of typical bark canker into the scion trunk from the rootstock.

[a] From Steiner and Lightner (1992); reprinted with permission.
[b] These estimates may vary within different climates

1992, van der Zwet *et al.,* 1994). The inaccuracies of *MARYBLYT* are partially attributed to how *MARYBLYT* factors in 'average temperature' and its lack of consideration of inoculum pressure. The model only generates qualitative values of infection potential. The infection potential does not change relative to which risk factor has been exceeded nor to what degree it has exceeded its minimum. Steiner observed: "When environmental conditions meet these criteria only marginally, the incidence of blossom blight infections is usually low with severity varying due to individual site differences (variation in bloom, elevation, local dews, blight history, grower management practices, etc.). By contrast, severe epidemics affecting large areas are most likely to occur when all or several of the criteria are well above the minimum activity thresholds" (Steiner 1990a). This presents problems when growers attempt to factor in the influence of varietal resistance, orchard age, or inoculum pressure. These factors are known to play a role in fire blight susceptibility but are not taken into account by *MARYBLYT* (Aldwinckle and Beer 1979, van der Zwet and Beer 1995). However, research is currently underway to address these problems which, incidentally, are not unique to *MARYBLYT*.

Cougarblight is a recent fire blight forecaster developed by Tim Smith at Wash-

ington State University during the 1980's, and it is the standard model used in the Pacific Northwest states of the US (Smith 1999). The model was developed in response to the poor performance of other fire blight risk assessment models when used in the Pacific Northwest. As with *MARYBLYT*, this model uses the bacterial growth curve of *E. amylovora* from laboratory tests. The Cougarblight system consists of a set of tables where the user looks up the number of degree-hours, in either Celsius or Fahrenheit, that have accumulated in the previous four days. With the four-day sum of degree-hours, an assessment of risk can be made relative to the inoculum pressure at that site. Inoculum pressure is quickly estimated by such criteria as whether there was fire blight present in the area in the last year (Smith 1999). If the risk is high or extreme then an antibiotic spray is recommended especially if a wetting event such as rain or dew is predicted. Using three years of weather data, Breth *et al.,* (2000) compared the performance of *MARYBLYT* and Cougarblight under New York conditions and found that they performed similarly. Whether this is true in all areas where fire blight is a problem should be assessed individually. One of the main advantages of Cougarblight is that it is very simple to use and is available, free of charge, to any user over the internet. However, Cougarblight does share some of the same shortcomings of *MARYBLYT*, for example varietal susceptibility is not explicitly defined in the model.

The Billing's revised system (BRS) was one of the first comprehensive forecasters for predicting blossom blight in apples, pears, and hawthorns. The original system (Billing's original system), from which the model is revised, was developed in England in the 1980's (Billing 1992). Like other models, knowledge of flowering period is essential and overwintering inoculum is helpful. Infection risks are calculated using weather data, inoculum levels and host susceptibility. The observed data are converted into scores from which infection risk is calculated and the decision to spray or not is made. The system also predicts when symptoms are expected to appear. A comprehensive overview of the BRS is given in Billing (1992). This model is not applied widely, although it is used in England and France. The main drawback of this system is that it is relatively complicated to use but is less so than the Billing's original system. However, BRS incorporated ideas that were novel when developed and eventually served as the basis of models that followed it.

The Penn State Fire Blight Management Model was developed in 1993. It consists of three modules; pre-season, bloom and post-bloom. In addition, an orchard risk value is calculated from an orchard profile that includes fire blight history, cultivar and rootstock. It is similar to *MARYBLYT* in the manner that it calculates blossom infection risk except that the infection risk is combined with a measure of orchard risk (Clarke *et al.,* 1993, Travis *et al.,* 1993). The model provides management options such as when a copper or an antibiotic application is needed, and when is the appropriate time for pruning. When compared with *MARYBLYT* in the field, it was found that *MARYBLYT* predicted more infection events than the Penn State model under high risk conditions.

7.1.3.5 Managing fire blight after bloom

The focus after bloom is on minimizing shoot blight (especially if blossom blight was severe) and the development of overwintering cankers that can serve as next year's

inoculum source. Minimizing shoot blight damage begins by pruning out infected limbs as soon as symptoms are detected and before extensive necrosis develops. Growers should use management systems that promote early cessation of tree growth without adversely affecting tree vigor. Excessive vigor is an important component of orchard risk for fire blight. When tree growth continues past mid summer, the likelihood that late season or trauma blight infections will overwinter increases. Nitrogen fertilizer should be applied based on foliar analysis but not after July. In young blocks, it is possible to use Apogee as means to terminate growth and possibly minimize the damage due to fire blight.

Trauma events (hail, high winds) can put any orchard block at risk because of damage to the tree's natural defenses such as bark. Varieties that are considered relatively resistant to blossom blight and shoot blight can suffer severe blight under trauma conditions. If a trauma event occurs when trees are actively growing, application of antibiotics within 12-24 hours after the trauma may limit the severity of trauma blight. After mid-summer when trees have hardened-off for the season, antibiotic protection following trauma events may be unnecessary because trees are thought to be fairly resistant to fire blight. Applications of antibiotics may be not be possible after mid-summer anyway because of the days-to-harvest limitations on the label.

7.2 Crown gall

Crown gall is a disease of worldwide distribution. Although the disease can affect both nursery stock and mature trees, it is much more problematic in nurseries and in young orchards. The disease has been reported to cause losses of 80-100% in many European and Pacific northwest nurseries (Garrett 1987, Canfield and Moore 1991). The disease is caused by the bacterium *Agrobacterium tumefaciens* (Smith & Townsend) Conn. It was first reported on apple in California in 1892 by Wickson and Woodworth but it was not until fifteen years later when Smith and Townsend identified that the galling was caused by a bacterium. Historically, the disease was manageable in the nursery using chemical compounds containing mercury (Grimm 1987). However, the use of mercury compounds is no longer permitted and, since then, a rise in losses due the disease has occurred.

7.2.1 Symptoms

The characteristic symptom of the disease is swelling along the roots or on the trunk just above the soil line. Young galls are smooth, soft and globular to elongated. The tissue is often pale in comparison to the surrounding healthy but darkens as the gall ages. The internal tissue of young galls is light in color and porous but, as they age, the tissue darkens and deep fissures form. The galls may completely surround the root or crown or may appear as a growth off to one side (Fig. 33). Galls start small and can grow to a typical 0.6 to 10 cm in diameter; in extreme cases galls can reach to over 30 cm in diameter. Infected trees are often stunted and may produce small, chlorotic leaves. These are symptoms typical of a tree under stress, similar to the symptoms encountered with tress infected with *Phytophthora*. In the nursery, apple rootstock infected with

crown gall is often too weak to be budded. In this case, budding may be delayed for 1 year or the rootstock may be discarded.

7.2.2 Disease cycle

The bacteria causing crown gall are a natural resident of many soils. The bacteria can enter the tree through wounds caused by machinery (particularly tractor wounds), insects, freeze damage, or even hail damage. The process of propagation, where larger wounds are made, is one of the most common methods in which trees become infected with the crown gall bacterium. Once the bacteria enters the tree, it incorporates a small piece of its DNA, called the Ti-plasmid, into the apple trees DNA. The Ti-plasmid carries on it genes that, among other things, induces the tree to produce growth factors

Figure 33: Rootstock infected with crown gall (Courtesy of Tom Burr).

which cause rapid and uncontrolled cell division. This is where the gall forms. As galls enlarge, the vascular tissue of the plant is crushed by the proliferation of cells disrupting the flow of water to the above ground part of the tree. Secondary spread of the bacteria occurs from the movement of soil by wind and splashing rain, or through direct movement of the bacteria from insects, pruning tools, or though propagation.

7.2.3 Disease management

The disease is best managed in the orchard by implementing practices that minimize wounding. As the bacteria are a natural resident of many soils, this prevents not only

infection, but the multiplication of the bacteria in its host. The use of chemical fumigants, such as methyl bromide, has proven to provide minimal reduction in disease (Utkhede and Smith 1993). Soil solarization, the procedure in which soil is heated under large plastic tarps, has been shown to reduce the resident *Agrobacterium* population by greater than 90%; however, this did result in a decrease in crown gall incidence (Raio *et al.,* 1997). Serious epidemics often result from the introduction of infected material. Therefore, carefully inspecting trees for the disease before planting can avoid tremendous losses. Dipping the root system of nursery trees in a 1% copper sulfate solution for 2 hour can eliminate the bacteria from the root system, as well as provide some limited protection. Infected trees should be removed from the field, including the removal of infected root system. The diseased plant material should be removed from the orchard and burned

In the nursery, sterilized soil must be used to minimize disease. Propagation tools should be sterilized frequently during budding in order to minimize the spread of the disease through the wounds made during propagation. For some crops infected by this bacterium, their rootstocks can be dipped into a solution containing the antagonistic bacteria *Agrobacterium radiobacter* strain K84. This product has been commercialized and sold under such trade names as Gall-Trol and has been reported to give up to 99% control of crown gall against sensitive strains. However, for some unknown reason, this is product is ineffective in preventing crown gall in apple.

7.3 Blister spot

Blister spot is caused by the bacterium *Pseudomonas syringae* pv. *papulans* (Rose) Dhanvantari, and is a disease of minor importance because it only causes significant problems on the cultivar 'Mutsu' , 'Fuji', and few less popular varieties. It is most commonly reported in New York State, USA and Ontario, Canada. The disease was first described in Missouri in 1916 (Rose 1917). It was later reported in several other states including New York, Michigan and Virginia as well as the Province of Ontario (Burr and Hurtwitz 1979, Dhanvantari 1969, Jones *et al.,* 1991; Yoder *et al.,* 1981). The disease has spread to other areas including western Canada and Italy (Sholberg and Bedford 1997). *P.s.* pv. *papulans* most recently has been found epiphytically on apple trees in France, although there have been no reports of disease to date (Kerkoud *et al.,* 2000).

The disease is not present in Japan where 'Mutsu' is an especially important cultivar. The disease is problematic mainly on 'Mutsu' (Fig.34). However, Sholberg and Bedford (1997) isolated *P.s.* pv. *papulans* from 'Jonagold' and 'Fuji' from a natural field infection. It was found that one of the British Columbian strains was more virulent on 'Fuji' than strains from Michigan and New York.

7.3.1 Symptoms

In its early phase, blister spot is a relatively unobtrusive disease. The most economically important phase of the disease is the fruit infections. Most infections occur on the lower half of the apple. From early to late June, the first infections are observed as small darkened water-soaked areas generally around lenticels (Smith 1944). From there, small

raised blisters are formed. The blisters at first start with a light color but eventually become purplish-black as they expand towards the end of the growing season. The epidermal layer covering the blister dies and will often flake off the surface. This stage is the most obvious symptom of blister spot and can be mistaken for tiny lesions caused by *Venturia inaequalis* (Sholberg and Bedford 1997, Smith 1944). The lesions, generally circular although are sometimes lobed, rarely become larger than 4-5 mm in diameter. The infections are shallow, not extending more than 1-4 mm into the fruit flesh. Fruit quality can be greatly reduced because of the number of lesions that can occur, between 10 and 200 per fruit (Burr and Hurtwitz 1979). *P.s.* pv. *papulans* also causes mid-vein necrosis of young succulent leaves which is suspected to occur under specific conditions. It is relatively rare phase of the disease, having been reported only on 'Mutsu'. The disease is generally associated with warm temperatures, high relative

Figure 34: 'Mutsu' fruit infected with blister spot (Courtesy of Megan Dewdney).

humidity, precipitation, wind and an elevated epiphytic population of *P.s.* pv. *papulans*. The lesions are a light tan to rust color. The crusty lesions occur on the under surface of the leaves of shoots and spurs causing puckering and curling generally misshaping them. Leaf symptoms can normally be found before those on the fruit (Bonn and Bedford 1986).

7.3.2 Disease cycle

P.s. pv. *papulans* overwinters in leaf scars and dormant buds (Burr and Katz 1981). It

has been found in apparently healthy buds but is often associated with internal necrosis. It is unclear whether *P.s.* pv. *papulans* is responsible for the necrosis or is merely colonizing the weakened tissue but there is a clear pattern of only the center structures of the bud being colonized (Burr and Katz 1984). The pathogen is most commonly located in the terminal buds but can be found down to the distal end of the shoot in the spring. High populations of the bacteria can also be found in leaf scars which may be the entry site into the shoot (Bedford *et al.,* 1984). *P.s.* pv. *papulans* is an epiphyte on both leaves and fruit of 'Mutsu'. It can be isolated throughout the summer growing season, sometimes being the only bacterial organism present. Historically very low populations of *P.s.* pv. *papulans* have been found on other cultivars (Bedford *et al.,* 1988). This could be the explanation for why 'Mutsu' is more readily and severely infected than other cultivars.

Fruit are most susceptible from 2-2.5 weeks after petal fall and become increasingly susceptible for another two to four weeks. After this period, the level of susceptibility sharply declines. Variability in the period of susceptibility can occur with weather conditions that affect growth rates of the apple (Burr and Hurwitz 1981). The fruit are infected through the stomates and it is assumed that the leaves are infected in a similar manner (Burr and Hurwitz 1979, Bonn and Bedford 1986). Symptom expression on fruit is associated with high epiphytic populations of the pathogen but it is unclear how long the latent period is or the conditions required for infection. It has been suggested that warm wet weather is associated with infection periods but this is unconfirmed (Bedford *et al.,* 1988).

7.3.3 Disease management

Applications of streptomycin starting 2-2.5 weeks after petal fall and continuing for another 2 to 4 weeks is the standard program for control of blister spot. (Burr 1991, Burr and Hurwitz 1981). This program worked fairly well in New York State from 1979 until 1986, when resistant strains of the bacteria became prominent. Resistance was associated with a plasmid that can be transferred from a resistant donor to a susceptible recipient (Burr *et al.,* 1988). Similarly, resistance became problematic in Michigan with resistant strains harboring the New York plasmid plus a new plasmid that appeared to be unrelated to the other indicating that selection was independent (Jones *et al.,* 1991). Unlike the fire blight bacterium, however, resistance is not a stable trait in the blister spot bacteria. In other words, resistance to streptomycin declines sharply in the absence of streptomycin. Therefore, growers can opt not to use streptomycin for a season when faced with loss of efficacy and then return to its use the following season and expect appreciable control. Fosetyl-Al is another option for managing blister spot. Like streptomycin, fosetyl-Al should be applied 10 to 14 days after petal fall followed by two additional sprays at weekly intervals.

Currently, streptomycin and fosetyl-Al are the two best materials available for managing blister spot. Both products will give about the same level of control when applied alone and at the appropriate timings. Slightly better control can be achieved if the two products are tank-mixed; this mixture may also be a useful for resistance management (Ellis *et al.,* 2000). Unfortunately, the level of control can be quite variable. In

years when disease pressure is high, you should expect less than 50% control with the most effective treatment. Alternative control options are somewhat limited. Some tests have been under taken with copper applications and other antibiotics with mixed results. Nonetheless, an application of fixed copper at green tip has is commonly used to reduce disease severity, presumably by knocking back epiphytic populations of the bacteria (Burr 1991, Burr *et al.,* 1988).

8. Postharvest Diseases

After harvest, apples are marketed to processors or to the fresh market through packers and shippers, depending upon the variety. A portion of these apples are placed into a storage facility where they will be marketed in the future on an 'as needed' basis. Apples destined for storage are stored for an average of 6-10 months in either cold storage or in controlled-atmosphere (CA) environment prior to being sold.

Prior to be being stored, apples are drenched with fungicide(s) to prevent decay in storage. Apples destined for CA storage may also be drenched with diphenylamine (DPA) to prevent apple scald, a physiological disorder associated with cold storage. In the United States, just under half of the apples sent to a packinghouse were treated with DPA for control of apple scald in 1996. Also, about 10% of apples were treated with sodium-*o*-phenyl-hypochlorite (SOPP), another 30% with sodium hypochlorite (bleach) and over 60% were treated with thiabendazole (TBZ) to manage storage decay. In most cases, TBZ was included in both the drench and in the flotation tank; sodium hypochlorite and SOPP are almost exclusively used in the flotation or dump tank.

A number of storage decays or postharvest diseases have been reported. The two major postharvest diseases are blue mold, caused primarily by *Penicillium expansum* Link, and gray mold, caused by *Botrytis cinerea* Pers.:Fr. Mucor rot, although a serious disease, does not occur as consistently as either blue mold or gray mold. Bull's eye rot is another important storage rot; however, this disease is managed primarily in the orchard (*see* above).

8.1 Blue mold

Blue mold is the most economically important postharvest disease of apples (Rosenberger 1990b). The disease, also known as soft rot or wet rot, causes substantial losses in the United States, Canada, England, Israel, South Africa, and many other countries. Before the wide usage of postharvest fungicides and controlled atmosphere (CA) storage, blue mold accounted for nearly 90% of the postharvest losses in storage facilities. Losses due to postharvest diseases are particularly costly because the loss represents the cumulative value of growing, harvesting, and storing the product. Losses to blue mold are often less than 1% in modern storage facilities, but it is still the leading cause of fruit decay.

Blue mold is caused by fungi in the genus *Penicillium* (Sanderson and Spotts 1995). At least 11 species of *Penicillium* have been reported to cause soft rot, but *P. expansum* Link is by far the most commonly isolated organism (Borecka 1977, Heald and Ruehle 1931). There are numerous synonyms of *P. expansum* and these include *P.*

glaucum Link, *Coremium glaucum* Link, *Floccaria glauca* Grenville, and *Coremium vulgare* Corda. The other species of *Penicillium* reported as causing postharvest rot are: *P aurantiogriseum* Dierckx (Borecka 1977) with synonyms *P. cyclopium* Westling (Borecka 1977), *P. martensii* Biourge (Heald and Ruehle 1931) and *P. solitum* Westling (Koffman and Penrose 1987); *P. brevicompactum* Dierckx (Barkai-Golan 1974); *P. crustosum* Thom (Prusky and Ben-Arie 1985); *P. diversum* Raper & Fennel (Borecka 1977); *P. funiculosum* Thom (Combrink *et al.,* 1985); *P. puberulum* Bainier [syn. *P. commune* Thom](Koffmann and Penrose 1987); *P. rugulosum* Thom (Barkai-Golan 1974); *P. spinulosum* Thom (Borecka 1977); *P. verrucosum* Dierckx (Heald and Ruehle 1931); and *P. viridicatum* Westling [syn. *P. olivinoviride* Biourge](Heald and Ruehle 1931).

8.1.1 Symptoms
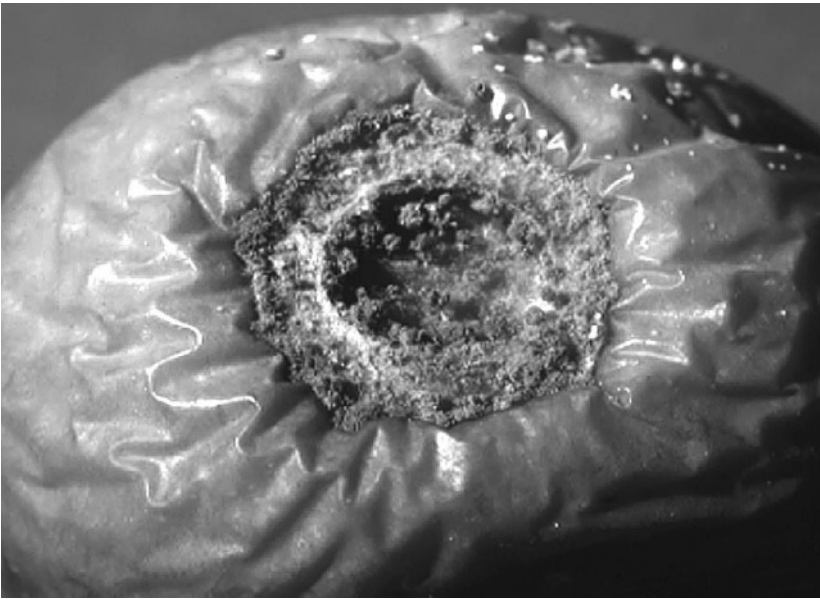
Figure 35: Blue sporulation characteristic of blue mold infected fruit (Courtesy of David Rosenberger).

Blue mold can enter the fruit through wounds, stem-end invasion, or as a core rot. Infection has also been reported to occur through lenticels but the importance of this mode of infection has yet to be determined (Rosenberger 1990b). Wound invasion is the most common form of entry in to the fruit. Infection is first visible as a soft and sunken, yellow to pale-brown, circular lesion on the surface of the fruit. Lesions expand rapidly and can quickly macerate the fruit under ideal conditions. It has been shown that the maceration is caused by a pectolytic enzyme caused by the fungus (Cole and Wood 1961). As the rot expands and tissue decays, the tissue becomes watery and the

skin above the rot takes on a wrinkled appearance.

A diagnostic symptom of this rot is a strong earthy or musty odor and unpleasant taste. If left under humid conditions, the fungus produces numerous blue-green tufts of spores on the surface of the fruit (Fig. 35). Observation of these tufts is considered a positive identification of the disease. However, sporulation typically does not occur under CA conditions, so the fruit must be left under ideal conditions to induce sporulation or the pathogen must be isolated from the fruit in order to positively identify the disease.

8.1.2 Disease cycle

Although *Penicillium* spp. can be readily isolated from the orchard, the disease is not considered to be a serious problem in the field. Incidence of blue mold in the field is almost strictly confined to apples which have fallen to the ground. In a survey of eight orchards in Lleida, Catalonia, only 0.2% of the fungi isolated were of the genus *Penicillium*; compare this to 65% of the total fungi isolated in packinghouses in the same area (Viñas *et al.,* 1991).

Blue mold is, however, a major disease in packing houses. Contaminated storage bins, picking boxes or decaying fruit lying on the floor or stuck to the walls of storage bins serve as the primary source of inoculum in this environment. Conidia are produced on the decaying fruit or on the surfaces bin walls present in packinghouses and storage facilities. Infection occurs when either air- or water-borne conidia enter the wounds of fruit damaged in either harvesting or handling, through insect injuries, scab lesions, or open lenticels (Tepper and Yoder 1982). Airborne inoculum is almost always present in packinghouse and storage facilities. Waterborne infections occur when fruit contact conidia in postharvest drench solutions and in water flumes used to float the fruit onto packing lines. The water used for drenching and in the water flumes harbor numerous spores as a result of successive washings of infected fruit and contaminated bins. In studies conducted in the Pacific northwestern United States, nearly 50% of samples of dump tank water contained between 10 to 100 conidia per milliliter of *P. expansum* and this number could exceed 4000 spore per milliliter (Spotts and Cerevantes 1993). Spotts (1986) found a direct relationship between inoculum concentration and decay in pears.

8.1.3 Disease management

Sanitation is the primary method for reducing the incidence of blue mold in the packinghouse environment. Storage bins often have decaying fruit stuck to the sides of the walls and these should be scraped from the bins. The bins should be scrubbed and rinsed with chlorinated water (1000 ppm) to sterilize their surfaces and reduce the inoculum potential. Moreover, mashed or decaying fruit should be removed from the floors of the packinghouse as soon as discovered. A single rotting fruit is capable of producing millions, if not billions of spores. A few of these rotting fruit can raise spore counts to over 300,000 spores per cubic foot of air. Although hard on machinery, chlorine solution (1000 ppm) should be used routinely to wash machinery, bins, and packinghouse walls and floors. Temperature and humidity in the packinghouse should not be too high as

these conditions promote the growth and sporulation of postharvest pathogens.

Fruit destined for cold storage should be moved into storage as quickly as possible to remove the "field heat". Storage facilities should promote good air movement for rapid cooling and bins should be stacked appropriately to allow good air flow over the fruit. When fruit are destined for CA storage, CA conditions should be established as quickly as possible; CA conditions provide a particularly poor environment for fungal growth.

Drenching apples with fungicides and scald-preventing chemicals is a common practice. However, certain varieties of apples and apples that are to be stored for less than 3 months are typically not treated. Many warehouse managers now use drive-through drenchers to apply fungicides and scald chemicals. Several factors should be considered when using drive-through drenchers. First, the tanks containing the chemicals should be agitated continuously to assure adequate mixing of the solution. The nozzles applying the solutions should be oriented to be sure that the chemicals are spraying the fruit and not just the sides of the bins. The bins receiving the treatment need to remain stationary for 30-60 seconds to assure adequate coverage. The drench solution should either be filtered and/or discarded when the solution is dirty.

Drenches can be treated with fungicide to reduce waterborne infections. The most commonly used fungicides in drenches are the benzimidazoles and include benomyl, thiophanate-methyl, thiabendazole, bleach, SOPP and imazalil. Imazalil is not registered as a postharvest treatment on apples in the United States. Captan is also registered as a postharvest treatment. Its use in the United States is small because of the zero-residue tolerance in place in many export markets. The use of captan with DPA is not recommended. SOPP is highly effective against *Penicillium* and *Botrytis*. SOPP does not bind with soil, as does chlorine, and is not as damaging to machinery. However, SOPP may cause fruit injury if the fruit is in contact with the chemical for too long.

The effectiveness of many postharvest treatments is limited due to the occurrence of resistance strains (Rosenberger *et al.,* 1991). Interestingly though, where benzimidazole-resistant strains existed, treatment with benzimidazole fungicides continued to provide about 10 days of protection against these strains. Furthermore, it was shown that DPA has fungicidal effects against benzimidazole-resistant strains of blue and gray mold (Rosenberger and Meyer 1984). Over time, though, strains resistant to both DPA and benzimidazoles have emerged and losses began to accumulate.

Spotts and Cervantes (1993) proposed the use of filtration to remove conidia from flotation water. A triple-stage filter unit with a 0.45-ìm terminal cartridge removed 92% of the conidia from drenching suspensions. Unfortunately, the system lacked the capacity to provide continuously filtered water that would provide commercially acceptable levels of control of blue mold.

When apples come out of storage, the bins are emptied by floating the apples from the bins in a larger "dump tank¨. Since the apples are not sorted or culled prior to being emptied, flotation water or dump tank and flumes often harbor high concentrations of spores. To prevent fruit infection from exposure to the spore-laden flotation water, chlorine or SOPP is often added to the water. To a lesser extent fungicides are added. Spotts (1986) reported that both chlorine and SOPP reduced the level of *Penicillium* spores. Chlorine effectively reduced spore germination but had no residual activ-

ity and should only be used in solutions where the pH is in the range of 6.5-7.5. When the pH drops below 6.5, chlorine is released as a gas into the atmosphere; when the pH rises above 7.5, it loses its efficacy. It is important that dump tank solutions be free of particulate matter as chlorine can be bound to soil particles.

8.1.3.1 Biological control

Over the past 10 years or so, a substantial research effort has been directed to find and commercialize biological control options for management of postharvest diseases. During this period, a number of organisms have been tested and have shown good efficacy against blue mold and/or gray mold. Yet, only a few are commercially available and it is difficult or perhaps to early to assess whether or not these products will be widely accepted by producers. Two products, BioSave-10 and BioSave-11 have been registered by EcoScience Corporation. BioSave-10 and BioSave-11 are formulations of the bacteria *Pseudomonas syringae*. BioSave-11 is targeted for pears, whereas BioSave-10 has better activity on apples. A third biocontrol formulation of the yeast *Candida oleophila* and is marketed as Decco I-182 (formally Aspire by Ecogen Inc., Langhorne, PA). However, a number of other potential organisms including various strains of *Aureobasidium pullulans, Rhodotorula glutinis,*, *Bacillus subtilis*, *Sporobolomyces roseus* have shown efficacy against blue mold, gray mold, and *Pezicula malicorticis* in field trials but are not yet (or may never be) available commercially (Janisiewicz *et al.,* 1994, Leibinger *et al.,* 1997)

    Biological control is difficult to achieve for a number of reasons. Primarily, the mechanism behind control is through competitive exclusion. The biocontrol agent simply has to colonize potential entry sites of the pathogen before the pathogen does. Many antagonistic microorganisms have exhibited good postharvest control under laboratory conditions and sometimes under semi-commercial conditions. However, under commercial conditions, they often do not perform as well as standard fungicides. This is unacceptable in commercial production and perhaps the primary reason why biocontrol has not been accepted in industry for control of postharvest diseases of apple.

    Another downfall to biological control is that biological control organisms tend to be organism specific. Packinghouses must contend with numerous diseases, thus it is not realistic for packinghouse managers to purchase individual products for each pest that must be controlled. Essentially, this becomes too complicated to manage and, more importantly, too costly.

8.2 Gray Mold

Gray mold is caused by the fungus *Botrytis cinerea* Pers. The sexual state of the fungus is *Botryotinia fuckeliana* (de Bary) Whetzel. Gray mold is the second most important postharvest disease on apple next to blue mold; it is considered to be the most important postharvest disease of pears (Rosenberger 1990c). In apples that are moved to storage without treatment, decays caused by *B. cinerea* often predominate. The pathogen attacks numerous crops worldwide and causes significant losses in crops such as strawberry, raspberry, onion, and several ornamentals. Gray mold is perhaps the single

most important postharvest disease worldwide.

8.2.1 Symptoms

Symptoms usually develop or start at the calyx or stem end of the fruit or at wound sites. Lesions on the fruit begin as small water-soaked areas that enlarge with time turning from greyish-brown, to light brown, and eventually to a darker brown. On some red varieties, a dark ring about 2-3 mm in diameter may form around the lenticels and this has led to the name "spot rot" (Adams and Tamburo 1957). The rotted tissue tends to be firmer than tissue infected with blue mold and does not separate easily from healthy tissue. Unlike blue mold, gray mold-rotted fruits have a relatively pleasant odor rather than a musty odor (Fig. 36). White or grayish-white mycelium will form on the surface of
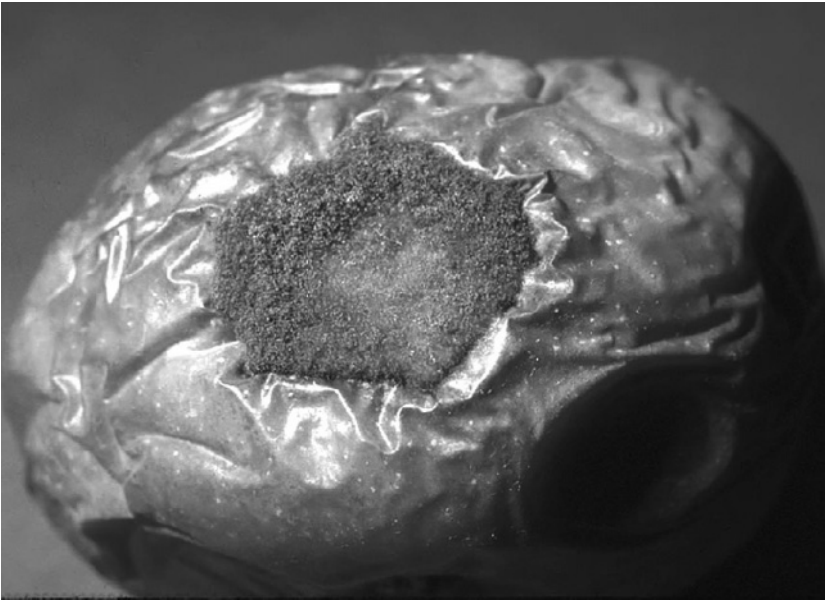
Figure 36: Fruit infected with *Botrytis* (Courtesy of David Rosenberger).

the rot under humid conditions. The production of spores gives the mycelium the characteristic gray color, however, little sporulation occurs at cold-storage temperatures. Apples with *Botrytis* decay coming out of CA storage appear firm and tan, and when completely-decayed, look very much like baked apples.

8.2.2 Disease cycle

Like blue mold, gray mold is rarely seen in the field (although it does cause dry eye rot [*see* above]). However, *B. cinerea* is a common saprophyte and can be readily isolated from rotting or decaying tissue lying on the orchard floor. Spores produced from these

sources may colonize apple tissue wounded during harvest and subsequently be transported to the packinghouse or storage facility. Infections can then progress while in storage and the disease can spread to healthy adjacent fruit by simple contact. This often results in a mass of infected fruit, termed nesting, leading to the production of spores. These spores are then spread to other fruit when they are mixed with fruit in dump tanks or, less frequently, by exposure from airborne conidia.

8.2.3 Disease management

Gray mold can be effectively treated with postharvest applications of benzimidazole fungicides or a combination of benzimidazole and captan. Some strains of *B. cinerea* have developed resistance to the benzimidazoles but these strains are often controlled by DPA.

Therefore, *Botrytis* decays are uncommon on fruit receiving postharvest treatments because relatively few strains of *Botrytis* have developed resistance to both DPA and TBZ, whereas such resistance is common in *Penicillium.*

8.3 Mucor rot

Mucor rot is caused by the fungus *Mucor piriformis* Fischer. It was first reported as a decay of apples in Washington by Heald and Ruehle (1931). It is a disease of worldwide importance occurring in the United States, Canada, Australia, South Africa, and Europe but it is less problematic than both blue mold and gray mold.

8.3.1 Symptoms

Apple tissue infected with *Mucor piriformis* appears light brown, soft, and watery. The infection usually develops at wound sites or at the calyx or stem end of the fruit. Complete decay occurs rapidly under packinghouse conditions and in about 2 months in cold storage.

Fully rotted apples release large amounts of liquid containing the sporangiophores or infective propagules of the fungus, which may spread the disease, but this is relatively uncommon in apples.

8.3.2 Disease cycle

M. piriformis survives in the soil and is associated with organic debris, including fallen fruit. Spores of the fungus come in contact with susceptible fruit through rain splash, directly when fruit fall to the ground, in the dump tank, or by rodents, birds, and insects that can move spores from rotting fruit. Fruit are most susceptible to infection from approximately 1 month prior to harvest through all its storage life. Populations of the fungus decline rapidly when soil temperatures exceed 33°C. The fungus survives best at temperatures around 20°C and where ample organic matter exists; the fungus is not a good competitor in the soil.

8.3.3 Disease management

In the orchard, the disease is managed by harvesting fruit carefully. That is, bins are placed in areas of the orchard that minimize the collection of soil and debris, fruit are harvested in dry weather, and those fruit which fall to the ground during harvest should not be placed inside the bin. Fungicides applied prior to harvest are generally ineffective. After harvest, removing fallen fruit from the orchard floor will help to reduce the build-up of the pathogen in the field. In the packinghouse, dump tanks and flume water should contain chlorine or SOPP to reduce spore levels.

9. Virus and Virus-like Diseases

Diseases caused by viruses are perhaps the most difficult to diagnose and identify. Symptoms can often be confused with other conditions, such as nutrient deficiencies, because visible signs of the pathogen are never present. Complicating diagnosis is that symptoms can be quite variable depending on the variety infected. Nonetheless, over the last 60 years or so, plant pathologists have begun to recognize that several maladies, once gone undiagnosed, are caused by viruses. This has happened because laboratory procedures and diagnostic methods have greatly improved. Even still there are many problems that are believed to be caused by viruses or virus-like organisms that have yet to be shown conclusively.

Many of the important viral diseases are transmitted through grafting. Thus, for the grower, it is important that trees be purchased from a reputable nursery that certifies their trees free of known viruses. Similarly, in propagation it is important that propagation material, mother trees, be routinely tested to assure that they are free of any threatening viruses. Yet, certification assures only that trees are free of the viruses that were being screened against. Unknown viruses, viruses that are not considered threatening, and viruses for which detection methods are unavailable are not found during these processes.

Since most viral diseases are managed as a process of propagation and not in the field, I will give only a brief description of a few of the most common viruses. Indeed, an entire chapter could be devoted to viral diseases of apple and I do not intend to diminish the importance of viral diseases in apple production.

9.1 Apple mosaic virus (AMV)

Apple mosaic is one of the most commonly occurring viruses of apple. The virus is also known as the common apple mosaic virus, apple infectious variegation virus, rose infectious chlorosis virus, rose mosaic virus, plum line pattern virus, and European plum line patter virus (Ogawa and English 1991). The disease is known to occur in the United States, Canada, England, New Zealand, and throughout much of the apple growing region of the world. In the United States it was first observed by Stewart in 1910 on apple trees in Long Island, New York and later in Maine by Morse (1916). On the west coast, Blodgett (1923) determined that AMV could be transmitted through grafting. Historically, this was apparently the first instance in that a virus was shown ca-

pable of being transmitted through grafting (Ogawa and English, 1991).

Young leaves of infected apple trees develop pale to bright cream colored spots as they expand (Fig. 37). The spots generally become necrotic and turn brown as they age. Infected trees can still produce a good crop. However, yield loss is dependent on the severity of infection and the variety infected and can vary from 0 to 50% (Welliver and Podleckis 1995). Almost all varieties are susceptible to the virus but some express symptoms more readily than others. 'Golden Delicious' and 'Jonathan' are very susceptible, 'McIntosh' is only moderately affected.

9.2 Apple union necrosis and decline (AUND)

Apple union necrosis and decline (AUND) is caused by tomato ringspot virus (TmRSV).

Figure 37: Foliar symptoms of apple mosaic virus (AMV) (Courtesy of David Rosenberger)

Tomato ringspot virus causes disease in numerous fruit crops including stem pitting of peach and nectarine and constriction disease in Stanley prune. The disease is only a problem on grafted trees where the scion variety is resistant to TmRSV and the rootstock is tolerant. 'Delicious', 'Quinte', 'Tydemans's Red', 'Jerseymac', and 'Jonathan' are varieties resistant to TmRSV; 'Golden Delicious', 'Empire', and 'York Imperial' are tolerant varieties. Tolerant rootstocks include MM.106, EM7A, EM26, EM9, MAC39, MAC9, P2, and Budogovosky 9; resistant rootstocks include M.4, M.7, Ottawa 3, and Novole.

The symptoms of AUND do not appear until trees reach bearing age. In this

sense, budbreak is often delayed in the spring, and young leaves appear dull, pale-green and are small and sparse. The growth of terminal shoots is reduced and this manifested in shortened internodes. Infected trees tend to flower heavily and bear a heavy load of small, highly colored fruit. Leaves are off-color and leaf fall occurs earlier in infected trees. Infected trees tend to produce numerous water sprouts at their base and there is often a swelling above the graft union. Infected trees are typically weak-ened at their graft union. As a result, trees may snap at this point, particularly under high winds. Peeling the bark at the graft union will show a distinct necrotic line at the scion/rootstock union. The infected rootstock often appears thicker, spongy, and may be orange-colored (Fig. 38).

TmRSV can survive in a number of broadleaf weed hosts, such as dandelion. In nature, the virus is spread short-distance from weeds to apples via the dagger nema-

Figure 38: Symptoms of apple union necrosis decline at the graft union (Courtesy of Turner Sutton)

tode (*Xiphinema americanum* Cobb and *X. rivesi* Dalmasso). TmRSV is capable of long-distance movement in the seeds of infected hosts, *e.g.* , dandelion seeds; weed management can help prevent the movement from in the field. Planting on sandy soils, or particularly on soils that harbor populations of the dagger nematode, should be avoided. The virus can also be transmitted through grafting.

9.3 Apple latent viruses

Apple chlorotic leaf spot virus, apple stem grooving, and apple stem pitting virus are three common latent viruses. Latent viruses are those viruses which survive without causing symptoms in most cultivars, but may cause symptoms in other varieties or

scion/rootstock combinations. There are number of latent viruses that have been described in apple, but these three are perhaps the most common.

Apple chlorotic leaf spot virus (CLSV) may cause chlorotic leaf spots or rings, leaf distortion, or leaf stunting. Apple stem grooving virus (SGV) produces chlorotic leaf spots, stem grooving and pitting, union necrosis, and swelling above the graft union (Podleckis and Welliver 1995). Apple stem pitting virus (SPV) causes pitting and grooving, as well as epinasty and decline. Again, these symptoms are not expressed in most cultivars, although many varieties may indeed harbor these viruses. It is only in those varieties that do express symptoms where significant losses are possible. No natural vector is known for any of these three viruses as diseased trees can be found growing next to healthy trees in orchards.

9.4 Apple proliferation

Apple proliferation is caused by a phytoplasma called the Apple Proliferation Phytoplasma (APP). It is the most important graft-transmissible and vector-borne disease in southern Europe. The phytoplasma is endemic to apple in southern Europe and is not known to infect other fruit trees. The disease is not known to occur in either North or South America, but this is probably a result of the restricted distribution of the leafhopper vectors rather than a result of environmental constraints.

APP induces a range of symptoms including witches' broom, reduced fruit size or inhibited fruit development, reduced leaf size but continued growth of the stipules, reduction in flowering and phyllody (*i.e.*, transformation of flower petals into green leaflets), growth of dormant auxiliary buds in summer, and formation of abundant but thin root hairs. All of these symptoms may not develop at once or on the same tree.

APP is not seed transmissible. It is, however, graft transmissible and is spread naturally by several leafhoppers including *Philaenus spumarius*, *Aphrophora alni*, *Lepyronia coleoptrata*, *Artianus interstitialis*, and *Fieberiella florii* (Seemüller 1990). Insecticides can limit transmission by these vectors. Infected trees often recover, so the benefit of roguing infected trees is not clear. Trunk injections of oxytetracycline between harvest and leaf drop has been used to reduce symptoms, and appears to be effective for 1 or 2 years. APP colonizes and overwinters in the root systems of infected trees. Thus, the use of resistant rootstock is critical in regions where APP survives. In the establishment of new orchards, only certified APP-free trees should be used.

10. References

Abeln, E.C.A., de Pagter, M.A. and Verkley, G.J.M. 2000. Phylogeny of *Pezicula*, *Dermea* and *Neofabraea* inferred from partial sequences of the nuclear ribosomal RNA gene cluster. Mycologia, 92:685-693.

Adams, R.E. and Tamburo, S.E. 1957. The West Virginia spot-rot complex of apple in 1956. Plant Disease Reporter, 41:760-765.

Agnello, A., Kovach, J., Nyrop, J., Reissig, H., Rosenberger, D. and Wilcox, W. 1999. Timing sprays to control flyspeck. In: "Apple IPM: A guide for sampling and managing major apple pests in New York State". NY State IPM Program, Geneva, Publication 207. pp 22-23.

Agnello, A.M., Landers, A.J., Turechek, W.W., Rosenberger, D.A., Robinson, T.L, Schupp, J.R., Cheng, L., Curtis, P.D., Breth, D.I. and Hoying, S.A. 2002. Pest Management Guidelines for Commercial Tree-Fruit Production. Cornell Cooperative Extension Publications, Ithaca, NY. 222 p.

Aldwinckle, H.S. 1974. Field susceptibility of 51 apple cultivars to apple scab and apple powdery mildew. Plant Disease Reporter, 58:625-629.

Aldwinckle, H.S. and Beer S.V. 1979. Fire blight and its control. Horticultural Reviews, 1:423-474.

Aldwinckle, H.S., Pearson, R.C., and Seem R.C. 1980. Infection periods of *Gymnosporangium juniperi-virginianae* on apple. Phytopathology, 70:1070-1073.

Arthur, J.C. 1885. Proof that the disease of trees known as pear blight is directly due to bacteria. New York State Experiment Station Bulletin, 2:1-4.

Arauz, L.F. and Sutton, T.B. 1989a. Temperature and wetness duration requirements for apple infection by *Botryosphaeria obtusa*. Phytopathology, 79:440-444.

Arauz, L.F. and Sutton, T.B. 1989b. Influence of temperature and moisture on the germination of ascospores and conidia of *Botryosphaeria obtusa*. Phytopathology, 79:667-674.

Arauz, L.F. and Sutton, T.B. 1990. Protectant and after-infection activity of fungicides against *Botryosphaeria obtusa* on apple. Plant Disease, 74:1029-1034.

Baines, R.C. 1939. *Phytophthora* trunk canker and collar rot of apple trees. Journal of Agricultural Research, 59:159-184.

Baines, R.C. 1940. Pathogenicity and hosts of the fly-speck fungus of apple. (Abstr.) Phytopathology, 30:2.

Baines, R.C., and Gardener, M.W. 1932. Pathogenicity and cultural characters of the apple sooty-blotch fungus. Phytopathology, 22:937-952.

Barkai-Golan, R. 1974. Species of *Penicillium* causing decay of stores fruits and vegetables in Israel. Mycopathologia and Mycologia Applicata, 54:141-145.

Bartlett, D.W., Clough, J.M., Godwin, J.R., Hall, Alison, A.H., Hamer, M. and Parr-Dobrzanski, B. 2002. The strobilurin fungicides. Pest Management Science, 58:649-662.

Becker, C.M., Burr, T.J. and Smith, C.A. 1992. Overwintering of conidia of *Venturia inaequalis* in apple buds in New York orchards. Plant Disease, 76:121-126.

Becker, C.M. and Burr, T.J. 1994. Discontinuous wetting and survival of conidia of *Venturia inaequalis* on apple leaves. Phytopathology, 84:372-378.

Bedford, K.E., MacNeill, B.H. and Bonn, W.G. 1984. Survival of a genetically marked strain of the blister spot pathogen *Pseudomonas syringae* pv. *papulans* in leaf scars and buds of apple. Canadian Journal of Plant Pathology, 6:17-20.

Bedford, K.E., MacNeill, B.H., Bonn, W.G. and Dirks, V.A. 1988. Population dynamics of *Pseudomonas syringae* pv. *papulans* on Mutsu apple. Canadian Journal of Plant Pathology, 10:23-29.

Beisel, M.B. and Hendrix, F.F. 1982. Infestation and infection of apple buds by *Botryosphaeria obtusa.* Phytopathology, 72:987.

Beisel, M.B., Hendrix, F.F. and Starkey, T.E. 1984. Natural inoculation of apple buds by *Botryosphaeria obtusa.* Phytopathology, 74:335-338.

Billing, E. 1992. Billing's revised system (BRS) for fire blight risk assessment. Bulletin OEPP/ EPPO Bulletin, 22:1-102.

Blodgett, F.M. 1923. A new host for mosaic. Plant Disease Reporter, 7:11.

Bonn, W.G. and Bedford, K.E. 1986. Midvein necrosis of Mutsu apple leaves caused by *Pseudomonas syringae* pv. *papulans*. Canadian Journal of Plant Pathology, 8:167-169.

Bonn, W.G. and van der Zwet, T. 2000. Distribution and economic importance of fire blight, In: "Fire Blight: the Disease and its Causitive Agent" (ed. Vanneste, J.L.) CAB International, Wallingford, UK. pp. 37-53.Borecka, H. 1977. Fungi of the genus *Penicillium* on apples and pears during the storage period. Acta Agrobotanica, 30:213-227.

Breth, D.I., Reddy, M.V.B., Norelli, J. and Aldwinckle, H. 2000. Successful fire blight control is in the details. New York Fruit Quarterly, 8(1):10-16.

Brown, E.M. and Sutton, T.B. 1986. An empirical model for predicting the first symptoms of sooty blotch and flyspeck of apples. Plant Disease, 79:1165-1168.

Brown-Rytlewski, D.E. and McManus, P.S., 2000a. Outbreak of Leucostoma canker caused by *Leucostoma cincta* on McIntosh apple trees in Wisconsin. Plant Disease, 84:923.

Brown-Rytlewski, D.E. and McManus, P.S., 2000b. Virulence of *Botryosphaeria dothidea* and *Botryosphaeria obtusa* on apple and management of stem cankers with fungicides. Plant Disease, 84:1031-1037.

Burr, T.J. 1991. Blister spot. In: "Compendium of Apple and Pear Diseases" (eds. Jones, A.L. and Aldwinckle, H.S.) APS Press, St-Paul, Minnesota, USA. pp. 63-64.

Burr, T.J. and Hurwitz, B. 1979. The etiology of blister spot of 'Mutsu' apple in New York State. Plant Disease Reporter, 63:157-160.

Burr, T.J. and Hurwitz, B. 1981. Seasonal susceptibility of Mutsu apples to *Pseudomonas syringae* pv. *papulans*. Plant Disease, 65:334-336.

Burr, T.J. and Katz, B.H. 1981. Survival of *Pseudomonas syringae* pv. *papulans* in dormant apple buds (Abstr.). Phytopathology, 71:864.

Burr, T.J. and Katz, B.H. 1984. Overwintering and distribution pattern of *Pseudomonas syringae* pv. *papulans* and pv. *syringae* in apple buds. Plant Disease, 68:383-385.

Burr, T.J., Norelli, J.L., Katz, B., Wilcox, W.F. and Hoying, S.A. 1988. Streptomycin resistance of *Pseudomonas syringae* pv. *papulans* in apple orchards and its association with a conjugative plasmid. Phytopathology, 78:410-413.

Butt, D.J., Santen, G. Van, Xu, X.M. and Stone, K.B. 1992. VentemCan apple scab (*Venturia inaequalis*) infection warning system. Version 3.1. Computer Software and Manual. HRI, East Malling, UK.

Butt, D.J. and Xu, X.M. 1996. ADEMCan integrated apple diseases warning system. Version 2.0a. Programme and Users Manual. HRI, East Malling, UK.

Canfield, M.L. and Moore, L.W. 1991. Isolation and characterization of opine-utilizing strains of *Agrobacterium tumefaciens* and fluorescent strains of *Pseudomonas* spp. from rootstocks of *Malus*. Phytopathology 81, 440-443.

Carisse, O., Philion, V., Rolland, D. and Bernier, J. 2000. Effect of fall application of fungal anatagonists on spring ascospore production of the apple scab pathogen, *Venturia inaequalis*. Phytopathology, 90:31-37.

Carisse, O. and Dewdney, M. 2002. A review of non-fungicidal approaches for the control of apple scab. Phytoprotection 83, 1-29.

Childers, N.F., Morris, J.R. and Sibbett, G.S. 1995. Modern Fruit Science. Horticultural Publications, Gainesville, Florida. 632 p.

Clarke, G.G. , Hickey, K.D., Travis, J.W. and Kleiner, W.C. 1993. Development and validation of the Penn State fire blight management model. Pennsylvania Fruit News, 73:57-60.

Cooley, D.R., Gamble, J.W. and Autio, W.R. 1997. Summer pruning as a method for reducing flyspeck disease on apple fruit. Plant Disease, 81:1123-1126.

Cooke, L.R. 1999. The influence of fungicide sprays on infection of apple cv. Brambley's seedling by *Nectria galligena*. European Journal of Plant Pathology, 105:783-790.

Cordley, A.B. 1900a. Apple-tree anthracnose. Oregon State Board of Horticulture Biennial Report, 6: 405-409.

Cordley, A.B. 1900b. Some observations on apple-tree anthracnose. Botanical Gazette, 30:48-58.

Cordley, A.B. 1900c. Apple-tree anthracnose: A new fungus disease. Oregon State Agricultural Experiment Station Bulletin, 60:8.

Coyier, D.L. 1968. Effects of temperature on germination of *Podosphaera leucotricha* conidia. Phytopathology, 58:1047-1048.

Creemers, P. and Vanmechelen, A. 1998. Managing fungal diseases on pome fruits in relation with anti-resistance strategies for modern fungicides. In: "Modern fungicides and antifungal compounds II" 12th International Reinhardsbrunn Symposium, Friedrichroda, Thuringia, Germany. pp. 257-266.

Daines, R., Weber, D.J., Bunderson, E.D. and Roper, T. 1984. Effect of early sprays on control of powdery mildew fruit russet on apples. Plant Disease, 68: 326-328.

Denning, W.M. 1794. On decay of apple trees. Proceedings Society for the Promotion of Agricultural Science, 1:219-222.

Dhanvantari, B.N. 1969. Bacterial blister spot of apple in Ontario. Canadian Plant Disease Survey, 49:36-37.

Dugan, F.M., Grove, G.G. and Roger, J.D. 1993 Comparative studies of *Cryptosporiopsis curvispora* and *C. perennans*. 1. Morphology and pathogenic behavior. Mycologia, 85:551-564.

Dullahide, S.R., Stirling, G.R., Nikulin, A. and Stirling, A.M. 1994. The role of nematodes fungi, bacteria, and abiotic factors in the etiology of apple replant problems in the granite belt of Queensland. Australian Journal of Experimental Agriculture, 34:1177-1182.

Ellis, M.A., Ferree, D.C., Funt, R.C. and Madden, L.V. 1998. Effect of an apple scab-resistant cultivar on use patterns of inorganic and organic fungicides and economics of disease control. Plant Disease, 82:428-433.

Ellis, M.A., Madden, L.V., and Burr, T.J. 2000. Effectiveness of fosetyl-aluminum and streptomycin alone and in combination for control of blister spot on 'Mutsu' apples in Ohio and New York. Online. Plant Health Progress doi:10.1094/PHP-2000-1204-01-RS.

Evans, J.R., Evans, R.R., Regusci, C.L. and Rademacher, W. 1999. Mode of action, metabolism, and uptake of BAS 125W, prohexadione-calcium. HortScience, 34:1200-1201.

Filajdic, N. and Sutton, T.B. 1991. Identification and distribution of *Alternaria mali* on apples in North Carolina and susceptibility of different apple varieties to Alternaria blotch. Plant Disease, 75:1045-1048.

Fischer, C., 2000. Apple Breeding in the Federal Centre for Plant Breeding Research, Institute for Fruit Breeding at Dresden-Pillnitz, Germany. Acta Horticulturae, 538:225-227.

Fisher, E.G., Parker, K.G., Luepschen, N.S. and Kwong, S.S. 1959. The influence of phosphorus, potassium, mulch, and soil drainage on fruit size, yield, and firmness of the Bartlett pear and on development of the fire blight disease. American Society for Horticulture Science Proceedings, 73:78-90.

Gadoury, D.M. and MacHardy, W.E. 1982a. Preparation and interpretation of squash mounts of pseudothecia of *Venturia inaequalis*. Phytopathology, 72:92-95.

Gadoury, D.M. and MacHardy, W.E. 1982b. A model to estimate the maturity of ascospores of *Venturia inaequalis*. Phytopathology, 72:901-904.

Gadoury, D.M. and MacHardy, W.E. 1986. Forecasting ascospore dose of *Venturia inaequalis* in commercial apple orchards. Phytopathology, 76:112-118.

Gadoury, D.M. MacHardy, W.E. and Rosenberger, D.A. 1989. Integration of pesticide application schedules for disease and insect control in apple orchards of the Northeastern United States. Plant Disease, 73:98-105.

Gadoury, D.M., Seem, R.C. and Stensvand, A. 1994. Ascospore discharge in *Venturia inaequalis.* Norwegian Journal of Agricultural Science Supplement, 17:205-219.

Garrett, C.M.E. 1987. Problems of *Agrobacterium tumefaciens* in planting material and its control. EPPO Bulletin 17, 263-268.

Gilpatrick, J.D. and Szkolnik, M. 1978. Maturation and discharge of ascospores of the apple scab fungus. In: "Proceedings Apple and Pear Scab Workshop" (eds. Jones, A.L. and Gilpatrick, J.D.), July 11, 1976, New York State Agricultural Experiment Station Special Report 28. pp. 1-6.

Grimm, R. 1987. Control of crown gall in Swiss apple nurseries. EPPO Bulletin, 17:269-272.

Grove, G.G. 1990a. Anthracnose and perennial canker. In: "Compendium of Apple and Pear Diseases" (eds. Jones, A.L. and Aldwinckle, H.S.) APS Press, St-Paul, Minnesota, USA. pp. 36-38.

Grove, G.G. 1990b. Nectria canker. In: "Compendium of Apple and Pear Diseases" (eds. Jones, A.L. and Aldwinckle, H.S.) APS Press, St-Paul, Minnesota, USA. pp. 35-36.

Grove, G.G., Dugan, F.M. and Boal. R.J. 1992. Perennial canker of apple: Seasonal host susceptibility, spore production, and perennation of *Cryptosporiopsis perennans* in infected fruit in eastern Washington. Plant Disease, 76:1109-1114.

Gu, Y.H. and Mazzola, M. 2001. Impact of carbon starvation on stress resistance, survival and biocontrol ability of *Pseudomonas putida* strain C28. Soil Biology and Biochemistry, 33:1155-1162.

Gubler, W.D., Rademacher, M.R., Vasquez, S.J. and Thomas, C.S. 1999. Control of powdery mildew using the UC Davis powdery mildew risk index. APSnet Feature Story, January 1999, http://www.apsnet.org/online/feature/pmildew/Top.html.

Heald, F.D. 1933. Manual of Plant Diseases. McGraw-Hill Book Company, Inc., New York, NY, USA. 953 p.

Heald, F.D. and Ruehle, G.D. 1931. The rots of Washington apples in cold storage. Washington Agricultural Experiment Station Bulletin, 253. 48 p.

Hickey, K.D. 1990. Calyx-end rot. In: "Compendium of Apple and Pear Diseases" (eds. Jones, A.L. and Aldwinckle, H.S.) APS Press, St-Paul, Minnesota, USA. pp. 29.

Hickey, K.D. and Travis, J.W. 1995. Evaluation for predictive models and antagonistic bacteria for control of fire blight blossom blight. Pennsylvania Fruit News, 75:41-43.

Horner, I.J. and Wilcox, W.F. 1995. SADAMCAP, a technique for quantifying populations of *Phytophthora cactorum* in apple orchard soils. Phytopathology, 85:1400-1408.

Horner, I.J. and Wilcox, W.F. 1996. Temporal changes in activity and dormant spore populations of *Phytophthora cactorum* in New York apple orchards. Phytopathology, 86:1133-1139.

Isutsa, D.K. and Merwin, I.A. 2000. Malus germplasm varies in resistance and tolerance to apple replant disease in a mixture of New York orchard soils. Hortscience 35:262-268.

Jackson, H.S. 1911. Apple-tree anthracnose. Oregon Agricultural Experiment Station Circular, 17. 4 p.

Jackson, H.S. 1912. The development of *Gloeosporium malicorticis* Cordley (abs.). Phytopathology, 2:95.

Jackson, H.S. 1913. Apple-tree anthracnose: A preliminary report. Oregon State Agricultural Experiment Station Biennial Crop Pest and Horticultural Report, 1911-12: 178-197.

James, J.R. and Sutton, T.B. 1982. Environmental factors influencing pseudothecial development and ascospore maturation of *Venturia inaequalis*. Phytopathology, 72:1073-1080.

Janisiewicz, W.J., Peterson, D.L. and Bors, R. 1994. Control of storage decay of apple with *Sporobolomyces roseus*. Plant Disease, 78:466-470.

Jeffers, S.N. 1992. Preplant root treatments to reduce the incidence of *Phytophthora* species on dormant apple rootstocks. Plant Disease, 76:12-19.

Jeffers, S.N. and Aldwinckle, H.S. 1988. Phytophthora crown rot of apple trees: sources of *Phytophthora cactorum* and *P. cambivora* as primary inoculum. Phytopathology, 78:328-335.

Jeffers, S.N. and Wilcox, W.F. 1990. Phytophthora crown, collar, and root and rots. In: "Compendium of Apple and Pear Diseases" (eds. Jones, A.L. and Aldwinckle, H.S.) APS Press, St-Paul, Minnesota, USA. pp. 43-45.

Jeger, M.J. 1984. Relating disease progress to cumulative numbers of trapped spores: apple powdery mildew and scab epidemics in sprayed and unsprayed orchard plots. Plant Pathology, 33:517-530.

Jeger, M.J. and Butt, D.J. 1986. Epidemics of apple powdery mildew (*Podosphaera leucotricha*) in a mixed cultivar orchard. Plant Pathology, 35:498-505.

Johnson, E.M., Sutton, T.B. and Hodges, C.S. 1996. *Peltaster fruticola*: A new species in the complex of fungi causing apple sooty blotch disease. Mycologia, 88:114-120.

Jones, A.L. 1990. Brown rot disease. In: "Compendium of Apple and Pear Diseases" (eds. Jones, A.L. and Aldwinckle, H.S.) APS Press, St-Paul, Minnesota, USA. pp 32.

Jones, A.L. 1992. Evaluation of the computer model MARYBLYT for predicting fire blight blossom infection on apple in Michigan. Plant Disease, 76:344-347.

Jones, A.L., Lillevik, S.L., Fisher, P.D. and Stebbins, T.C. 1980. A microcomputer based instrument to predict primary apple scab infection periods. Plant Disease, 64:69-72.

Jones, A.L., and Aldwinckle, H.S. (eds.) 1990. Compendium of Apple and Pear Diseases. The American Phytopathological Society, St. Paul, Minnesota, USA. 100 p.

Jones, A.L., Norelli, J.L. and Ehret, G.R. 1991. Detection of streptomycin-resistant *Pseudomonas syringae* pv. *papulans* in Michigan apple orchards. Plant Disease, 75:529-531.

Jones, A.L., Fernando, W.G.D. and Ehret, G.R. 1999. Controlling secondary spread of fire blight with prohexadione calcium. Phytopathology, 89:S37.

Keitt, G.W. and Jones, L.K. 1926. Studies of the epidemiology and control of apple scab. Wisconsin Agricultural Experiment Station Research Bulletin 73. 104 p.

Kemp, H. and van Dieren, M. 2001. Resistant cultivars should taste. Fruitteelt (Den Haag) 91:20-22.

Kerkoud, M., Manceau, C., Gardan, L., Samson, R. and Paulin, J.-P. 2000. Epiphytic occurrence of *Pseudomonas syringae* pv. *papulans* (Rose) in France, where blister spot has never been seen. European Journal of Plant Pathology, 106:481-485.

Kienholz, J.R. 1939. Comparative study of the apple anthracnose and perennial canker fungi. Journal of Agricultural Research, 59:635-65.

Kienholz, J.R. 1951. The bull's eye rot (*Neofabraea*) problem of apples and pears. Oregon State Horticultural Society Proceedings 66:75-77.

Kienholz, J.R. 1956. Control of bull's eye rot on apples and pears. Plant Disease. Reporter, 40:872-877.

Kim, K.W., Park, E.W., Kim, Y.H., Ahn, K.K., Kim, P. G. and Kim, K. S. 2001. Latency- and defense-related ultrastructural characteristics of apple fruit tissues infected with *Botryosphaeria dothidea*. Phytopathology, 72:165-172,

Koffmann, W. and Penrose, L.J. 1987. Fungicides for the control of blue mould (*Penicillium* spp.) in pome fruits. Scientific Horticulture, 31:225-232.

Köller, W. 1999. Chemical approaches to managing plant pathogens. In: "Handbook of Pest Management" (ed. Ruberson, J.R.) Marcel Dekker, Inc., New York, NY, USA. 842 p.

Köller, W., Wilcox, W.F., Barnard J., Jones, A.L. and Braun , P.G. 1997. Detection and quantification of resistance of *Venturia inaequalis* populations to sterol demethylation inhibitors. Phytopathology, 87:184-190.

Köller, W. and Wilcox, W.F. 1999. Evaluation of tactics far managing resistance of *Venturia inaequalis* to sterol demethylation inhibitors. Plant Disease, 83:857-863.

Köller, W. and Wilcox, W.F. 2001. Evidence for the predisposition of fungicide-resistant isolates of *Venturia inaequalis* to a preferential selection for resistance to other fungicides. Phytopathology, 91:776-781.

Kozlovskaya, Z.A., Marudo, G.M. and Ryabtsev, A.S. 2000. Some results of the apple breeding program in Belarus. Acta Horticulturae, 538:219-223.

Latham, A.J. and Hollingsworth, M.H. 1973. Incidence and control of sooty blotch and flyspeck on apples in Alabama. Auburn University Agricultural Experiment Station Circular 208.

Lalancette, N. and Hickey, K.D. 1985. Apple powdery mildew disease progress on sections of shoot growth: an analysis of leaf maturation and fungicide effects. Phytopathology, 75:130-134.

Lalancette, N. and Hickey, K.D. 1986. An apple powdery mildew model based on plant growth, primary inoculum, and fungicide concentration. Phytopathology, 76:1176-1182.

Latorre, B.A., Rioja, M.E. and Wilcox, W.F. 2001. *Phytophthora* species associated with crown and root rot of apple in Chile. Plant Disease, 85:603-606.

Lawrence, W.H. 1904. Black-spot canker. Washington State Agricultural Experiment Station Bulletin, 66. 35 p.

Leibinger, W., Breuker, B., Hahn, M. and Mendgen, K. 1997. Control of postharvest pathogens and colonization of the apple surface by antagonistic microorganisms in the field. Phytopathology, 87:1103-1110.

Lerner, S.M. 1999. Studies on the biology and epidemiology of *Schizothyrium pomi*, causal agent of flyspeck disease on apple. M.S. thesis. University of Massachusetts, Amherst.

Lieberman, P.B. and Wootan, M.G. 1998. Protecting the crown jewels of medicine. Center for Science in the Public Interest Newsletter, http://www.cspinet.org/reports/abiotic.htm.

Lightner, G.W. and Steiner, P.W. 1990. Computerization of blossom blight prediction model. Acta Horticulturae, 273:159-162.

Lolas, M. and Latorre, B.A. 1997. The efficiency of chemical control against European canker of apple by *Nectria galligena*. *Fitopatologia* 32: 131-136.

Lyr, H. (ed.). 1995. Modern Selective Fungicides: Properties, Applications, Mechanisms of Action. Jena: New York, Gustav Fischer. 595 p.

MacHardy, W.E. 1996. Apple Scab: Biology, Epidemiology, and Management. The American Phytopathological Society, St. Paul, Minnesota, USA. 545 p.

MacHardy, W.E. 2000. Action thresholds for managing apple scab with fungicides and sanitation. IOBC/WPRS Bulletin, 23:123-131.

MacHardy, W.E. and Gadoury, D.M. 1989. A revision of Mills' criteria for predicting apple scab infection periods. Phytopathology, 79:304-310.

MacHardy, W.E., Gadoury, D.M. and Rosenberger, D.A. 1993. Delaying the onset of fungicide programs for control of apple scab in orchards with low potential ascospore dose of *Venturia inaequalis.* Plant Disease, 77:372-375.

MacHardy, W.E., Gadoury, D.M. and Gessler, C. 2001. Parasitic and biological fitness of *Venturia inaequalis*: Relationship to disease management strategies. Plant Disease, 85:1036-1051.

Mai, W.F. and Abawi, G.S. 1981. Controlling replant disease of pome and stone fruits in Northeastern United States by preplant fumigation. Plant Disease, 65:859-864.

Massie, L.B. and Szkolnik, M. 1974. Prediction of ascospore maturation of *Venturia inaequalis* utilizing cumulative degree days. Proceedings American Phytopathological Society, 1:140 (Abstr.)

Mazzola, M. 1998. Elucidation of the microbial complex having a causal role in the development of apple replant disease in Washington. Phytopathology, 88:930-938.

Mazzola, M. and Gu, Y.H. 2000. Impact of wheat cultivation on microbial communities from replant soils and apple growth in greenhouse trials. Phytopathology, 90:114-119.

Mazzola, M., Granatstein, D.M., Elfving, D.C. and Mullinix, K. 2001. Suppression of specific apple root pathogens by *Brassica napus* seed meal amendment regardless of glucosinate content. Phytopathology, 91:673-679.

McManus, P.S., and Stockwell, V. 2000. The use of antibiotics in agriculture: silver bullet or rusty saber. APSnet Feature Story, June 2000, http://www.apsnet.org/online/feature/Antibiotics/Top.html

Mills, W.D. 1944. Efficient use of sulfur dusts and sprays during rain to control apple scab. Cornell Extension Bulletin, 630:1-4.

Morse, W.J. 1916. Spraying experiments and apple diseases in 1915. Maine Agricultural Experiment Station Bulletin, 252:186-187.

Nannfeldt, J.A. 1932. Studien über die Morphologie und Systematik der Nichtlichenisierten Inoperculaten Discomyceten. Nova acta Regiae societatis scientiarum upsaliensis, 48:1-386.

Nasu, H., Fujii, S. and Yokoyama, T. 1985. *Zygophiala jamaicensis* Mason, a causal fungus of flyspeck of grape, Japanese persimmon, and apple. Annals of the Phytopathological Society of Japan 51, 536-545.

Norelli, J., Aldwinckle, H., Momol, T., Johnson B., DeMarree, A. and Reddy, M.V.B. 2000. Fire blight of apple rootstocks. New York Fruit Quarterly, 8:5-8.

Norelli, J.L., Jones, A.L., and Aldwinkle, H.S. 2003. Fire blight management in the twenty-first century: Using new technologies that enhance host resistance in apple. Plant Disease 87:756-765.

Northover, J. and Schneider, K.E. 1993. Activity of plant oils on diseases caused by *Podosphaera leucotricha*, *Venturia inaequalis*, and *Albugo occidentalis*. Plant Disease, 77:152-157.

Ocamb-Basu, C.M., Sutton, T.B. and Nelson, L.A. 1988a. Effect of temperature and relative humidity on germination, growth, and sporulation of *Zygophiala jamaicensis*. Phytopathology, 78:100-103.

Ocamb-Basu, C.M., Sutton, T.B. and Nelson, L.A. 1988b. The effects of pruning on incidence and severity of *Zygophiala jamaicensis* and *Gloeodes pomigena* infections of apple fruit. Phytopathology, 78:1004-1008.

Ogawa, J.M. and English, H. 1991. Diseases of Temperate Zone Tree Fruit and Nut Crops. University of California, Division of Agriculture and Natural Resources, Oakland, CA. Publication 3345. 461 p.

Olivier, J.M. 1984. Evolution de la lutte contre la tavelure du pommier. La Défense des Végétaux, 225:22-35.

Parker, K.C. and Sutton, T.B., 1992. Susceptibility of apple fruit to *Botryosphaeria dothidea* and isolate variation. Plant Disease, 77:385-389.

Parker, K.C. and Sutton, T.B., 1993. Effect of temperature and wetness duration on apple fruit infection and eradicant activity of fungicides against *Botryosphaeria dothidea*. Plant Disease, 77:181-185.

Pierson, C.F. 1958. Forecasting bull's eye rot in northwestern grown apples in storage. Plant Disease Reporter, 42:1394-1396.

Pierson, C.F., Ceponis, M.J. and McColloch, L.P. 1971. Market diseases of apples, pears, and quinces. USDA-ARS Agricultural Handbook 376:21-23.

Podleckis, E.V. and Welliver, R. 1995. Common Latent Viruses of Apple. In: "Mid-Atlantic Orchard Monitoring Guide" (ed. Hogmire, Jr., H.W.) Northeastern Regional Agricultural Engineering Service, Cornell University, Ithaca, NY, USA. pp.110.

Proffer, T.J. and Jones, A.L. 1989. A new canker disease of apple caused by *Leucostoma cincta* and other fungi associated with cankers on apple in Michigan. Plant Disease, 73:508-514.

Prusky, D. and Ben-Arie, R. 1985. Effect of imazalil on pathogenicity of *Penicillium* spp. causing storage rots of pome fruits. Plant Disease, 69:416-418.

Raio, A., Zoina, A. and Moore, L.W. 1997. The effect of solar heating of soil on natural and inoculated agrobacteria. Plant Pathology, 46:320-328.

Reddy, M.V.B., Norelli, J.L. and Aldwinckle, H.S. 2000. Control of fire blight infection of apple blossoms, 1999. Fungicide and Nematicide Tests, 55:22.

Reuveni, M., Oppenheim, D. and Reuveni, R. 1998. Integrated control of powdery mildew on apple trees by foliar sprays of mono-potassium phosphate fertilizer and sterol inhibiting fungicides. Crop Protection, 17:563-568.

Rose, D.H. 1917. Blister spot of apples and its relation to a disease of apple bark. Phytopathology, 7:198-208.

Rosenberger, D.A. 1990a. Dry eye rot. In: "Compendium of Apple and Pear Diseases" (eds. Jones, A.L. and Aldwinckle, H.S.) APS Press, St-Paul, Minnesota, USA. pp. 28-29.

Rosenberger, D.A. 1990b. Blue mold. In: "Compendium of Apple and Pear Diseases" (eds. Jones, A.L. and Aldwinckle, H.S.) APS Press, St-Paul, Minnesota, USA. pp. 54-55.

Rosenberger, D.A. 1990c. In: "Compendium of Apple and Pear Diseases" (eds. Jones, A.L. and Aldwinckle, H.S.) APS Press, St-Paul, Minnesota, USA. pp. 55-56.

Rosenberger, D.A. 1994. Summer disease control on apples. In: "124thAnnual Report of the State Horticultural Society of Michigan" (eds. Hull, J. Jr. and Perry R.) Dec. 6-7, 1994, Michigan State Horticultural Society, East Lansing, MI. pp 80-86.

Rosenberger, D.A. 2001a. Fungicide strategies for control of apple scab and mildew in 2001. Scaffolds Fruit Journal (newsletter), 10(2):1-3.

Rosenberger, D.A. 2001b. Fungicide strategies for control of apple scab and mildew in 2001, Part II. Scaffolds Fruit Journal (newsletter), 10(3):1-3.

Rosenberger, D.A. 2001c. Adjusting fungicide programs to compensate for SI resistance. Scaffolds Fruit Journal (newsletter), 10(4):5-7.

Rosenberger, D.A. and Meyer, F.W. 1984. Negatively correlated cross-resistance to diphenylamine in benomyl-resistant *Penicillium expansumi.* Phytopathology, 75:74-79.

Rosenberger, D.A., Wicklow, D.T., Korjagin, V.A. and Rondinaro, S.M. 1991. Pathogenicity and benzimidazole resistance in *Penicillium* species recovered from flotation tanks in apple packinghouses. Plant Disease, 75:712-715.

Rosenberger, D.A., Meyer, F.W., Ahlers, C.A. and van Camp, K.L. 2002. Post-infection activity of Sovran, Flint, Benlate, and Topsin-M for control of flyspeck and sooty blotch, 2001. Fungicide and Nematicide Tests, 57:PF23.

Rossi, V., Ponti, I., Marinelli, M., Giosue, S. and Bugiani, R. 1999. Field evaluation of some models estimating the seasonal patterns of airborne ascospores of *Venturia inaequalis.* Journal of Phytopathology, 147:567-575.

Rossi, V., Ponti, I., Marinelli, M., Giosue, S. and Bugiani, R. 2001. Environmental factors influencing the dispersal of *Venturia inaequalis* ascospore in the orchards air. Journal of Phytopathology, 149:11-19.

Rytter, J.L. and Travis, J.W. 1994. Canker causing fungi of apple wood in Pennsylvania. Norwegian Journal of Agricultural Sciences Supplement, 17:303-307.

Sanderson, P.G. and Spotts, R.A. 1995. Postharvest decay of winter pear and apple fruit caused by species of *Penicillium.* Phytopathology, 85:103-110.

Schwabe, W.F.S. 1980. Wetting and temperature requirements for apple leaf infection by *Venturia inaequalis* in South Africa. Phytophylactica, 12:69-80.

Seem, R.C. and Gilpatrick, J.D. 1980. Incidence and severity relationships of secondary infections of powdery mildew on apple. Phytopathology, 70:851-854.

Seemüller, E. 1990. Apple proliferation. In: "Compendium of Apple and Pear Diseases" (eds. Jones, A.L. and Aldwinckle, H.S.) APS Press, St-Paul, Minnesota, USA. pp. 67-68.

Sharma, I.M., and Bhardwaj, S.S. 1999. Canker and foliar diseases of apple. In: "Diseases of Horticultural CropsC-Fruits" (eds. Verma, L.R. and Sharma, R.C.) Indus Publishing Co., New Delhi, India. pp. 15-53.

Sholberg, P.L. and Bedford, K.E. 1997. Characterization of blister spot [*Pseudomonas syringae* pv. *papulans*] in British Columbia and its potential for spread to new apple cultivars.

Canadian Journal of Plant Pathology, 19:347-351.

Sholberg, P.L., Haag, P., Boulé, J. and Bedford, K. 2001. Efficacy of two biological control agents for control of fire blight on apple. Biological and Cultural Tests for Control of Plant Diseases, 16:N57.

Shtienberg, D., Zilberstaine, M., Oppenheim, D., Herzog, Z., Manulis, S., Shwartz, H. and Kritzman, G. 2001. Efficacy of oxolinic acid and other bactericides in suppression of Erwinia amylovora in pear orchards in Israel. Phytoparasitica, 29:143-154.

Siegel, M.R. and Sisler (eds.) 1977. Antifungal Compounds. Marcel Dekker, New York, USA.

Smith, M.A. 1944. Blister spot, a bacterial disease of apple. Journal of Agricultural Research, 68:269-298.

Smith, T.J. 1999. Report on the development and use of Cougarblight 98C - A situation-specific fire blight risk assessment model for apple and pear. Acta Horticulturae, 489:429-436.

Smock, R.M. and Neubert, A.M. 1950. Apples and apple products. Wiley Interscience, New York, NY, USA. 486 p.

Spotts, R.A. 1986. Relationship between inoculum concentration of three decay fungi and pear fruit decay. Plant Disease, 70:386-389.

Spotts, R.A. 1990a. Moldy core and core rot. In: "Compendium of Apple and Pear Diseases" (eds. Jones, A.L. and Aldwinckle, H.S.) APS Press, St-Paul, Minnesota, USA. pp. 29-30.

Spotts, R.A. 1990b. Bull's-eye rot. In: "Compendium of Apple and Pear Diseases" (eds. Jones, A.L. and Aldwinckle, H.S.) APS Press, St-Paul, Minnesota, USA. pp. 56-57.

Spotts, R.A., and Cerevantes, L.A. 1993. Use of filtration for removal of conidia of *Penicillium expansum* from water in pome fruit packinghouses. Plant Disease, 77:828-830.

Spotts, R.A., Cervantes, L.A. and Niederholzer, F.J.A. 1997. Effects of dolomitic lime on production of asci and pseudothecia of *Venturia inaequalis* and *V. pirina*. Plant Disease, 81:96-98.

Steiner, P.W. 1990a. Predicting apple blossom infections by *Erwinia amylovora* using the *MARYBLYT* model. Acta Horticulturae, 273:139-148.

Steiner, P.W. 1990b. Predicting canker, shoot, and trauma blight phases of fire blight epidemics using the *MARYBLYT* model. Acta Horticulturae, 273:149-158.

Steiner, P.W. and Lightner, G. 1992. *MARYBLYT*: A Predictive Program for Forecasting Fire Blight Diseases in Apples and Pears. University of Maryland, College Park, MD, USA. 55 p.

Stevens, F.L., Ruth, W.A. and Spooner, C.S. 1918. Pear blight wind borne. Science, 48:449-450.

Stensvand, A., Gadoury, D.M., Amundsen, T., Semb, L. and Seem, R.C. 1997. Ascospore release and infection of apple leaves by conidia and ascospores of *Venturia inaequalis* at low temperatures. Phytopathology, 87:1046-1053.

Stephan, S. 1988. Research into the sporulation and dispersal of spores of apple mildew (*Podosphaera leucotricha* (Ell. et Ev.) Salm.). Archiv für Phytopathologie und Pflanzenschutz, 24:491-501.

Stewart, F.C. 1910. Notes on New York plant diseases. New York Agricultural Experiment Station Geneva Technical Bulletin, 328: 318.

Stewart, T.M., Knight, J.D., Manktelow, D.W.L. and Mumford, J.D. 1998. SpraycheckCa model for evaluating grower timing of black spot (*Venturia inaequalis*) fungicides in apple orchards. Crop Protection, 17:65-74.

Sutton, T.B. 1990a. Bitter rot. In: "Compendium of Apple and Pear Diseases" (eds. Jones, A.L. and Aldwinckle, H.S.) APS Press, St-Paul, Minnesota, USA. pp. 15-16.

Sutton, T.B. 1990b. White rot. In: "Compendium of Apple and Pear Diseases" (eds. Jones, A.L. and Aldwinckle, H.S.) APS Press, St-Paul, Minnesota, USA. pp. 16-18.

Sutton, T.B. 1990c. Black rot. In: "Compendium of Apple and Pear Diseases" (eds. Jones, A.L. and Aldwinckle, H.S.) APS Press, St-Paul, Minnesota, USA. pp. 18-20.

Sutton, T.B. 1996. Changing options for the control of deciduous fruit tree diseases. Annual Review of Phytopathology, 34:527-47.

Sutton, T.J. and Jones, A.L. 1979. Analysis of factors affecting dispersal of *Podosphaera leucotricha* conidia. Phytopathology, 69:380-383.

Sutton, T.B. and Arauz, L.F. 1991. Influence of temperature and moisture on germination of ascospores and conidia of *Botryosphaeria dothidea*. Plant Disease, 75:1146-1149.

Sutton, D.K., MacHardy, W.E. and Lord, W.G. 2000. Effects of shredding or treating apple leaf litter with urea on ascospore dose of *Venturia inaequalis* and disease buildup. Plant Disease, 84:1319-1326.

Sutton, T.B. and Anas, O. 2002. Treatments for fire blight in blossoms and terminals, 2001. Fungicide and Nematicide Tests, 57:PF27.

Tepper, B.L. and Yoder, K.S. 1982. Postharvest chemical control of Penicillium blue mold of apple. Plant Disease, 66:829-831.

Thakur, V.S. and Sharma, R.D. 1999. Apple Scab and its Management. In: "Diseases of Horticultural CropsCFruits" (eds. Verma, L.R. and Sharma, R.C.) Indus Publishing Co., New Delhi, India. 724 p.

Thomas, T.M. and Jones, A.L. 1992. Severity of fire blight on apple cultivars and strains in Michigan. Plant Disease, 76:1049-1052.

Torgeson, D.C. (ed.). 1967. Fungicides; An advanced treatise. Academic Press, New York, USA.

Trapman, M.C. and Polfliet, M. 1997. Management of primary infections of apple scab with the simulation program RIMpro: review of four years field trials. IOBC Bulletin 20(9): 241-250.

Travis, J.W., Clarke, G.G. and Hickey, K.D. 1993. Development of a computerized decision support system for fire blight management in Pennsylvania apple orchards. Pennsylvania Fruit News, 73:22-25.

Travis, J.W., Rytter, J.L. and Biggs, A.R. 1995a. Black rot. In: "Mid-Atlantic Orchard Monitoring Guide" (ed. Hogmire, Jr., H.W.) Northeastern Regional Agricultural Engineering Service, Cornell University, Ithaca, NY, USA. pp. 112.

Travis, J.W., Rytter, J.L. and Biggs, A.R. 1995b. White rot. In: "Mid-Atlantic Orchard Monitoring Guide" (ed. Hogmire, Jr., H.W.) Northeastern Regional Agricultural Engineering Service, Cornell University, Ithaca, NY, USA. pp. 114.

Tsc, L.I. and Utkhede, R.S. 1991. Effects of soil pH and nutrients on growth of apple seedlings grown in apple replant disease soils of British Columbia. Canadian Plant Disease Survey, 71:29-32.

Utkhede, R.S. 1986. Biology and control of apple crown rot caused by *Phytophthora cactorum*: a review. Phytoprotection, 67:1-13.

Utkhede, R.S., Smith, E.M. and Palmer, R. 1992. Effect of root-rot fungi and root lesion nematodes on the growth of young apple trees grown in apple replant disease soil. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz, 99:414-419.

Utkhede, R.S. and Smith E.M. 1993. Evaluation of biological and chemical treatments for control of crown gall on young apple trees in the Kootenay Valley of British Columbia. Journal of Phytopathology, 137:265-271.

Utkhede, R.S. and Smith, E.M. 1996. The effect of three irrigation practices on phytophthora crown and root rot of apple trees under field conditions. European Journal of Plant Pathology, 102:507-510.

Vinas, I., Usall, J. and Sanchis, V. 1991. Tolerance of *Penicillium expansum* to postharvest fungicide treatments in packinghouses in Lerida (Spain). Mycopathologia 113:15-18.

van der Zwet, T. and Keil, H.L. 1979. Fire Blight a Bacterial Disease of Rosaceous Plants. U.S. Department of Agriculture, Agricultural Handbook 510. 200 p.

van der Zwet, T., A. R. Biggs, R. Heflebower, and G. W. Lightner. 1994. Evaluation of the MARYBLYT computer model for predicting blossom blight on apple in West Virginia and Maryland. Plant Disease, 78:225-230.

van der Zwet, T. and Beer, S.V. 1995. Fire Blight - Its Nature, Prevention, and Control: A Practical Guide to Integrated Disease Management, U.S. Department of Agriculture, Agriculture Information Bulletin No. 631. 97 p.

Welliver, R. and Podleckis, E.V. 1995. Apple Mosaic Virus. In: "Mid-Atlantic Orchard Monitoring Guide" (ed. Hogmire, Jr., H.W.) Northeastern Regional Agricultural Engineering Service, Cornell University, Ithaca, NY, USA. pp. 110.

Westwood, M.N. 1993. Temperate-Zone Pomology Physiology and Culture, third edition. Timber Press, Portland, OR, USA. 523 p.

Whetzel, H.H. 1906. The blight canker of apple trees. Cornell University Agricultural Experiment Station Bulletin, 236:103-138.

Wilcox, W.F. 1993. Incidence and severity of crown and root rots on four apple rootstocks following exposure to *Phytophthora* species and waterlogging. Journal of the American Society for Horticultural Science, 118:63-67.

Wilcox, W.F., Wasson, D.I. and Kocvach, J. 1992. Development and evaluation of an integrated reduced-spray program using sterol demethylation inhibitor fungicides for control of primary apple scab. Plant Disease, 76:669-677.

Williamson, S.M. and Sutton, T.B. 2000. Sooty blotch and flyspeck of apple: Etiology, Biology, and Control. Plant Disease, 84:714-724.

Xu, X. M. 1996. The effects of constant and fluctuating temperatures on the length of the incubation period of apple powdery mildew (*Podosphaera leucotricha*). Plant Pathology, 45:924-932.

Xu, X. M. 1999. Modelling and forecasting epidemics of apple powdery mildew (*Podosphaera leucotricha*). Plant Pathology, 48:462-471.

Xu, X. M. and Butt, D.J., 1994. The biology and epidemiology of *Nectria galligena* and an infection warning system. Norwegian Journal of Agricultural Sciences Supplement, 17:317-324.

Xu, X. M., Butt, D.J. and Ridout, M.S. 1995. Temporal patterns of airborne conidia of *Podosphaera leucotricha*, causal agent of apple powdery mildew. Plant Pathology, 44:944-955.

Xu, X. M. and Butt, D.J. 1996. Tests of fungicides for post-germination activity against *Nectria galligena*, causal agent of canker and fruit rot of apple. Crop Protection, 15:513-519.

Xu, X. M. and Butt, D.J. 1998. Effects of temperature and atmospheric moisture on the early growth of apple powdery mildew (*Podosphaera leucotricha*) colonies. European Journal of Plant Pathology 104:133-140.

Xu, X. M., Butt, D.J. and Ridout, M.S. 1998. The effects of inoculum dose, duration of wet period, temperature and wound age on infection by *Nectria galligena* of pruning wounds on apple. European Journal of Plant Pathology, 104:511-519.

Yoder, K.S. 1990. Brooks fruit spot. In: "Compendium of Apple and Pear Diseases" (eds. Jones, A.L. and Aldwinckle, H.S.) APS Press, St-Paul, Minnesota, USA. pp. 25-26.

Yoder, K.S. 2000. Effect of powdery mildew on apple yield and economic benefits of its management in Virginia. Plant Disease, 84:1171-1176.

Yoder, K.S., Lacy, G.H. and Tepper, B.L. 1981. Blister spot of 'Mutsu' apples in Virginia (Abstr.). Virginia Journal of Science, 32:81.

Yoder, K.S., Miller, S.S. and Byers, R.E. 1999. Suppression of fireblight in apple shoots by prohexadione-calcium following experimental and natural inoculation. HortScience, 34:1202-1204.

Zeller, S.M. and Childs, L. 1925. Another apple-tree anthracnose in the pacific northwest and a comparison with the well-known apple-tree anthracnose (abs.). Phytopathology, 15:728.

Diagnosis and Management of Virus and Virus like Diseases of Citrus

C.N. Roistacher

Department of Plant Pathology, University of California, Riverside, 92521,California, USA

Abstract : This chapter covers fifteen virus and virus like diseases of citrus plus thermotherapy, with emphasis on the citrus tristeza disease. Included in this chapter are the citrus disease of psorosis, cristacortis, citrus chlorotic dwarf, impietratura, satsuma dwarf, vein enation, leprosis, tatter leaf, infectious variegation, exocortis, cachexia, gum pocket, stubborn and concave gum. Epidemiology of tristeza is presented in some detail where the disease has ravaged the citrus industries of so many countries. Tristeza cross protection has helped the citrus industries of Brazil, South Africa and Australia overcome the devastation of some of the more virulent isolates of the citrus tristeza virus.

Psorosis was once the major citrus virus disease problem in California. However, with certification program this disease has virtually disappeared in that State. The concave gum, impietratura and cristacortis diseases are a family, which cause separate and distinct symptoms in field trees. However, on indexig show the typical and classical oak leaf pattern. These diseases can be eliminated by thermotherapy, or shoot tip grafting. Satsuma dwarf and related diseases as found in Japan, and the citrus infectious variegation disease both are caused by ilar viruses. Indexing and certification can limit spread of these ilar virus diseases. In general, the citrus vein enation disease is not a serious threat to citrus worldwide. However, once introduced, is difficult to eradicate. Quarantine against introduction is important. The disease will not do well or exist where temperatures are warm and thermotherapy or shoot tip grafting will readily eliminate the disease. The citrus leprosis disease is a most serious problem in Brazil, and is now spreading to countries of the Caribbean region. Leprosis is difficult to manage and strict quarantine is the only effective way of preventing the entry into new areas. The citrus tatter leaf disease is present worldwide wherever the Meyer lemon has been introduced. The disease can easily be detected by indexing and can be eliminated by thermotherapy. The viroid diseases of exocortis and cachexia are widespread everywhere citrus is grown. The viroids can readily be detected by indexing to citron or by sPAGE and can be eliminated by shoot tip grafting. Thermotherapy is not effective in eliminating viroids from citrus. Gum pocket of trifoliate orange, present mostly in South Africa in now known to be caused by a citrus viroid -III. With this knowledge, indexing can be now be done by sPAGE and the viroid eliminated by shoot tip grafting. Stubborn disease of citrus is caused by Spiroplasma citri, a mycoplasma-like organism located in the sieve tubes. The disease is found in the warmer and drier citrus growing regions of the world and has been quite destructive. Some history and background of each disease is given with some of the early pioneering work. Symptoms are described and the means of detection given including new technologies.

1. Introduction

Citrus is a very important crop in many tropical and sub-tropical countries throughout

the world. In addition to its economic rewards, the fruit is an important source of vitamin C, and the juice and fruit provide a welcome addition to the diet of many developing countries. However, certain virus and virus-like diseases, together with fungal and bacterial diseases can limit production and in some cases are destroying and ravaging citrus as an industry or eliminating backyard trees. Unknowingly, infected budwood is being introduced into new citrus growing areas and new diseases are appearing where they were not previously known. Some of these diseases are proving to be destructive and costly to the citrus industry and devastating to individual growers. Examples of such a new disease are citrus variegated chlorosis costing Brazil over 90 million dollars annually, the witches' broom disease of limes which has destroyed the small fruited lime trees in Oman and is ravaging the lime industry of Iran and the stem pitting tristeza disease in Peru which has virtually destroyed their once thriving navel orange industry. A knowledge of these diseases might be helpful to growers, nurserymen and research workers in formulating certification programs, and the reviews and references in this chapter could provide some assistance in identifying and understanding these citrus diseases.

When a citrus tree shows a new peculiar symptom, or suddenly dies prematurely, there is usually a cause. The pioneer workers who searched for answers as to why the trees were showing certain symptoms or were dying, spent many years and in some cases a lifelong effort in exploration, research and discovery. Their efforts opened a path of understanding and sometimes they discovered clues for the eventual identification and control of the disorder. It is therefore fitting that their efforts and achievements be acknowledged and hopefully their methods and techniques will help others who are the current pioneers in the discovery and cure of new and old diseases.

In addition to fifteen of the major graft-transmissible diseases of citrus, the thermotherapy in elimination of citrus viruses is also included in this chapter. All of the material and photographs are taken from the extensive slide shows which can be readily accessed on the internet at http://ecoport.org.

2. Tristeza disease

2.1 Early history and background

The tristeza caused by the citrus tristeza virus (CTV) is perhaps the most serious and one of the most devastating diseases of citrus. This disease has been the most extensively studied among the entire citrus virus and virus-like diseases throughout the world. It is safe to predict that tristeza is present in all citrus growing counties. However, in some countries, serious spread as yet has not occurred. Tristeza may be present in these countries symptomless in varieties such as mandarins or sweet orange on tolerant rootstocks. The spread and movement of tristeza depends upon the distribution of infected budwood, the vectors present, the strains of the virus present and temperatures. China is probably the home of many species of citrus and also of the origin of the citrus tristeza virus. The probable routes of tristeza through movement of infected plants and vector was from China to Japan, to the Philippines, India, Australia, and South Africa. CTV and its principal vector *Toxoptera citricida* became endemic in

these countries. Tristeza was present in California and Florida in the 1880's with the importation of satsuma mandarin trees from Japan (Tanaka, 1952). Wallace *et al.,* (1956a) writes: "Records found in South Africa indicate that 1,400 Lue Gim Gong orange trees on rough lemon rootstock were exported to the Argentine in July, 1930, followed by a further exportation of 250 navels, 500 Valencias, 350 Lue Gim Gong and 100 Ruby blood on rough lemon rootstock about one year later." It is almost certain all were infected with CTV and most likely the vector *T. citricida* was present on the exported trees. Fraser and Broadbent (1979) in reviewing the history of tristeza in Australia indicated the presence of the disease and its most efficient vector well before 1890 and perhaps earlier than 1870. They also indicated that there is evidence that in 1933, large shipments of citrus trees were sent from Australia to estates being developed in Argentina. An excellent reviews on the history of tristeza is given by Bar-Joseph *et al.,* (1981).

There has been a direct relationship between the great *Phytophthora* epidemics of the 19th century and the ensuing tristeza epidemics, beginning in the 1930's and directly related to the sour orange as a rootstock. The spread of *Phytophthora* species detrimental to citrus was epidemic between 1836 and 1916. The destruction of seedling citrus trees was devastating. The worldwide epidemics by Phytophthora induced a change in citrus culture from primarily growing trees as seedlings to budded scions on sour orange rootstock. The sour orange was found to be tolerant to citrus Phytophthora species and also was found to be a superb rootstock. The sour orange was tried as a rootstock in Australia prior to 1870 (Fraser and Broadbent, 1979) and in South Africa about 1895. Its failure in both countries was thought to be due to incompatibility, but was in fact due to tristeza. It is apparent that tristeza and its prime vector *T. citricida* were well established in Australia and South Africa at that time. In South Africa, when sweet orange was put on sour orange rootstock, the trees did not grow and ultimately died. All varieties of sweet orange, mandarins or lemons budded to sour orange died in a relatively short time (Powell, 1930). Zeman (1931) reported the death of sweet orange trees on sour orange rootstock in Argentina in 1930. Bitancourt (1940a) reported a similar death of trees on sour orange rootstock in Brazil occurring in 1937. Toxopeus (1937) correctly indicated that the incompatibility of sweet orange on sour orange rootstock was due to a substance, which was formed, in the sweet orange canopy and when transported across the bud union to the sour orange was lethal to the sour orange rootstock. The combination of sweet orange budded to sour orange, as a rootstock failed in all parts of Java and symptoms were identical to the failure reported for this combination in South Africa. Yet, Toxopeus cites that this combination of sweet on sour was highly successful in so many other countries. Webber (1925) visited South Africa and observed that the sour orange as a rootstock failed everywhere in the country. He clearly felt that the failure was not due to off type seedlings, incompatibility or soil or climate differences. Webber (1943) was the first to postulate a theory suggesting this new disease was vector-transmitted and caused by a virus. Based on Toxopeus' studies (Toxopeus, 1937), Webber concluded that: the disease was most likely caused by some virus that might be carried to the sweet orange by an unknown vector. A virus not noticeable in its effect on the sweet orange but when introduced into the sour orange, is toxic to that species. His analysis, made over 60 years ago, was prophetic and accurate. The new devastating disease which began to ravage the citrus of Argentina

and Brazil was appropriately named 'tristeza' by Prof. S. Moreira. In Spanish or Portuguese tristeza means sadness (Moreira, 1942). Drs. Knorr and Ducharme visited Argentina in 1950 as part of a joint USDA project with Argentina and Brazil to study this new disease. They reported on the devastation they observed (Knorr and Ducharme 1951) and poetically wrote as follows: "When for the first time we looked down on the citrus acreage between Buenos Aires and Concordia we saw a sight, that in its desolation exceeded all anticipation. It was so calamitous, so appalling and heart-rendering, that we felt no previous account ever pictured tristeza's rapacity." Dead trees killed by tristeza in Argentina were piled up and the wood carted away in the mid 1930's and the 1940's.

The destruction of sweet orange on sour orange rootstock in California began in 1939. It was called 'quick decline' because the trees declined very rapidly during the springtime. This decline was first noted in the Covina-Azusa area of Los Angeles County in 1939 and by 1945 had increased to about 20,000 collapsed trees. The cause of this decline was not known at that time. All research resources of the State were mobilized and personnel from the University of California at Riverside and the California Department of Food and Agriculture began intensified studies of this problem. Death of trees by the new 'quick decline' of citrus was related to the death or necrosis of the phloem cells in the cambium tissue of the sour orange (Schneider, 1954). This effectively girdled the tree at the bud union. With death of these phloem cells, the starch produced in the leaves of the canopy was prevented from being transported to the roots; the roots died and when the weather warmed up in the spring the trees quickly declined. Thus the name 'quick decline' was given to this disease in California. Meneghini (1946) showed transmission of an apparent virus by the aphid *T. citricida*, the brown citrus aphid (called *Aphis traversi* in this publication). Costa and Grant (1951) showed that a single aphid of *T. citricida* could transmit the tristeza disease.

The possible relationship of 'quick decline' with a disease called tristeza in Brazil and Argentina was recognized by many workers (Halma *et al.*, 1944, 1945); Stout, 1945; Fawcett, 1947). The historical discovery that 'quick decline' in California was possibly the same as the tristeza disease in South America (Halma *et al.,* 1944). Fawcett and Wallace (1947) proved the disease was transmissible. The evidence which finally and conclusively proved the similarity between a number of various diseases came with the discovery of the seedling lime index simultaneously developed in Brazil, California and South Africa (Costa *et al.*, 1950; Wallace and Drake, 1951; McClean, 1950). Their work was based on the discovery by Hughes and Lister (1949) that graft or aphid transmission from diseased lime trees in the Gold Coast of Africa induced vein clearing in leaves and stem pitting in stems of acid lime seedlings. The new seedling index proved that the tristeza disease in South America, quick decline in California, stem pitting of grapefruit in South Africa and Australia and the decline and stem pitting of limes in the Gold Coast of Africa were all caused by one virus - the citrus tristeza virus.

2.2 Early pioneering work

 The first test for tristeza was the iodine test. A Potassium Iodide solution applied to the bud union area after cutting a window exposing the bud-union of a declining tree will

show black above the bud union - but not below the bud union. This indicated starch accumulation above the bud union and starch depletion in the rootstock. This test, plus observation for inverse stem pitting in field trees were the very first field tests for suggesting the presence of tristeza (Bitancourt, 1944). Prof. A. A. Bitancourt was an eminent early pioneer in tristeza research in Brazil. With the first outbreak of the new disease in Argentina he gave an excellent review of the disease which laid the foundation for other discoveries (Bitancourt, 1940b). He developed the starch test - the first index for the disease (Bitancourt, 1944). He was also a teacher and Professor at the Instituto Biologico in Sao Paulo, and was the guiding teacher of many eminent citrus virologists including Victoria Rossetti.

Dr. Gilbert Stout with the California Department of Food and Agriculture was a pioneer in surveying for tristeza in California during the mid 1940s. Armed only with determination and a bottle of potassium iodide solution, he organized some 50 State and County inspectors and trained them in special classes. In a 3-month period approximately 240,000 acres of citrus in California in 12 Counties were inspected. "Equipment consisted of a car, a shovel, a trowel, a pair of snap-cut pruners, a dropper bottle of iodine solution and materials necessary for making uniform records." One must admire the dedication and tenacity of these workers in their search to find the extent of this new spreading disease. Previous to this survey, quick decline had been known only in parts of Los Angeles county (Covina-Azusa) and on property in west San Bernardino county. Diagnosis for quick decline in 1945 was not definitive. "Trees injured by gophers, trees in heavy soil, or some trees on trifoliate stock would not react to the iodine test." However, over 7,600,000 trees were inspected outside of the decline area and 457 new positive and suspect trees recorded. This survey was the beginning. The disease ultimately spread rapidly and its destruction is now history. Over three million trees on sour orange rootstock were killed in southern California. One significant outcome of this survey was the finding that the disease was not present in certain areas of the State. Therefore, quarantine laws were subsequently enacted to prevent further spread of the disease. Also significant was the relatively rapid dispersion of the disease from the period of the surveys in early 1945 to the 1960s where almost all trees in Los Angeles, Orange, Ventura, San Bernardino and Riverside counties became infected. In an excellent history and review of tristeza and rootstock compatibility by Bitters and Parker (1951) showed that grapefruit top worked to tristeza-infected sweet orange on sour orange rootstock would fail. They also presented the first indication that Troyer citrange was a tolerant rootstock to tristeza decline. They indicated that stem pitting by tristeza was present in many kinds of citrus and was severest in limes trees.

Dickson *et al.,* (1956a) summarized his extensive research on the transmission of CTV by *Aphis gossypii*. This aphid was found to be the primary vector in California even though it represented only 4% of the aphid population. The transmission rate was less than 5%. Norman and Grant (1953) showed that *A. spiraecola* and *A. gossypii* would transmit CTV in Florida, also at low rates of transmission. Mendel (1956) warned of the possible presence of tristeza in old citrus varieties in Israel and in the Mediterranean area. He also warned that it could spread. He accurately predicted a change in transmissibility of CTV in Israel. Tristeza was in fact found to be present in Israel (Wallace *et al.*, 1956b; Reichert *et al.*, 1956). Wallace and Drake (1955) demonstrated

that all Meyer lemon trees in California indexed positive for CTV. However, there was no apparent spread from the infected Meyer lemon trees to nearby sweet/sour. This research led to the massive eradication program of all Meyer lemon trees in central California, where, Stout (1945) had found little or no tristeza during his many surveys.

2.3 Detection of Tristeza disease

The first test for the detection of tristeza in the early years was the iodine test for starch accumulation above the bud union and starch depletion below the bud union. This, combined with inverse pitting or honeycombing in the sour orange rootstock just below the bud union was somewhat diagnostic, but not certain. This was later followed by the use of the Mexican lime as an indicator and finally the use of ELISA with its various procedures including direct tissue blot immunoassay. Also, the visualization of the virus particles in the electron microscope was also helpful for confirmation during the early years. Kitajima *et al.*,(1963) reported thread-like virus particles associated with tristeza infected tissue. This was the first report indicating an association of a specific virus particle with the tristeza disease. These virons are the largest known among RNA plant viruses, measuring 10 x 2,000 nm at 80,000 magnification. High yields of partially CTV were obtained by Bar-Joseph *et al.,* (1970). CTV belongs to the Closterovirus genus (Bar-Joseph *et al.*, 1979a) and the Closteroviridae family.

2.3.1 Biological Indexing

The discovery of the small fruited lime as an index plant for the citrus tristeza virus (CTV) integrated many separate diseases as just one disease 'tristeza' all caused by CTV. The small fruited lime is commonly known as the 'Mexican' or 'West Indian' lime, the 'Kaghzi' in India, the 'Baladi' in Egypt, the 'Doc' of Morocco, the Omani in Oman and the 'Gallego' in Brazil. In all citrus growing countries it is a popular fruit and given a local name, but they are all very similar. Biological indexing is still required for detection of the severe seedling yellows and stem pitting isolates of CTV. Most tristeza will usually induce typical vein clearing and cupping in leaves of the small fruited lime (*Citrus aurantifolia*). In addition to vein clearing, the cupping of the leaf in Mexican lime is helpful for diagnosis in greenhouse indicator plants grown under relatively cool conditions. However, the vein enation virus may also induce a leaf cupping reaction in Mexican lime indicator seedlings. Symptoms due to CTV when viewed on the underside of a leaf of Mexican lime by reflected light are dark green to black broken lines in the leaf veins. These are the same areas that show up as translucent when viewed from the underside of the leaf in transmitted direct.sunlight. Most isolates of CTV can be observed within 4 to 8 weeks in Mexican lime seedlings. Vein clearing for most isolates of CTV can be detected within 8 weeks in healthy vigorous plants of Mexican limes grown under proper temperature conditions (Fig.1). Symptoms of vein clearing in lime seedlings are masked at warm temperatures. (Roistacher *et al.*, 1974). Certain CTV isolates will induce only a mild vein clearing in seedlings of Mexican lime.Observing for tristeza vein clearing should be done in strong light, preferably through direct sunlight, or symptoms can be missed. CTV isolate T-519 is used as a standard very mild positive

control at the University of California Rubidoux indexing facility. This isolate induces a very mild vein clearing and stem pitting reaction in Mexican lime seedlings.

With very few exceptions, possibly all tristeza isolates will induce stem pitting in Mexican lime seedlings. Most reactions are very distinct (Fig.2). However, at times only one or two pits may be seen with milder reacting isolates. For critical indexing in a certification program, both biological and ELISA should be used. Gillings *et al.,* (1996) reported: "To date, glasshouse indexing is the only method available to reliably determine the biological properties of a given field isolate of CTV. However, it is time consuming and labor intensive". This remains a true statement to this date.Vein clearing may appear in greenhouse grown leaves of seedlings of sweet orange inoculated with severe seedling yellows or sweet orange stem pitting isolates of CTV. However, most CTV isolates will not affect sweet orange indicator seedlings. Similarly, Vein clear-

Figure 1: Vein clearing in Maxican

ing in leaves of sour orange seedlings may occur with severe CTV isolates but most isolates of CTV will NOT induce vein clearing in leaves of sour orange indicator seedlings. The seedling yellows reaction in grapefruit seedlings was also observed with some isolates of CTV but most CTV isolates found in the United States and the Mediterranean region will not affect grapefruit seedlings or induce yellows reaction. Normally the sour orange, sweet orange and rough lemon are resistant to stem pitting when inoculated with many isolates of tristeza. However, there are tristeza isolates which can stem pit these hosts. The vein corking reaction in Mexican lime, sour orange and sweet orange is also associated with severe isolates of CTV.

Fraser (1952) first reported the seedling yellows reaction in seedlings of lemon

with a severe CTV isolate found in Australia. Eureka or Lisbon lemon seedlings are good indicators for seedling yellows. However, they produce extremely low numbers of true-to-type nucellar seedlings (below 7%). The sour orange is a preferred indicator for the seedling yellows reaction.

2.3.2 The detection of severe CTV isolates by ELISA

With unparalleled efforts a team of scientists reported on the purification of the citrus tristeza closterovirus. They then successfully developed the enzyme linked immunosorbent assay (ELISA) for the rapid detection of tristeza. This was a momentous breakthrough (Bar-Joseph *et al.,* 1979b). In 1982 the members of this team of M. Bar-Joseph, S. Garnsey, D. Gonsalves, M. Moskovitz, D.E. Purcival, M.F. Clark and G.
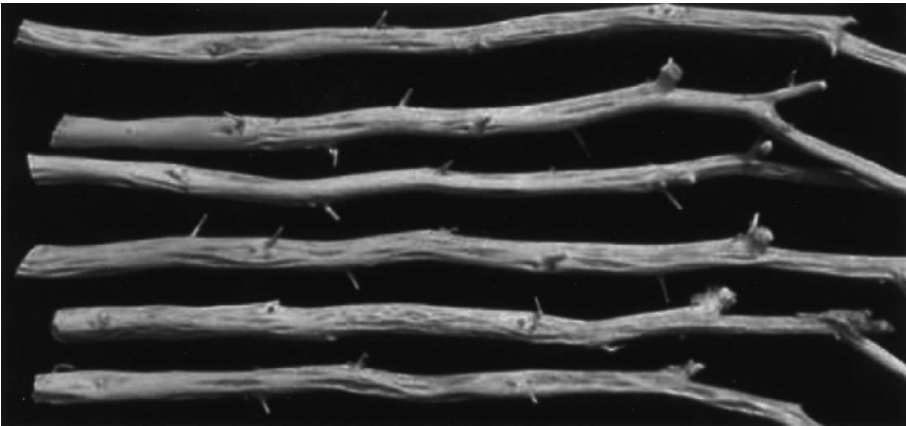
Figure 2: Typical stem pitting reaction in Mexican lime

Loebenstein were given the coveted Hutchinson award by the American Phytopathological Society for this outstanding contribution (Phytopathology 72:29, 1982).
Detection of CTV by ELISA opened the door for large scale indexing for determining the distribution of CTV within a grove, area, region or country. New technology using imprinting proved innovative and valuable for the rapid detection of CTV (Garnsey *et al.*, 1993). Temperature plays an important part in the effective use of ELISA technology (as well as in biological indexing, and ELISA alone should not be used in the critical indexing for foundation block candidate trees in a certification program.

The search continues for a laboratory method to detect severe and exotic isolates of CTV using ELISA. Permar and Garnsey (1991) reported on a new monoclonal antibody which could detect the more severe isolates of CTV which were associated with the severe stunting of sweet orange on sour orange rootstocks in Florida. Although MCA-13 is excellent for detecting this severe sweet/sour decline isolate, it has failed to detect 10 out of 22 exotic severe CTV stem pitting and seedlings yellows

isolates in the citrus virus bank at the University of California at Riverside. Also, it did not detect many severe CTV isolates in other Asiatic countries (Kano *et al.*, (1991). Plants are still needed for the verification of severe and exotic isolates of CTV (Roistacher and Moreno, 1991); Gillings *et al.*, (1996). To prevent confusion, when the term "severe" is used, care should be exercised in defining severe and a full description of the basis upon which this determination was made should be presented. Garnsey *et al.*, (1987) gives a working basis for defining severity.

The time of year for ELISA testing is critical. Most isolates of CTV can be missed if temperatures are too warm. Studies by Dodds *et al.,* (1987) reported that during the hot periods of August, September and October in Riverside, California the titer of CTV in the trees was low and the virus could not be detected by ELISA. In a certification program, both biological procedures and ELISA should be used for indexing. A side shoot permitted to grow on the Mexican lime indicator seedling under cooler greenhouse conditions is suitable for CTV detection. The tissue from this side shoot grown under cool conditions should then be used for an ELISA test while the Mexican lime seedling is for biologically testing, Such testing should be done, not only for CTV, but for other graft transmissible pathogens of citrus. A comprehensive pictorial review of the ELISA technique is given by Garnsey and Cambra in Graft Transmissible Disease of Citrus – Handbook for Detection and Diagnosis' (Roistacher, 1991). The handbook, is now the standard reference for detection techniques of CTV and other citrus graft-transmissible pathogens of citrus.

The acceptance and use of ELISA for determining the presence and spread of CTV is now worldwide and universal. In Israel Bar-Joseph *et al.*, (1983) tested one million citrus trees for CTV by ELISA in the three years following the initial development of the ELISA technique. ELISA has been used for epidemiology studies throughout the world (Cambra *et al.*, 1988, 1993), Gottwald *et al.*, 1993, Lastra *et al.*, 1988, Permar *et al.*, 1990, Kano *et al.*, 1991; Aubert *et al.*, 1992; Grisoni and Riviere, 1993; Kyriakou *et al.*, 1993; Skaria, 1993; Chakraborty *et al.*, 1993); Dodds, 1994). These are but some of the many publications on the use of ELISA for detecting the presence and spread of CTV.

2.3.3 New innovative technology for detection of CTV

Dodds and Bar-Joseph (1983) reported on double stranded Ribonucleic acid (dsRNA) from plants infected with closteroviruses. The research paper by Dodds *et al.*, (1984) on the dsRNA technique won the Wallace award as the outstanding paper for the 9th conference of the International Organization of Citrus Virologists. The band at .05 of typical DsRNA profiles has been found associated with the more exotic, severe CTV isolates. Garnsey *et al.*, (1993) introduced a direct tissue blot immunoassay, which is rapid, requires little sample preparation and the membranes can be stored for as long as 30 days prior to assay. Also, blotted membranes can be sent to another location for testing.

Garnsey and Hilf (2000) reported marked differences among isolates of CTV found in different citrus-growing countries and molecular studies show extensive genetic divergence among isolates. They further reported systematic comparisons of the

biological properties of isolates from numerous different countries and comparison of genetic profiles by PCR with selective primers has provided a preliminary assessment of the global range of variation among CTV isolates. Broad and isolate-specific forms of resistance have also been observed. The variability of symptom severity is both isolate and host specific. The extensive divergence in sequence among isolate groups, and variability in host range suggest that the current global population of CTV isolates in commercial citrus reflects multiple ingress of divergent CTV isolates into cultivated cultivars from wild citrus or another host. Widespread, but erratic dispersion of various CTV isolates to secondary centers of citriculture via infected plants or budwood has occurred. Subsequent vector dispersion has resulted in new citrus germplasm - CTV isolate exposure combinations. Clonal propagations of tolerant host-virus isolate combinations are apparently stable, however, vector transmission, exposure to different hosts and replication in severely affected hosts presumably create selection pressures that could expose new genetic variants.

The CTV genome is a positive sense, single-stranded RNA composed of 19,296 nucleotides, and it contains the potential for encoding of 17 proteins.

2.3.4 A look at exotic isolates of CTV worldwide and their potential for destruction

The loss of millions of trees of sweet orange, mandarin and grapefruit on the sour orange rootstock is but one very important means of destruction of citrus by CTV. This has been offset by the use of CTV tolerant rootstocks not subject to phloem necrosis. However, there are isolates of CTV which can destroy citrus regardless of rootstock and which can attack the scions or rootstock directly (Roistacher and Moreno, 1991). The small fruited lime trees are highly susceptible to CTV infection and will decline in the presence of most isolates of the virus. Cross protection has been effective in Brazil and in South Africa for the small fruited lime. Natsudaidai sour orange on trifoliate orange rootstock, a favorite fruit in Japan may show severe CTV stem pitting. The fruit size on infected trees were small, stunted and unproductive (Omori and Matsumoto, 1972).

A new severe stem pitting isolate of CTV (12B CTV isolate) was found in the fields at the University of California (UCR) orchards in Riverside (Calavan *et al.*, 1980). This discovery prompted a testing program of all citrus trees at UCR for stem pitting or seedlings yellows CTV. Trees found infected were discarded or given therapy treatments. This 12B CTV isolate severely affected Madam Vinous and other sweet orange seedlings when they are bud or vector inoculated. All of 100 typical CTV isolates found in California had no effect in protecting against the 12B isolate (Roistacher and Dodds, 1993).

Sweet orange seedlings normally do not react to most CTV graft-transmitted isolates. However, when budwood from tangelos in field 12B containing the severe stem pitting isolate were inoculated into Madam Vinous sweet orange seedlings, a severe reaction occurred.

The financial losses due to stem pitting tristeza in red grapefruit in South Africa were studied by Marais and Breyten (1996). They evaluated 660,000 trees on over 5,500 acres and calculated a total loss of 5 million US dollars each year.

2.4 Epidemiology

The citrus tristeza virus probably originated in China and with movement of budwood and trees is probably present in every citrus growing country in the world. There are two primary vectors of CTV: *T. citricida* and *A. gossypii*. *T. citricida* is an extremely efficient vector of CTV. The first major and massive disaster of the killing of trees on the sour orange rootstock occurred in Argentina and Brazil beginning in the 1930's. Many millions of trees were killed. Epidemics followed in Florida in 1951, Spain in 1956, Israel in 1970 and Venezuela in 1980. New outbreaks of tristeza occurred as new more transmissible and devastating isolates appeared. The spread of *T. citricida*, the primary aphid vector, moving from South America into all of the countries in the Caribbean region and into Mexico and Florida will ultimately kill all of the trees on sour orange rootstock. This is now occurring in most of the island countries in the Caribbean. Without a certification program, exocortis disasters usually follow when replanting is done on CTV-tolerant rootstocks, most of which are susceptible to citrus viroids. Warm temperatures are conducive to severe viroid reactions. There are CTV stem pitting and seedlings yellows isolates which destroy the scion directly. The loss of the navel orange industry in Peru was the direct result of the importation of severe exotic CTV isolates from Japan in symptomless Satsuma mandarin on trifoliate rootstock. Other exotic and destructive CTV isolates were found in the Capao Bonito region in southern Brazil and also in Queensland, Australia. In both cases, these isolates were illegally imported. The epidemiology of the disease and its direct and indirect effects on the citrus industries of various countries will be discussed in this part.

2.4.1 CTV in Florida

CTV was present in Florida for many years, but outbreaks occurred only as new strains of the virus emerged or were introduced and were efficiently transmitted by *A. gossypii*. Knorr (1956) suggested that Florida had a milder form of CTV and indicated that tristeza had been present in Florida for over 50 years, citing the presence of the Meyer lemon as an example. He reported that a 65-year old navel orange tree was found infected while nearby Valencia orange trees were found free of CTV. Grant (1959) described the presence of many isolates of CTV in Florida in different citrus varieties and these ranged from mild to quite severe. Burnett and Boring (1960) reported that tristeza had spread very fast in Florida between 1957 and 1959. Bridges and Youtsey (1972) documented how rapidly CTV spread in one Florida County. In the mid-1970's Garnsey and Jackson (1975) reported on a destructive outbreak of CTV in Florida, indicating the appearance of a new CTV isolate which would destroy sweet orange on sour orange rootstock. Previous to this new decline the sour orange was still being used as the preferred rootstock in Florida because of its tolerance to blight.

Yokomi *et al.,* (1991) reported that there was an increase in the natural spread of CTV in grapefruit in Florida between 1976 and 1983. Trees on sour orange rootstock were badly affected. Brlansky *et al.,* (1986) described a new and severe CTV epidemic in south Florida with extensive tree losses of sweet/sour. They reported extensive tree losses within a 100 square mile area west of Fort Pierce and within a 200 square mile area

T
declined rapidly and often died within several months. This destruction was caused by release of propagations of budwood containing a severe stunting isolate of CTV.

Once *T. citricida*, the brown citrus aphid (BrCA) enters a country, transmission of tristeza would be at much faster rate as compared to *A. gossypii*. An isolate of CTV can spread and infect an entire new citrus planting within one to two years whereas the spread of CTV by *A. gossypii* may take many years to achieve 100% infection. Yokomi and Damstreegt (1991) studied single aphid transmission for *T. citricida* compared to *A. gossypii* and the results showed a remarkable difference in transmission of various exotic isolates of CTV from different countries. The average transmission for *T. citricida* using single aphids was 16% compared to 1.4% for *A. gossipii.*

The BrCA can be identified by its four distinguishing characteristics which separates it from the other black or brown citrus aphid *T. aurantii* . i) it is much larger; ii) there is a fork in the midvien of the wing (M-2) whereas *T. aurantii* shows no forked midvein. iii) The pterostigma on the upper wing of *T. citricida* is light in color whereas the pterostigma on the upper wing of *T. aurantii* is dark in color, and iv) the 3rd segment of the antenna is dark in *T. citricida* and light in *T. aurantii.*

There have been accelerated losses of trees on the sour orange rootstock in Florida. As of December, 2000, the third year for the presence of the BrCA in Florida,. Tsai *et al.,* (2000), using single aphids of *T. citricida*, was able to extract stem pitting isolates from a non-stem pitting declining tree of sweet/sour. This suggested that sweet orange stem pitting strains are present in non-stem pitting sources. Moreno *et al.,* (1993) was also able to extract severe stem pitting and vein corking isolates in sweet orange from a declining tree of sweet/sour tree which showed no stem pitting. This was done by single aphid transmissions using *A. gossypii*.

2.4.2 The spread of tristeza in Spain

Navarro (1993), in a history of the orange in Spain, reported that the first general manifestation of tristeza occurred in Spain after the severe freeze of 1956. Beginning in 1957, decline of trees on the sour orange rootstock was noted in the Alcira region. Trees became defoliated with a gradual loss of vigor making them completely unproductive. In some cases a quick decline was noted. However, some farmers reported a similar decline prior to the freeze of 1956. Other focuses of decline were noted as probably due to dissemination of infected trees. Navarro suggested that tristeza was probably present in Spain much earlier with importation of infected material from the United States. Indexing tests done at the old experiment station at Burjassot in 1959 were positive for CTV. However, this was not accepted and the widespread belief was that the problem was physiological and was due to the freeze damage of 1956 followed by heavy rainfall in the following years after the freeze. However, in 1962, there was a severe outbreak of the disease with many dead and dying trees and again another severe outbreak in 1968. The primary cause of distribution of the disease throughout Spain was by propagation of infected budwood and its distribution throughout the country. Further spread was then by aphid transmission. Observation of decline of mandarins on sour orange rootstock in Spain in 1975 revealed that tristeza began destroying trees in Spain about 1956.

Currently over 35 million trees have been killed, and are still dying on sour orange rootstock. The industry was regenerated using shoot-tip grafted virus-indexed budwood grafted to CTV tolerant rootstocks. Cambra *et al.,* (1988) monitored the incremental spread of CTV in a new planting of 832 citrus trees in one orchard in Spain over a 6-year period using ELISA. At the end of 6 years, 67% of the trees were infected. The vector was primarily *A. gossypii*. Ballester *et al.,* (1993) surveyed many citrus areas in Spain and studied the symptomatology and transmissibility of a number of isolates. They reported the range of transmissibility of 30 to 100% from their various CTV sources.

2.4.3 Outbreak of tristeza in Israel

About 1970, citrus trees were found dying on a farm in the Hibet Zion region of Israel. The cause was not known, but tristeza was suspected. Electron microscopy revealed the presence of particles of CTV (Bar-Joseph *et al.*, 1974). Many individual trees were tested, first using electron microscopy and later using the Mexican lime indicator plant. The transmissibility of CTV by *A. gossypii* in Israel had changed from the low rates of 4 to 6% typical for the ST, BT and CT isolates. This was typical of the low transmissibility found in California by Dickson *et al.,* (1956b). However, in the Hibet Zion region the new spread of the VT and HT isolates had changed and was as high as 40 to 60%. This was a new discovery and was probably responsible for the rapid dissemination of tristeza in the Hibet Zion area. Bar-Joseph (1983) described cross-protection incompleteness of CTV in a concept as internal cross-protection where one strain of CTV protected a more transmissible strain. He suggested that this protection breaks down after about 30 years resulting in increased transmissibility of CTV by *A. gossypii.*

A new severe outbreak of CTV occurred in Israel in the Morasha Junction region near Tel Aviv in Shamouti sweet orange on sour orange rootstock. This strain of CTV moved rapidly from the point of aphid infection in the outer crown of the tree down to the bud union destroying the phloem cells in the sour orange rootstock. This effectively girdled the tree and caused large scale death and destruction of the Shamouti orange industry in the region (Bar-Joseph and Nitzan, 1991). Graft transmission to sour orange or lemon seedlings from a dying tree in the Morasha region of Israel showed that the isolate responsible for this severe decline was a seedling yellows tristeza isolate. The eradication program failed in Israel because the disease was widespread in coastal areas and the eradication program was discontinued there. The disease now spreads to new plots probably through infected nursery plants.

The change in transmissibility of the citrus tristeza virus in Israel led to a series of experiments at Riverside, California to determine if transmissibility of CTV had changed in California. Studies on transmissibility of CTV showed that transmissibility of CTV in California during the early spread of tristeza was at the relatively low rate of 4 to 6 % as shown by Dickson *et al.*, (1956a) but was later found to be at 100% for many isolates. ELISA and biological indexing failed to recognize tristeza. Citrus trees were killed even before CTV could be detected by either.

2.4.4 Tristeza epidemic in Venezuela

In the 1970's the citrus industry of Venezuela was primarily on the sour orange root-

stock and there had been only one report of the presence of tristeza in that country (Knorr *et al.*, 1960). *T. citricida* was not present and this South American country had escaped the ravages of tristeza. Roistacher visited Venezuela in 1979 and observed the presence of the BrCA in every orchard he visited in the citrus growing regions around Maracay and Valencia. However, he found no evidence of tristeza decline in any grove visited.

In his report, he warned of the potential future destruction of sweet orange trees on the predominant sour orange rootstock and suggested that a massive indexing program be undertaken using ELISA. He outlined such a program to the Minister of Agriculture and warned that unless this was done, there would be massive destruction of their sweet orange industry. This was ignored and the death of trees on the sour orange rootstock began and continued throughout Venezuela. By 1983 four million trees had been killed (Mendt *et al.*, 1984). During a visit to Venezuela in 1992, he observed that trees on sour orange rootstock were still massively dying from tristeza. Also, the quality of the trees observed in many new orchards, which were on the tristeza tolerant rootstocks of Cleopatra mandarin and Volkamer lemon appeared poor. In 2000, it was estimated that over 10 million trees had died on sour orange rootstock with a loss exceeding over 50 million dollars.Slowly but inevitably the brown citrus aphid spread from Venezuela into Central America, Mexico, the many islands of the Caribbean and into Florida, with serious consequences for the growing of citrus in the entire region. The BrCA invaded Costa Rica about 1989.

In a survey of that country in 1991 the aphid was found everywhere throughout the country. Once the BrCA enters a country, the sour orange will eventually disappear as rootstock. The timing depends upon the vector, the strains or isolates of the CTV present and the temperature.

2.4.5 A severe destructive isolate of CTV in southern Brazil

The Capao Bonito severe CTV isolate is believed to be of Japanese origin since it was first discovered in the Registro region where a large Japanese colony is located (Rossetti, 1975). The symptoms in the Capao Bonito County of Brazil are very similar to the Peru navel orange stem pitting isolate and the '12B sweet orange stem pitting isolate in Riverside, California. By strict quarantine measures, this severe CTV has been restricted to the Capao Bonito district in Brazil.

2.4.6 An outbreak in Australia

Owen-Turner (1990), and Broadbent *et al.*, (1992) reported on a new and exceptionally severe isolate of CTV which stem pitted Washington navel orange trees in Australia. This isolate was similar to the 12B stem pitting isolate in California, the Capao Bonito isolate in Brazil and the severe navel stem pitting isolate in Peru.

Evidence indicated that there was an illegal import of budwood which may have been responsible for this outbreak. Effective isolation and a severe tree removal program has effectively diminished potential epidemic damage from this isolate.

2.5 Cross Protection

When tristeza destroyed millions of trees on the sour orange rootstock in South America, California, Spain, and elsewhere, the fortunate fact that certain rootstocks could be used to grow citrus successfully allowed for the replanting and rehabilitation of citrus industries. New rootstocks were tested and found to be resistant to the bud union phloem necrosis induced by the citrus tristeza virus (CTV). Some of the rootstocks found tolerant to CTV are the trifoliate orange and its hybrids *i.e.* Troyer or Carrizo citrange and citrumelo. Also the rough lemon, Volkamer lemon, Rangpur lime and Cleopatra mandarin are tolerant to CTV. All were successful in there tolerance to CTV, and the sour orange was replaced as the primary rootstock where tristeza epidemics had occurred. However, there are strains of CTV which can attack the scion directly regardless of the rootstock. These strains induce severe stem pitting in trunks and branches of lime, grapefruit and sweet orange trees resulting in the production of small fruit and tree decline. Since the scions and even rootstocks may be severely pitted, changing to a new rootstocks has little or no effect, and this course of action could not be used to resolve this serious problem. The only solution was to search for and find mild strains of CTV which might protect against the severe stem pitting strains.

2.5.1 Theory of Cross protection

Lee *et al.,* (1987) theorized that mild strain cross protecting isolates of CTV reaches higher concentrations in its host plant than does the run of the mill mild strain isolates. These cross protecting isolates will overshadow the new introduced severe isolate. Normally, the rule of the thumb is, the more severe the CTV isolates, the higher the concentration of the isolate. They also theorized that the distribution of the mild protective isolate is important and it should have the ability to quickly invade all new flushes. Targon *et al.,* (2000) showed that the mild protective CTV isolate in Brazil, replicated faster and at a higher titer than the severe isolate in the tissues of all samples evaluated.

There are three general types of tristeza which can cause tree decline. i. Sweet orange, mandarin or grapefruit on sour orange rootstock, ii. Seedling yellows tristeza (SYT) and iii. Stem pitting tristeza (SPT). In an excellent three part series reviewing his lifelong experiences with tristeza, McClean 1977a, 1977b, 1977c) described the three types of decline induced by tristeza. 1) the necrosis of the phloem cells in the sour orange rootstock just below the bud union causing girdle and death of trees. 2) seedling yellows tristeza - strains of tristeza which induce a seedling yellows reaction in certain seedlings such as lemon, grapefruit or sour orange and 3) stem pitting tristeza which induces stem pitting in the scion or rootstock of lime, grapefruit or sweet orange.

With over a 100 million trees of sweet orange, mandarin or grapefruit on the sour orange rootstock which have declined, no effective long term cross protection has been developed over a period of 60 years. It is questionable to expend funding and energy to derive protective CTV isolates for scions on the sour orange rootstock with the objective of reviving the sour orange as a rootstock? Even if a protective isolate were found could it be recommended and would it be worth the risk Also, most cross protection studies in different countries for protection of sweet orange on sour orange

decline have ultimately failed (Stubbs, 1964; Wallace and Drake, 1974; Cohen, 1972; Thornton *et al.*, 1980;Van Vuuren *et al.*, 1991; Pelosi and Powell, 1992).

Wallace and Drake (1972, 1974) working with recovered shoots from seedlings yellows tristeza infected plants, discovered that these recovered tristeza strains would provide protection of sweet orange on sour orange rootstock. Experimental trees were put in the field at the University of California at Riverside. For the first six years results were very promising. After a number of years Wallace and Drake (1976) reported that cross protection began to break down as new strains of the virus appeared. They concluded that though a number of SY-CTV isolates used provided good protection "a few immunized trees developed symptoms as severe as those of the healthy control trees". This experiment was observed by this author ten years after the report of Wallace and Drake (1976) and nearly all of the protected trees of sweet orange on sour orange rootstocks had declined.

A second type of CTV called seedling yellows will induce a severe reaction in seedlings of lemon, grapefruit or sour orange. Symptoms are a severe reduction in size of the inoculated seedling plus severe chlorosis and yellowing of the foliage. There is evidence that this more severe form of CTV can directly affect scions of citrus causing debilitation and even death of the trees.The seedling yellows CTV strains react on grapefruit seedlings in the greenhouse and also reacts similarly in sour orange seedlings. However, most CTV isolates found in the United States and the Mediterranean region will not affect seedlings of grapefruit or sour orange or induce the seedling yellows reaction. Currently, the sour orange is being used and is preferred as the standard method of indexing for seedling yellows at the Rubidoux indexing facility at the University of California, Riverside. Lemon seedlings, when inoculated with a severe CTV seedling yellows tristeza isolate, will also show typical seedling yellows reaction and are also not affected by most CTV isolates found in California or the Mediterranean countries. The reason the sour orange is preferred over lemon seedlings as indicators is due to the very low number of true to type seedlings produced (less than 7%) and the seedling yellows reaction in the sour orange is the same as that for lemon (Roistacher, 1991 – handbook).

A survey of declining trees in the field orchards at the University of California at Riverside (UCR) revealed the presence of severe seedling yellows and stem pitting isolates of CTV. There appeared to be a direct association between the presence of seedling yellows tristeza and decline (Roistacher, 1981a, 1981b).

2.5.2 Cross protection against grapefruit stem pitting

When severe isolates of CTV are present and especially when the brown citrus aphid (T. citricida) is endemic, the grapefruit is difficult to grow without some means of cross protection. It is subject to severe stem pitting resulting in a reduction of fruit size. In addition, the distribution of CTV in grapefruit is not uniform.

Although there have been effective cross protection of grapefruit as reported from Australia and South Africa, there is a tendency for the cross protection to eventually break down. This may be due in part to a general poor distribution of the protective CTV isolates within the grapefruit tree (Lee *et al.*, 1988). In truth, the mechanism of

tristeza cross protection in citrus is not understood, but breakdown of protective CTV isolates for grapefruit occurs and a search for newer and better protective isolates must be part of any good research program where grapefruit is grown and where the brown citrus aphid is endemic.

Protection was afforded to grapefruit for 13 years at Somersby, Australia at two locations, however, breakdown was reported at both locations but proceeded more rapidly in the cooler region (Broadbent *et al.*, 1991). Collins and Van Vuuren (1990) showed a gross return of $7,300 per ha over a 4-year period due to cross protection for Marsh grapefruit in South Africa protected with the Nartia strain compared to non-protected but virus free plantings. Cross protection paid. Van Vuuren, in a personal communication of June, 2001 reported that GFMS-12 is kept as the protective isolate for white grapefruit and pummelos. GFMS-35 has replaced GFMS-12 for all red grapefruit. It is almost certain that in time, protection for grapefruit will break down. Continued research is needed to look for and test new protective CTV isolates for grapefruit

2.5.3 Cross protection for the small fruited lime tree

The small fruited lime trees are highly susceptible to CTV infection and will decline in the presence of most isolates of the virus. Cross protection has been effective in India, Brazil and in South Africa for the small fruited lime. However, continued research must be done to find superior protective isolates since new strains of CTV will evolve and break down existing protection.When searching for protective isolates for lime trees, one must search in lime orchards to look for trees showing protection. Similarly, searching should be done in sweet orange orchards for finding protective strains for sweet orange (Costa and Muller, 1980). A protective isolate for sweet orange may not protect lime trees and visa versa.

In South Africa, a new effective protective isolate 'LMS-6' in was tested in cross protection research on lime trees. The yields over a three year period with 'LMS-6' were significantly better, averaging over 40 kg/tree compared to that of the non-protected and virus-free lime trees which averaged less than 5 kg/tree. In a personal communication from Van Vuuren (June, 2001) he reported that LMS-6 has replaced GFMS-12 in all sweet oranges and mandarin types (Clementines and satsumas) and LMS-6 remains as the protector for all limes. Van Vuuren *et al.,* (1991,1993) showed the superior performance of protective isolate LMS-6 against 10 selected isolates; two from South Africa, many imported from Florida, and one from Corsica. All were inferior to the LMS-6 isolate.

Balaraman and Ramakrishnan (1977) obtained 70 CTV isolates from a collection of 112 selections of the small fruited acid lime (called 'Kagzi' in India) to be used in cross protection studies.They showed cross protection of a mild reacting strain against one that induced corky vein in seedlings of Mexican lime. Cross protection research was done by Balaraman and Ramakrishnan (1980) from this collection of 112 budwood samples from trees of the small fruited acid lime 'Kagzi' which showed a mild CTV reaction. They reported good mild strain cross protection after five years and the persistence of the mild strains with the lime trees. However, a personal observation by the author made in November, 1992 of these experimental lime trees in the research plots at

the Indian Institute of Horticultural Research, showed that almost all of these experimentally protected lime trees were in decline. Ahlawat *et al.*, (1993) reported that the mild strains of CTV in India could not be discriminated from other strains using MCA-13 in several thousands of tests which had been made from various parts of the country.

Tsuj *et al.,* (1989) reported that after 19 years of mild strain cross protection of Hassaku, the number of trees found with none to mild stem pitting was over 200 compared to 40 trees showing moderate to severe stem pitting. This indicated that despite excellent success earlier, there was breakdown of protection. In any cross protection research program, adequate funding is required for searching and testing new protective isolates. Funds spent on such research will be returned many fold when new protective isolates are found.

2.5.4 A new technique for rapid development of protective isolates of CTV

After the destruction of orange, lime, grapefruit or Hassaku trees by severe stem pitting CTV, the solution for finding protective isolates has been to search for trees which will survive the destructive effects of CTV on the scions, or by searching for mild reacting CTV isolates after indexing to lime seedlings. This requires many years of searching and testing and as pointed out by Costa and Muller (1980) one "must start with a rather large number of mild isolates". Even after diligent searching and testing, perhaps only 1 or 2 out of 100 of the better selections may have broad spectrum protective ability.

Studies were initiated on a new approach for finding protective isolates by passing severe CTV isolates through *Passiflora* species by vector transmission. It was discovered that tristeza stem pitting isolates could be attenuated after passage through Passiflora and these attenuated isolates had the potential for cross protection (Roistacher and Bar-Joseph, 1987; Roistacher *et al.*, 1987; Roistacher *et al.*, 1988). *Passiflora gracilis* could harbor the citrus tristeza virus and induce striking symptoms in this herbaceous host (Muller *et al.,* (1974). CTV was transmitted from infected citrus to *P. gracilis* by *T. citricida*. They also tried vector transmission of CTV to many weeds but found that only Passiflora was infected.

Following up on the studies of Muller *et al.,* (1974) trials were started to transmit CTV to Passiflora from CTV-infected citrus using *A. gossypii*. Transmissions were made from CTV-infected Mexican lime to Passiflora and then back to Mexican lime. *P. gracilis* showed severe reaction with a severe CTV-seedling yellows isolate when transmitted by *A. gossypii*. This reaction was very similar to that obtained by Muller *et al.,* (1974). The technique for transmission of CTV through Passiflora includes certain steps: 1) The virus is vector transmitted by *A. gossypii* from a CTV infected sweet orange to Passiflora. 2)The virus is then transmitted by vector or by graft-transmission to Mexican lime or to other *Passiflora* species. 3) From Mexican lime the virus is then transmitted by bud graft to other citrus index plants. In this way and in these indexed plants, the attenuation of CTV isolates was observed. All of the CTV isolates which passed through Passiflora were found attenuated and would protect against severe CTV-Stem pitting isolates (Roistacher and Bar-Joseph, 1987).

Excellent protection against severe stem pitting of grapefruit was achieved by CTV isolates Code-37a and Code 40. Both of these protective isolates were derived by

passage of their severe stem pitting CTV through Passiflora at the Rubidoux indexing facility in Riverside, California. Attenuation and protection of codes 37 and 40 has held up over a number of years. The protective isolate Code 37 was derived from Brazil navel CTV-SY-663 by vector passage through *P. caerulea* (Roistacher *et al.*, 1988). Trials have been under way in Peru using Codes 37 and 40 against Duncan grapefruit and Madam Vinous sweet orange and are giving excellent results (Personal communication with photographs (2002) from Klas Bederski, citrus nurseryman in Peru).

Excellent protection was also afforded against the 12B severe sweet orange stem pitting CTV with code- Z-5, however, over a period of years, this protection of CTV isolate Code Z broke down. Thus, protection of sweet orange or grapefruit from severe stem pitting can be achieved by developing attenuated-protective isolates in a plant laboratory.

A complete four part slide show can be accessed on the internet at http://ecoport.org/ as slide shows 97, 98, 102 and 103 by C. N. Roistacher.

3. Psorosis and related viruses

The psorosis disease of citrus was first observed in Florida and California in the early 1890's and named psorosis by Swingle and Webber (1896) based on the Greek psora = ulcer or mange. It is the first of the citrus virus diseases described and the oldest researched citrus virus disease. Psorosis was also the first citrus disease to be shown as transmissible by Fawcett (1938) which led to the first eradication program for citrus viruses. Also, the first seedling index for a citrus virus was for psorosis (Wallace, 1945), which reduced detection time from 10 years to just a few weeks. Psorosis was once the most destructive citrus disease and is still highly destructive in certain regions of South America. The disease was commonly called scaly bark. It is believed to have originated in the orient and spread worldwide by the distribution of citrus species and varieties.

Fawcett (1933), designated the disease as psorosis-A to distinguish it from the more virulent form he called psorosis-B. Psorosis-A was linked with the concave gum-blind pocket and crinkly leaf-infectious variegation diseases of citrus as one complex (Fawcett and Bitancourt, (1943). However, research over the years has shown that these diseases separate diseases and not related to psorosis. The psorosis disease was comprehensively reviewed by Timmer and Benateña (1977), and by Roistacher (1993). Also, two comprehensive slide shows on psorosis can be viewed and downloaded on the http://ecoport.org website as slide shows 65 and 66.

3.1 Field symptoms

The classic field symptoms of psorosis are a distinct and diagnostic bark scaling on the trunk and limbs of sweet orange, mandarin or grapefruit (Fig. 3). Psorosis bark lesions can appear in as short a period as 3 years but may not appear even after 50 years. Thus, trees infected with psorosis can be symptomless for many years. The average time for the appearance of bark lesion symptoms is 7 to 14 years. Other symptoms of psorosis on field trees are wood staining in the cut branches, lesions of bark scaling on branches, and with severe psorosis-B leaf lesions and fruit lesions may be visible. Wood staining

seen in a cross section of a branch is diagnostic for psorosis-A.

Psorosis is one disease that can be diagnosed in the field by its distinct bark lesions, wood staining and occasional severe leaf symptoms. However, because psorosis may be totally symptomless in field trees for many years, indexing is an absolute requirement for verification that the tree is not infected with psorosis-A. This is extremely important for selecting source trees for propagation.

3.2 Diagnostics

Figure 3: Severe bark scaling in Sweet orange.

Until recently the only way to detect latent psorosis infection was by graft-transmission to indicator plants. Graft-transmission as a diagnostic tool for detection of psorosis was originated by Wallace (1945). This was a most important and innovative technology as it was the key for the means of rapid detection of other graft-transmissible diseases. With the discovery of the virus particles which were associated with the psorosis disease, new possibilities for indexing became evident. ELISA and PCR technology is now available and appears to have broad spectrum ability for detection of

perhaps all psorosis isolates.

3.2.1 Biological indexing

The glasshouse temperatures for indexing for psorosis should be kept relatively cool at 27-30°C maximum and 16-18°C minimum. If indexing is done under these temperature and plants are vigorous and in good growing condition, the first symptom of psorosis on the preferred sweet orange indicator varieties of Madam Vinous or Pineapple is a wilting and dieback of the new emerging shoots which is called 'shock'. The young leaves will dry up and drop off (Fig 4). It is important to state that sweet orange varieties will differ in their ability to show shock or leaf symptoms (Roistacher and Nauer, 1964). Psorosis leaf symptoms are highly variable and suggest great diversity and possible

Figure 4: Psorosis shock symptoms in inoculated young seedlings of Pineapple sweet orange

variation in virus strains. Warm temperatures may suppress or change the expression of symptoms. Shock symptoms will rarely be seen at warm temperatures and at times, no symptoms will be seen if the temperatures in the greenhouse are too warm.

Not all leaf spots which develop after indexing are caused by the presence of the psorosis virus. The environment (dust or air pollution) or spray injury may cause spotting. Some of these leaf symptoms may resemble those of psorosis - but are not due to the presence of the citrus psorosis virus. It is important to include non-inoculated control plants in all index tests.

3.2.1.1 Mechanical transmission

Most psorosis strains or isolates will not transmit mechanically. Thus, lack of mechanical transmission does not mean that psorosis is absent. All knives and clippers should be disinfected with sodium hypochlorite as routine procedure when working with all citrus viruses. Dipping tools in a 20% commercial bleach containing sodium hypochlorite is recommended as routine (Roistacher *et al.,* 1980).

3.2.2 Psorosis-A and Psorosis-B

Plants containing the psorosis-A virus will protect against a challenge with lesion psorosis-B inoculum. This becomes an important index for determining if the virus is truly psorosis. Wallace (1957) defines psorosis A and B where both A and B are related by cross protection. Both psorosis A and B are systemic in all infected trees. Wallace explained that the concentration of B becomes dominant in bark, thus forming bark lesions. Non-lesion psorosis-A protects against lesion psorosis-B and protection breaks down in 12 to 16 years. All psorosis-A contains psorosis-B.

3.2.2.1 Psorosis-B

The severe form of psorosis is called psorosis-B and comes from the bark lesions. It will induce a severe reaction in sweet orange indicator plants. Specifically, blisters on the stems and leaves.

If we cut a small section of lesion bark and inoculate it into a sweet orange seedling, we can get the blister or psorosis-B lesions. Distinct blisters are also seen on the upper leaf surface of a psorosis-B inoculated sweet orange. Psorosis-B also induces, blister-like lesions on the underside of a sweet orange leaf. Similar lesions may occasionally be found on leaves of field trees infected with severe psorosis.

Many virus diseases of citrus will induce leaf spots and these can be confused with psorosis. For example, all of the following citrus viruses and conditions have not been shown to be related to psorosis-A: Infection variegation, crinkly leaf, leaf rugose, concave gum, blind pocket, Indian citrus ringspot, Florida seed transmitted leaf spot, Dweet mottle, the psorosis like pathogen from Spain, the Bahia bark scaling disease and a bark scaling problem in Trinidad. These are separate diseases and most, when tested, have failed the cross protection challenge with psorosis-B (Roistacher and Blue, 1968). Bahia-type psorosis causes serious damage to grapefruit and sweet orange in Northeast Brazil, especially in the Bahia area and the symptoms strongly resemble the bark scaling of psorosis, not only in the trunks, but on twigs and branches. All indications and tests show that Bahia bark scaling is not related to psorosis-A. The bark scaling is very severe, especially on grapefruit and transmission trials to indicators have all been negative. Numerous trials over a period of 10 years offer evidence that it is etiologically distinct from classical psorosis (Nickel, Personal communication). A seed transmitted psorosis-like pathogen found in citranges in Florida is probably not related to psorosis-A since true psorosis-A has not been shown to be seed transmitted (Wallace, 1978). The identity of this seed transmitted disease is not known nor has its relationship to

psorosis-A ever been shown (Childs and Johnson, 1966, Bridges *et al.,* 1965).

Ringspot psorosis is often cited as a separate virus, but proven tests show that it is a part of the psorosis-A complex and not a separate virus. Ringspot psorosis is mechanically transmitted and necrotic local lesions develop on *Chenopodium quinoa.* Ringspot symptoms can also develop on grapefruit and sweet orange seedlings. (Pujol and Benetena, 1965). Transmission studies by Garnsey and Timmer (1988) from a ringspot infected tree to citron; then from citron to *C. quinoa*: from *C. quinoa* to Gomphorena; then from Gomphorena back to citron and finally from citron to sweet orange induced psorosis bark lesions in the sweet orange. This essentially completed Koch's postulates showing that ringspot is psorosis-A.

3.2.3 The psorosis organism
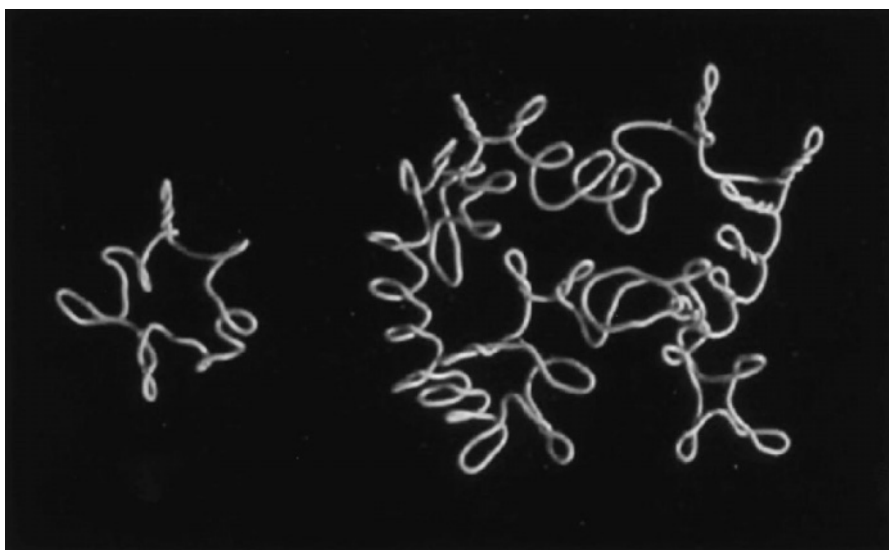
Figure 5: A wire hanger model of the particles of the citrus psorosis virus showing the short form or top component (left) and the long form or bottom component (right).

Derrick *et al.,* (1988) were the first to visualize the psorosis virus. They showed two linear components one large and one small 48kd proteins. These could be isolated and when mixed together were infectious. Derrick *et al.,* (1991) proposed a new classification calling it 'Spirovirus'.

However, through negative staining and electron microscopy, Milne *et al.*(1997) were able to observe the open form of the psorosis virus and showed its complex morphology. They proposed a new genus as 'Ophiovirus'. It was thus named and has been accepted as the new genus. Illustrations of this ophiovirus in the form of wire hanger model is shown in Fig.5 which depicts the short form or top component and the

long form or bottom component.

3.2.4 Detection of psorosis by PCR and ELISA

New means are now available for detection of psorosis using PCR, ELISA and development of monoclonal antibodies. Garcia *et al*., (1997) developed PCR for detection of the psorosis virus and they could detect the virus in leaves of field trees. D'Onghia *et al*., (1998), Djelouah and D'Onghia, (1998), reported a new antiserum and monoclonal antibodies (Djelouah *et al.,* (1998)) which were highly effective in detecting numerous strains of the psorosis virus from many sources and in many countries. Alioto *et al.,* (1999) have improved on the detection of citrus psorosis virus by using newly developed monoclonal and polyclonal antibodies with excellent broad spectrum results. In many tests worldwide, these new antiserums have been remarkably effective in detecting almost all isolates of the psorosis virus.

3.3   Natural spread

Natural transmission of psorosis has been noted in California, Texas, Argentina and Uruguay. Natural transmission of psorosis was shown by Benateña and Portillo (1984) at Concordia, Argentina. There is no valid research showing that psorosis is seed transmitted. Wallace (1978) reported that he and Dr. H. Fawcett critically observed 20,000 seedling trees derived from psorosis-infected sources and found no evidence of seed transmission. The natural spread of psorosis in Argentina and Uruguay is a most serious development and causes great damage to citrus. The vector for this spread is not known.

3.4 Management

Control of psorosis involves certification and indexing. The virus can be eliminated from propagative budwood by shoot tip grafting or by thermotherapy. Thermotherapy can be done in a chamber at 40° C for 16 hr with lights and 30° C for 8 hr in the dark for a period of 8 to 12 weeks. The rootstock used in thermotherapy is important. It is also important to remember that most of citrus varieties and rootstocks are symptomless carriers of the psorosis virus and the lack of field symptoms is no indication of presence or absence of psorosis. Indexing MUST be done to ascertain freedom of the propagative budwood from the virus. *See:* Ecoport.org slide lecture No. 42 on thermotherapy.

4. Citrus Cristacortis

4.1 Overview

Cristacortis can be recognized by the presence of numerous deep pits in both the scion and the rootstock. Diseased trees show oak leaf patterns (OLP) on leaves of the spring flush of growth when temperatures are cool.

This disease is found primarily in the Mediterranean basin *i.e.* Algeria, Corsica,

Italy, Morocco, Sardinia, Spain, and probably in the other countries in this region. Its presence elsewhere in the world is limited. However, it could become established wherever citrus is grown if diseased budwood is imported. Susceptible varieties are sweet orange, mandarin, tangelos, tangors, grapefruit, sour orange, rough lemon, siamelo, sweet lime, and occasionally lemon. It has not been found in Troyer citrange, trifoliate orange, citron, chinotto, Citrus hystrix or Mexican lime.

The cristacortis pathogen has never been isolated but is presumed to be a virus. It is very likely related to the concave gum pathogen since both induce OLP in leaves of

Figure 6: Showing typical deep sharp pits on both the mandarin scion and the sour orange
rootstock

field trees or index plants. The OLP symptom is partially diagnostic and can be used as a rapid index for determining the presence or absence of the pathogen after heat treatment or shoot tip grafting. Mild, moderate and severe forms of the disease exist and the Clementine mandarin is useful for strain identification (Vogel and Bove, 1976).

Mechanical, vector or seed transmission has not been demonstrated. Transmission has been accomplished be placing pollen from infected trees under the bark of

indicator plants (Vogel and Bove, 1980). Cristacortis-like symptoms were reported on mandarin in Vietnam (Le *et al.*, 1997). Vogel and Bove (1964) published the first account of cristacortis observed in Corsica.

4.2 Field symptoms

Cristacortis is characterized by deep pits in both the scion and the sour orange rootstock in field grown mandarins or tangelos (Fig. 6). The oak leaf patterns are prevalent in the spring flush of growth. All deep pitting symptoms in the rootstocks may not be due to cristacortis. A citrus viroid (CV-Ia) will induce deep pitting symptoms in inoculated trifoliate rootstock (Roistacher *et al.*, 1993). Indexing is always necessary to confirm this disease.

4.3 Indexing methods

Indexing is done primarily by graft-transmission to a sweet orange or mandarin indicator seedling for detection of an OLP, or to a tangelo seedling for symptoms of stem pitting.. The presence of an OLP in sweet orange of mandarin seedlings suggests the presence of either cristacortis, impietratura or concave gum diseases. The elimination of OLP after budwood has been treated by thermotherapy or shoot tip grafting would indicate the elimination of these pathogens. The Orlando tangelo is an excellent indicator for cristacortis and will show deep pits as in Fig 7.

      In the Mediterranean region, cristacortis, concave gum, impietratura, psorosis, and other graft-transmissible pathogens of citrus are spread mostly by top-working and by propagation of infected buds. Certification, which includes an indexing program, is a necessity for control of the disease.

5. Citrus Chlorotic Dwarf (CCD)

5.1 An overview

A whitefly transmitted citrus disease was found in the Adana region of Turkey in 1986 and reached epidemic levels in citrus nurseries in just a few years. In a 1988-1989 survey of the area around Adana, only a few trees were found infected. However, in September to March, 1993-1994, 40% of 2,800 trees of grapefruit, mandarin, lemon and sweet orange were showing symptoms (Çinar *et al.*, 1995). Lemons appeared to be most seriously affected but to some extent the disease attacked most citrus varieties. The satsuma mandarin appears to be somewhat tolerant in the field.

      Symptoms of the disease somewhat resemble that of citrus infectious variegation showing warping, pocketing and flecking on new leaves. However, no antigen relationship was found with the infectious variegation virus and symptoms on indicator plants were different for the two viruses.

      The Japanese Bayberry whitefly Parabemisia myricae (Kuwana) has been shown to transmit this disease. This vector was accidentally introduced into Turkey and first detected in the eastern Mediterranean region in 1982 and later spread to the western

region of Turkey. This is the first recorded whitefly-transmitted disease of citrus and "has to be considered as the most serious citrus disease in the Eastern Mediterranean region of Turkey". The organism, presumably a virus, could readily be eliminated from infected budwood by shoot tip grafting *in vitro*.

5.2 Field symptoms

Figure 7: Section of peeled bark from an Orlando tangelo showing typical cristacortis pegs (Corsica).

Field symptoms include a strong chlorosis and reduced leaf size as found on a variety of citrus hosts (Fig. 8). Severe symptoms on very small leaves have been reported on lemon, Minneola tangelo and Kutiken lemon in the Mersin area of Turkey. Symptoms have also been reported in Red Blush grapefruit. Various chlorotic patterns, crinkling and other types of leaf distortions are induced in young leaves by this virus. Sweet orange is least affected than other citrus species.

5.3 Detection by graft-transmission

Successful graft transmissions were made to eight mandarins, three grapefruit, two lemons, and two sweet orange varieties. When Eureka lemon was graft inoculated with bud chips obtained from a CCD infected field tree, the bent tip of the leaf and the V-notch at the end of the leaf were the first symptoms to appear (Fig. 9). These symptoms are diagnostic for CCD on indicator plants. Also, diagnostic on sour orange indicator seedlings is a distinct hook at the tip of the leaf after graft inoculation from a CCD infected plant. This usually is the first typical symptoms of CCD on an indicator plant.

5.3.1 The putative organism

Figure 8: Kutiken lemon showing typical symptoms of citrus chlorotic dwarf (CCD)

The agent that causes citrus chlorotic dwarf has been shown to be infectious by slow reproduction of disease symptoms in inoculated plants. The pathogen has not been fully characterized. Korkmaz *et al.,* (1994) found the virus extremely difficult to purify and showed that the virus was phloem limited and present in very low concentrations in infected citrus tissue. All attempts to find a non citrus host have been unsuccessful.

The rugosity caused by CCD virus somewhat resembles the rugosity of leaves caused by the citrus infectious variegation virus. Infectivity studies showed that CCD can be transmitted by knife slash to citron, but could not be transmitted mechanically to herbaceous hosts, whereas the citrus variegation virus can readily be mechanically

transmitted to a number of herbaceous hosts.

5.3.2 The vector

The vector for CCD is a whitefly, *Parabemisia myricae*. *P. myricae* infests only the very young tender shoots of citrus and cannot penetrate or oviposit on older leaves. Therefore it is always found on new shoots. In contrast, *Dialeurodes citri,* the other common whitefly on citrus in the Mediterranean region prefers older leaves for feeding and

Figure 9 : Showing bent tip and V notch on indicator plant

oviposition. *P. myricae* can produce up to 11 generation a year in Turkey, with its cold winters. From egg to adult it takes only 10 15 days and a single female deposits about 60 eggs. *P. myricae* has more than 46 host plants (citrus and noncitrus hosts) and within a very short time is capable of spreading and infesting an entire citrusgrowing region. Although it can infest all citrus varieties it prefers those which have many flushes producing tender new leaves such as lemon and grapefruit.

5.4 Control

Eremocerus debachi is a potent parasitoid of the whitefly *P. myricae* and an excellent biological control agent. Within two to three years after release of *E. debachi* it brought down the whitefly population to an almost zero level and has never allowed an increase in population. After four years it was difficult to find any non-parasitized *P. myricae* in the orchards. This is one of the very few examples of an almost complete biological control of an insect vector.

6. Citrus impietratura

6.1 An overview

Symptoms resembling that of impietratura were first described in Palestine by Reichert and Hellinger (1930) who believed it was a physiological problem. The fruit of affected trees showed gumming in the albedo of citron, grapefruit and oranges. Ruggieri (1955) described and named the disease impietratura since the fruit turned hard like a stone.

Impietratura is prevalent in all countries of the Mediterranean basin and also found in Iran, Venezuela, India South Africa and reported in Nepal. There is no reason why impietratura cannot exist anywhere citrus is grown if infected budwood is introduced and propagated. The fruit of sweet orange, grapefruit and Volkamer lemon are highly susceptible but symptoms have been reported on lemon, rough lemon, bergamot, tangelo, citron and mandarins. A complete slide show on impietratura can be seen on the internet at http://ecoport.org as slide show 55. http://ecoport.org.

6.2 Field symptoms

Symptoms are small fruit, one quarter to one third normal size and showing gum pockets and gumming in the rind (Fig.10). The fruit hardens and drops to the ground during the summer months. The infected fruit usually remain smaller in size as compared to normal fruits. Round protuberances and prematurely colored spots may appear on the rind of green fruit. Later, these spots may not color with the fruit and they remain green while the remainder of the rind turns orange. If the peel is sliced just below the protuberance, pockets of gum are distinctly visible in the rind and albedo (Fig. 11). Green spotting on the rind and gumming in albedo may be seen in infected grapefruit, navel orange, sweet orange and Volkamer lemon. Symptoms in the fruit are almost identical to those induced by boron deficiency, however analysis for boron will show normal levels in impietratura infected trees.

Transmission of the disease is primarily by man if infected budwood is propagated or topworked. There is no evidence for seed, mechanical or vector transmission.

6.3 Indexing

The pathogen is almost certainly a virus, but it has not been isolated or characterized. It may be related to or part of the concave gum family which produces a distinct oak leaf

pattern (OLP) on leaves on field trees in the spring flush of growth.

The OLP symptom in inoculated seedlings was shown to be diagnostic for impietratura. The absence of OLP symptoms in indicator plants inoculated with tissue from heat treated or shoot tip grafted plants would indicate elimination of the pathogen. Thus, for therapy purposes, a short term index using seedlings may be efficient for determining presence or absence of the disease (Bar-Joseph *et al.*, 1970).

Indexing may also be done by grafting inoculum buds just behind young immature fruit on the limb of a field tree in the spring. Symptoms of gum spots typical for impietratura will appear in the rind as the fruit matures (Scaramuzzi *et al.*, 1968).

The symptoms of impietratura vary with the rootstock being used. Rough lemon as the rootstock induced more fruit symptoms followed by sour orange, Cleopatra mandarin, sweet orange and Troyer citrange (Papasolomontos and Economides, 1967).
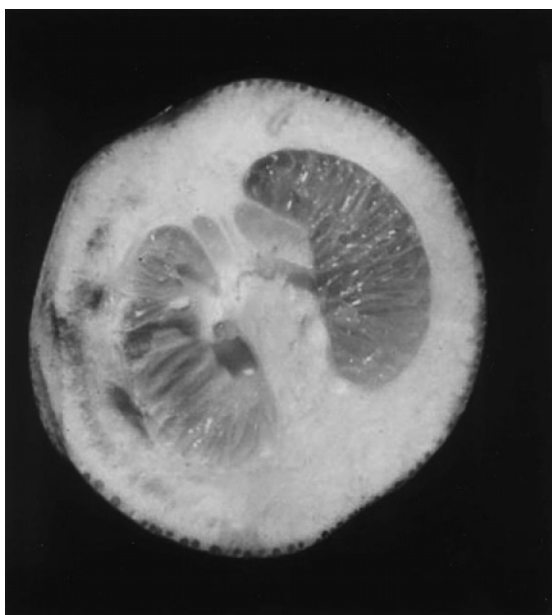
Figure 10 : Impietratura induced gumming in albedo of a sectioned young grapefruit

6.4 Management

The disease is spread primarily by citrus nurserymen using infected budwood. Top working is also a way the impietratura disease is spread in the Mediterranean region. The management strategies involves a certification program which includes detection of the disease by indexing followed by shoot tip grafting and/or thermotherapy and followed by indexing to verify that the symptoms of OLP are gone. The sweet orange, mandarin or Dweet tangor are excellent index plants for the OLP.

7. Citrus satsuma dwarf

7.1 Background

The satsuma dwarf family of virus diseases are found primarily in Japan and consist of the satsuma dwarf virus (SDV), navel infectious mottle virus (NIMV), citrus mosaic virus (CiMV) and natsudaidai dwarf virus (NDV). All are serologically related and are transmissible to herbaceous hosts.

  The dwarfing problem of satsuma mandarins was known since the early 1930's in Shizuoka prefecture. Yamada and Sawamura (1952) showed the disease to be infectious and named it the 'dwarf disease of satsuma' which has been popularized to satsuma dwarf. The disease is also present in Turkey and China. There is no reason
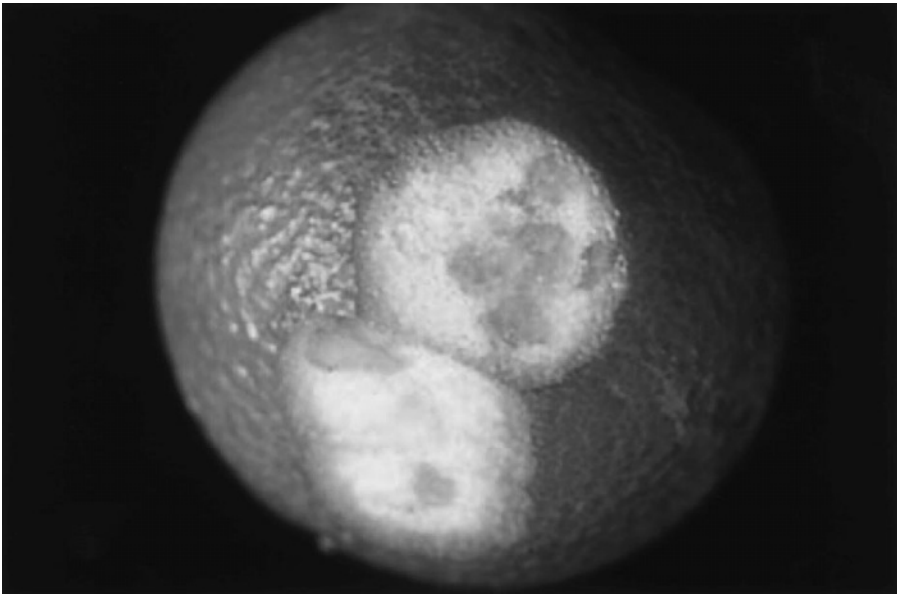
Figure 11: Slice deeper into the albedo, the gumming becomes more apparent and may extend into the rind.

why the disease could not develop anywhere citrus is grown if infected budwood is introduced and cool temperatures prevail. Miyakawa (1969) found 18 species of citrus plus 7 hybrids and 2 related genera to be susceptible when they were bud-inoculated with infected material.

  Satsuma dwarf is caused by a spherical virus, 26-27 nm diameter. The virus has been purified and characterized (Tanaka and Imada, 1974). A close relationship with other family members of NIMV, CiMV, and NDV are suggested because of particle size, reactivity to sesame (*Sesamum indicum*) and to citrus, and serological relationships. Despite the similarity of particle size and morphology of SDV and citrus infectious

variegation virus (CIVV) the reaction to sesame and lack of serological relationship to CIVV antiserum, suggest that SDV is not related to CIVV. The satsuma dwarf family of viruses are all readily mechanically transmissible to sesame.

Dr. K. Kishi was responsible for the early studies on the use of indicator plants for detection of SCV and was a co-discoverer of the use of sesame as an excellent indicator (Tanaka and Kishi, 1963; Kishi and Tanaka, 1964). SDV can be readily transmitted mechanically on tools. However, the primary means of spread is by man through propagation of infected budwood and spread through the soil. The soil borne nature of this virus disease of citrus may be an important aspect of its epidemiology since once a site is infected, the disease may be permanently established. There is no evidence for above ground vector transmission.

Studies by Zhou (1993a) indicated that the Gou tou Cheng 'sour orange' used as an indicator differentiated citrus mosaic virus from satsuma dwarf virus indicating they are possibly two different viruses. Sequencing of SDV and CMV suggest a very close relationship - but also suggest that they are distinct viruses (Iwanami and Kondo, 2000). A complete slide program of this disease can be found on the Ecoport.org website http://ecoport.org/ as slide show Number 61.

7.2 Field symptoms

Stunting and dwarfing of infected trees is characteristic for satsuma dwarf disease as the name implies. The infected trees develop boat or spoon shaped leaves and show peaked shaped fruit. However, it should be noted that all spoon or boat shaped leaves may not be due to SDV. Severe strains of the citrus tristeza virus can also induce boat shaped leaves under cool conditions (Zhou *et al.*, 1996). Infectious variegation virus and the tatter leaf virus can also induce boat shaped leaves in satsuma. Witches' broom symptoms showing bunched and tight branches may also appear in Wase satsuma in Japan. Boat shaped leaves are characteristic of shoots on field trees. Wase satsuma produced more severe symptoms than the ordinary type of satsuma. Affected fruit tend to show a peak at the stem end as compared to a normal fruit (Fig.12).

7.2.1 The virus

The satsuma dwarf disease is caused by an icosahedral virus 26-28 nm dia. It has been purified and an antiserum produced. A region of the satsuma dwarf virus was sequenced and it was suggested that it be classified as a distinct new plant virus group (Iwanami *et al.*, 1993; Iwanami and Ieki, 1997).

7.3 Indexing

7.3.1 ELISA

The satsuma dwarf virus had been purified and an antiserum produced (Saito *et al.*, 1963). Kuhara *et al.*,(1981) used SDV antiserum in a nation wide survey and campaign to certify satsuma to be free of CVV which is closely related to SDV. They tested 53,000

trees and found 38% infection. In order to eliminate the threat of nationwide contamination by this virus 17,410 field trees and 120,000 nursery trees were destroyed. ELISA is now used extensively as an index for the satsuma dwarf family of viruses in Japan.

7.3.2 Mechanical transmission

Indexing by mechanical transmission to white sesame (*S. indicum*) is the preferred

Figure 12: A satsuma dwarf affected fruit on a field tree showing the peaked stem end which occurs at times in satsuma dwarf-affected trees

index method for laboratory detection. Mechanical sap transmission of SDV to cotyledons of a sesame plant induces local lesions. Kishi and Tanaka (1964) were the first to transmit the virus to sesame. The infectious variegation virus will not transmit mechanically to sesame and this is one distinctive symptomatic difference between the two viruses. SDV mechanically transmitted to cowpea induces severe necrosis of the upper stem of the young emerging cowpea seedling.

7.3.3 Graft transmission to Satsuma

Indexing can be done by graft transmission to satsuma budded to trifoliate rootstock. Ten months after inoculation, symptomatic spoon or boat shaped leaves developed in the inoculated plants (Miyakawa, 1969). Koizumi *et al.,* (1988) reported soil transmission to China Laurestine, which is used as wind, breaks in Japan. This tree was found to be a symptomless carrier of the disease and accelerated natural transmission in the field.

7.3.4 Spread in China

Zhou Chang-yong *et al.,* (1993b) reported that SDV was found in two Miyamoto early satsuma mandarins collected from Huangyan County, Zhejiang Province and from Fengjie county, Sichuan Province, China. This mandarin was imported from Japan in the early 1980's whereas the other 82 samples collected from 8 Provinces of China were SDV-free. Zhou (personal communication) reported that in 1994 another SDV-positive sample of Okitsu early satsuma was found in Wuxian County in Jiangsu Province, and this was also imported from Japan.

Zhou Chang-yong *et al.,* (1993b) reported that the SDV was found in 84 samples from Huangyan County and Sichuan Province, China. They believed all were imported from Japan. and suggested that the SDV in China is of recent origin. Iwanami *et al.*, (1993) transmitted SDV to the citrus relatives of Fortunella, Atalantia, Clymenia, Severinia, Microcitrus, Feroniella, Eromocitrus, Swinglea and Aegle.

7.4 Relation to Citrus mosaic

CiMV is closely related to SCV. Antiserum made to SDV will detect CiMV and was as mentioned was used in a nation-wide campaign to locate and destroy citrus mosaic infected trees in Japan (Kuhara *et al.*, 1981, Yamamoto and Yamaguchi, 1980). Zhou *et al.,* (1993a) reported that the Gou tou cheng sour orange seedling was able to detect two CMV isolates of Japanese origin but showed no symptoms when inoculated with SDV. Thus, it appears that the Gou tou cheng is a specific indicator for CMV. He suggested that further index tests on other isolates of CMV should be done to verify this index. Also, sequencing of SDV and CMV suggested a very close relationship, but also suggested that they are distinct viruses (Iwanami and Kondo, 2000).

7.5 The current status in Japan

As of January, 2000, the satsuma dwarf disease is still spreading in the citrus growing areas of Japan primarily because of the use of uncertified budwood for propagation. This budwood is often taken from top worked trees to replace existing cultivars. The education of growers is a primary need if spread is to be slowed down. Also, the spread of the disease in contaminated soil as reported by Koizumi *et al.,* (1988) suggested use of chemical treatment of the infected soil before planting. This has been tried in some areas. Currently, there are some research trials are under way using heat treatment to

find protective strains to be used in cross protection.

8. Citrus Vein Enation/woody gall

The vein enation disease of citrus was first described from California by Wallace and Drake (1953) and was reported from South Africa by McClean (1954). In Australia, Fraser (1959) demonstrated transmission of a disease causing woody galls on rough lemon rootstocks. Wallace and Drake (1960, 1961) reported that the woody gall problem found on rough lemon or other rootstocks in Peru, South Africa and Australia was related to the vein enation disease. They were able to induce galls in pre-infected rough lemon seedlings by needle puncture. The term 'vein enation' will be used to designate both the vein enation and woody gall diseases. This disease has been reported or ob-

Figure 13: Vein enations on the underside of a Mexican lime seedling which had been graft inoculated with vein enation virus. A non-inoculated leaf is on the top.

served in Peru, Turkey, Spain, Brazil, Japan and China and is endemic throughout coastal Peru, Japan, and in the cooler areas of California. Studies indicated widespread presence of vein enation disease in Zhejiang province, China (Chen *et al.*, 1992) and China may be the home of the vein enation disease. The disease has not been reported in the warmer central valley or hot Imperial valley of California or from Florida, probably due to the warmer temperatures in these regions.

The disease is usually symptomless on leaves in the field in most citrus hosts. Transmission has been reported by the aphid vectors *Toxoptera citricida, Myzus persicae*, and *Aphis gossypii* and by dodder (*Cuscuta sublinclusa*). Laird and Weath-

ers (1961) showed that *A. gossypii* is a vector of CVEV in California and Maharaj and daGraca (1989) showed that *T. citricida* is the vector for CVEV in South Africa. There are indications that other vectors may spread the virus.

Leaves on seedlings of Mexican lime and sour orange will show diagnostic small pimple-like outgrowths or enations when they are graft-inoculated and these are the preferred indicators (Fig. 13).

The vein enation disease is caused by the citrus vein enation virus (CVEV). Rogers and daGraca (1986) showed virus-like particles measuring 22-24 nm in diameter in CVEV-infected tissue. A causal relationship between the particles and the disease was undetermined. There have been no reports of mechanical or seed transmission. CVEV is a cool temperature pathogen and is readily eliminated from citrus budwood by thermotherapy or even when infected citrus is grown in a warm room (Calavan *et al.*, 1972b; Roistacher and Calavan, 1974). Shoot tip grafting is effective in eliminating the pathogen from infected budwood (Roistacher and Kitto, 1977).

Weathers (1961) showed a synergistic effect between CVEV and the citrus yellow vein pathogen. This synergism is the only one known with citrus graft transmissible pathogens. Fraser and Broadbent (1979) indicated that the pathogen may not be limited to citrus but may be present in many other non-citrus species showing galls. Enation symptoms on leaves of field trees are difficult to see and are relatively rare, but can occasionally be seen on leaves of the vigorous growth of young lemon trees or young sour orange seedlings in the nursery. Indexing is the only certain method of diagnosis.

CVEV can readily be imported in introduced budwood, and because it is easily transmitted by a number of aphid species, could become widespread in countries with cooler climates. Studies in Japan show cross protection CVEV against the citrus tristeza virus. Field trees at Kutchinotsu Research Station in Japan were protected against severe CTV stem pitting strains by the vein enation virus.

8.1 Field symptoms

Symptoms in the field generally show a gall formation on rough lemon or Volkamer lemon rootstocks. Leaf enations, as mentioned, are very rare in the field, but are sometimes seen on leaves of field trees under cool conditions. Woody gall is also called as elephant's foot on Volkamer lemon in Brazil and can be very destructive on certain rootstocks (Fig.14).

Woody galls can be induced in rough lemon rootstock by puncturing a young seedling which had been pre-inoculated with CVEV (Wallace and Drake, 1961; Wallace and Drake, 1960).Not all galls are due to the CVEV. The Bonanza navel orange has a tendency to show galls on the sweet orange scion and these galls could be mistaken for woody gall. CVEV does not induce galls on sweet or sour orange.

8.2 Indexing

CVEV can be detected by indexing to Mexican lime or sour orange seedlings under cool temperature conditions in the index facility. Inoculated Mexican lime seedlings will

show vein enations or small pimple like pustules on the leaves (Fig 13). Sour orange seedlings are also an excellent indicator. Shock symptoms were found as a distinct symptoms associated with CVEV on inoculated Mexican lime at the indexing facility in Spain.

8.2.1 Detection of citrus vein enation virus (CVEV) using cereal yellow dwarf virus ELISA kits

Figure 14 : Close up of the trunk Volkamer lemon rootstock showing the severity of the galls of 'elephant foot' in Brazil.

Studies by South African researchers indicate that CVEV is a probable luteovirus with isometric particles averaging 28 nm in diameter and found in both infected plants and in the *T. citricida* aphid. These isometric particles were isolated from rough lemon by da Graca and Maharaj (1991). With the evidence that CVEV is possibly a luteovirus Maharaj and daGraca (1988), and daGraca, and (Maharaj) 1991 tested five commercially available kits for Luteoviridae (Clark and daGraca, 2000). Two ELISA kits designed for detection

of the citrus yellow dwarf virus were successful for detecting CVEV. This finding supported the proposal that CVEV is a member of the Luteoviridae family.

8.3 Management

The virus is readily eliminated by thermotherapy and also by shoot tip grafting in vitro. Quarantine measures are very important for prevention of the introduction of the disease in imported and infected citrus to new areas. Once the disease enters a country, it will spread rapidly in the cooler areas, but may not be found in the warmer regions of a country.

9. Citrus Leprosis Disease

9.1 Background

Leprosis is a destructive disease of sweet orange, incited by a virus, transmitted by a Brevipalpus mite and causes extensive damage to the citrus of Brazil with an estimated loss of over 90 million dollars in 1999. The disease has been recognized in Brazil since 1933. It was first reported in Brazil by Bitancourt (1937) who named it "A leprose dos citrus". Leprosis is epidemic in Brazil and had been reported in Argentina, Venezuela, Uruguay, Paraguay, and Peru. Leprosis was present in Florida prior to 1926, and had a serious negative impact on citrus production at that time. Then about 1926 the incidence of leprosis in Florida drastically declined and the decline coinciding with the introduction of sulfur as an effective miticide for controlling citrus rust mite (Knorr, 1968). . Searches for this disease over the past 20 years have resulted in no cases of CiLV in Florida (Lee & Timmer, personal communication.).

Much of the studies and research on the leprosis disease and its interaction with the Brevipalpus mite has been carried out by Dr. Victoria Rossetti.
The citrus leprosis virus (CiLV) is a plant rhabdovirus. The disease is transmitted by mites of the genus Brevipalpus.

Chagas and Rossetti (1984) reported that *B. phoenicis* was an efficient vector of CiLV with higher transmission occurring in the larval stage. CiLV is reported to be transmitted by *B. obovatus* in Argentina and by B. Californicus in Florida (Knorr *et al.*, 1968). Previously, leprosis was reported only from Argentina and Brazil, but has moved northward and is now in Venezuela (Fig.15), and Panama (Kitajima, personal comm.) There are unconfirmed reports that the disease is in Costa Rica and Guatemala (R. Lee, personal comm.). It appears that the disease is traveling northward with the spread of *B. phoenicis* and *B. abovatus*.Leprosis poses a major threat to all citrus industries in the Caribbean, including Florida. Trees are killed by expanding lesions which may girdle the trunk and which kill the branches. Mites must be continually controlled and multiple applications of miticides can result in development of tolerance to the pesticide. Young immature fruit will drop which results in greatly reduced yield. Leprosis is presently an exotic disease for Florida, although at one time the Florida citrus industry was severely threatened by it.

9.2 Field symptoms

Leprosis induces bark scaling on the tree trunk (Fig. 16). The bark scaling can be confused with that caused by psorosis. However, no wood straining will be found beneath the scaling in a leprosis infected trunk or limb. Indexing would readily differentiate psorosis from leprosis.

Leprosis symptoms develop in young twigs , fruit (Fig 17), and leaves of sweet orange (Fig. 18). The lesions on leaves and twigs are first chlorotic and then become necrotic in the center. Extensive lesions on twigs cause dieback. Leaves and fruit commonly abscise and fruit will drop prematurely. A combination of leprosis and blight can be devastating.

Figure 15: Distribution of Leprosis disease of citrus

9.3 Diagnosis

Diagnosis of CiLV is difficult as it is very poorly mechanically transmitted by graft-inoculation and by mites. Transmission by electron microscopy is currently the only certain available method for diagnosis except for the symptomatic lesions. A quick, reliable detection method for CiLV would facilitate diagnostic/quarantine measures in the Caribbean.

Partial purification of the virus, transmission to herbaceous hosts such as *C. quinoa* for diagnostic purposes has been studied by Colariccio *et al.,* (2000) and by Lovisolo *et al.,* (2000). The history, geographical distribution, properties, diagnosis,

and phytosanitary importance of CiLV have been recently discussed by Lovisolo (2001). Slide show #64 available on the internet at http://ecoport.org is available for further study of the disease at http://ecoport.org.

9.3.1 Preliminary purification of citrus leprosis virus

Colariccio *et al.,* (2000) partially purified citrus leprosis virus and observed a light scattering band formed after ultra-centrifugation in a cesium sulfate linear gradient of PEG-precipitated extract from local lesions of leprosis-infected Cleopatra mandarin

Figure 16 : Bark scaling on tree trunk

leaves. Virus particles observed under the electron microscope measured 45-50 by 80-120 nm (Colariccio *et al.,* 2000). Electron microscopic studies have also been carried out in cells of an experimentally infected leaf of *C. quinoa* (Lovisolo, 2001; Lovisolo *et al.*, 2000).

9.3.2 Mechanical transmission to various herbaceous hosts

Studies on mechanical transmission of CiLV to *C. quinoa* , *Atriplex hortensis* and *Gomphrena globosa* have been conducted and these herbaceous hosts expressed typical leaf necrosis and spots which were useful of diagnosis (Lovisolo *et al.*, 2000, and Lovisolo, 2001).

9.4 Control
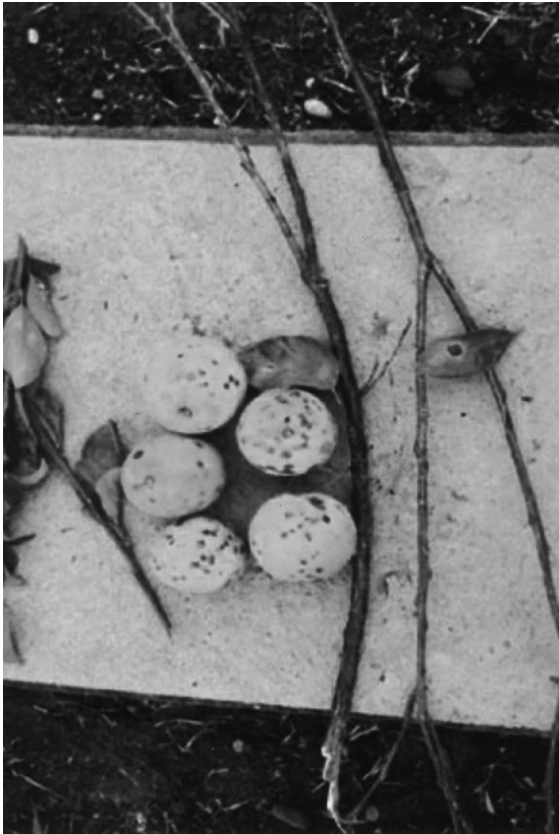
Figure17: Leprosis on young twigs and fruit

At present there is no other means of control except by spraying with a copper compound. This is expensive and when the price of citrus drops one can see many abandoned groves of citrus along the roadside in San Paulo State, Brazil. Strict quarantine measures are the only means of defense in preventing the disease from entering a country.

10. Citrus tatter leaf disease

The tatter leaf disease of citrus, induced by the citrus tatter leaf virus (CTLV) was first described by Wallace and Drake (1962) as a transmissible disease which caused mottling and tattering of the margins of leaves in *Citrus excelsa* indicator seedlings, hence the name was given as "tatter leaf". Calavan *et al.,* (1963) first showed the destructive potential of this disease to citrange rootstock when tatter leaf infected tissue was graft-inoculated to satsuma mandarin budded on Troyer citranges. Meyer (Beijing or Hsien Yuang) lemon trees, imported into the United States from Beijing, China in 1908, were later found to contain the citrus tatter leaf virus. Perhaps all Meyer lemon trees throughout the world which originated from the 1908 introduction contained the virus, including many propagations and plantings of the original Beijing or Hsien Yuang

Figure 18 : Zonate chlorosis on leaves of sweet orange at Maracay, Venezuela

lemon in China (Zhang *et al.*, 1988). The disease is endemic in mainland China and may be widespread (Zhang *et al.*, 1988). Tatter leaf disease is also widespread in Taiwan and Japan and is present elsewhere in the world where the Meyer lemon or other infected citrus had been imported. The tatter leaf disease has been reported from South Africa in declining Shamouti orange trees on citrumelo rootstock (Marais and Lee, 1986). CTLV is also present in Texas (Herron and Skaria, 1997), and was recently reported in Australia. Su and Tsai (1990) reported CTLV present in the Philippines, Thailand and Korea. Nishio *et al.*, (1989) indicated that CTLV is a cappilovirus and is closely and serologically related to the apple stem grooving virus.

10.1 Field symptoms

A tatter leaf infected Meyer lemon grafted on Troyer citrange rootstock shows with a severe rope-like condition of the rootstock (Fig.19). In general, most citrus stionic combinations are symptomless when infected with CTLV. Only where the rootstock is the trifoliate or hybrids of the trifoliate are symptoms evident. When the bark of the field infected Troyer citrange is removed, severe indentations and bud union crease typical for the disease are evident. The natural incompatibility of Meyer lemon scion with

Figure 19: The severe reaction on a Troyer citrange rootstock with a CTLV-infected satsuma mandarin scion.

trifoliate rootstock also shows a deep brown line in the bud union. However, when the tatter leaf virus is present, the situation is aggravated and the bud union is so severely affected that it can result in easy breakage in a wind storm.

It is important to note that CTLV is symptomless in most commercial citrus scions such as sweet orange, grapefruit, lemon and mandarin on the rootstocks of sour orange, rough lemon, gou tou and others. Only the trifoliate and trifoliate hybrid

rootstocks show the disease.

10.2 Indexing

Indexing can be done by graft-transmission to an indicator plant, by ELISA or by mechanical transmission to herbaceous hosts.

10.2.1 Indexing by graft transmission

The tatter leaf virus is readily detected by graft inoculation to seedlings of *Citrus excelsa*. In Fig. 20, the three leaves on the right show the tattered leaf symptoms for which the disease was named. The uninoculated control leaf is on the left. These

Figure 20 : Graft inoculation to seedlings of *Citrus excelsa*

symptoms may be difficult to see if the citrus tristeza virus is present, since *C. excelsa* is very susceptible to the tristeza virus and will show severe vein clearing symptoms it will mask the symptoms of tatter leaf. Citranges are also excellent seedling indicators for presence of CTLV (Fig. 21).

10.2.2 Mechanical transmission to herbaceous hosts

CTLV may be indexed by mechanical transmission to herbaceous hosts. Cowpea and *C. quinoa* are two good indicators for the virus. Mechanical transmission of the CTLV was

first shown by Fulton, (1966). Garnsey (1974) knife slashed from CTLV-infected Meyer lemon budwood to citron and then mechanically transmitted the virus from the citron to *Nicotiana clevlandii*. He then mechanically transmitted back to citron and from citron to *C. excelsa* inducing excellent symptoms of tatter leaf in the *C. excelsa.*

Necrotic brown local lesions are produced on the leaves of cowpea when mechanically inoculated by rubbing with a mild 0.1M KPO4 buffer at a pH of 7.0. Tatter leaf is highly mechanically transmissible from citron to citron by knife slash, and transmission can be prevented by a quick dip of the knife blade in a 1% sodium hypochlorite solution (one part commercial bleach to 3-4 parts water).

It is important to disinfect all tools when working with this virus. In an experiment reported by Roistacher *et al.,* (1980), knife blades first slashed into and infected citron and then dipped in a 1% sodium hypochlorite solution showed no transmission in 25/25 knife slashed citron plants whereas 23/25 plants were infected mechanically by

Figure 21: Mottle leaf reaction on three leaves of Troyer citrange on the right with the control
                 leaf on the left.

knife blades which were not disinfected.

10.2.3 ELISA

ELISA technology has been used to identify CTLV. A monoclonal antibody was developed by Su and Tsai (1990) in Taiwan and used successfully to show widespread presence of tatter leaf disease in that country.

However, ELISA can sometimes fail in detecting CTLV if temperatures are too warm during testing. This was noticed in Thailand and also reported from Texas in samples which showed positive by biological indexing but failed detection by ELISA (Herron and Skaria, 1997).

10.3 Management

Tatter leaf can be eliminated from citrus plants by thermotherapy in a heat chambers at 40°C day for 16 hours and 30°C night for 8 hours over 8-12 weeks (Roistacher *et al.*, 1972). Tatter leaf is difficult to eliminate by shoot tip grafting alone. In three separate experiments, CTLV could not be eliminated by shoot tip grafting in 64 heat treated buds (Roistacher *et al.*, 1976). However, Navarro *et al.,* (1991) succeeded in eliminating the virus from 1/24 buds of sweet orange where 6 leaf primordia was used and in 14/45 buds where 3 leaf primordia was used. They combined heat treatment with shoot tip grafting. Koizumi (1984) also reported the elimination of CTLV from citrus tissue by combining shoot tip grafting with thermotherapy. It is important that indexing for tatter leaf should be included in any certification program for citrus.

For a more complete slide show on the tatter leaf disease visit the http://ecoport.org website and click on slide show number 72 http://ecoport.org.

11. Citrus infectious variegation (Crinkly leaf, Citrus variegation)

11.1 Background

Citrus infectious variegation virus (CIVV), also called citrus variegation virus (CVV), was the first citrus virus to be transmitted experimentally. Fawcett (1936) illustrated a transmissible disease which they called 'crinkly leaf'. Later, Fawcett and Klotz (1939) transmitted a disease from infected lemon to sour orange and named it Infectious variegation and suggested it was caused by a transmissible virus. They found symptomatic lemon trees in Glendora, California and transmitted the disease by graft-transmission to sour orange. A similar disease found in Florida by Grant and Smith (1960) was graft-transmitted to many other citrus species. CIVV is of exceptional interest since it was the first citrus virus to be mechanically transmitted from citrus to citrus and from citrus to herbaceous hosts. In addition to its presence in the United States, the disease has been reported from many countries in the Mediterranean basin and from Australia. The disease was observed by this author in Uruguay.

CIVV purified is an icosahedral virus 26-30 nm diameter (Dauthy and Bove, 1968). Crinkly leaf, once thought to be a separate virus was shown to be a milder strain of CIVV. CIVV will be used to designate both the milder and severe strains. CIVV is serologically related to other ilarviruses such as asparagus virus II-P and II-S, citrus leaf rugose virus, elm mottle virus and Tulare apple mosaic virus (Uyeda and Mink, 1983). It is somewhat similar to the satsuma dwarf family of viruses in its morphology, particle size and reaction on certain citrus seedling indicator plants. However, there is no evidence for serological relationship and there are distinct differences in mechanical transmission to herbaceous hosts.

Citrus leaf rugose virus was described by Garnsey (1975) as a citrus crinkly leaf type, and found in one location in Florida. It has subsequently been found in Japan and Argentina. It induces flecking in Eureka lemon, leaf rugosity in Mexican lime and severe stunting of young Duncan grapefruit plants. It is an Ilarvirus 25-32 nm in diameter. Two strains which differ in their effect on Duncan grapefruit have been described. It appears

to be somewhat similar to the infectious variegation group but with some distinct serological differences (Garnsey, 1975; Gonsalves and Garnsey, 1976).

A variegation which occurs on leaves of sour orange called 'Petri's variegation' was reported from Italy by Petri (1931). Leaf symptoms are somewhat similar to those of CIVV, however Petri's variegation is non-transmissible and the variegation is probably the result of genetic sensitivity of the local sour orange seedlings to winter cold.

CIVV can be transmitted at low percentages through seed (Wallace, 1968). The primary means of spread of the disease is by man via infected budwood. CIVV can be eliminated from citrus tissue by thermotherapy and by shoot tip grafting in vitro. The purification and production of antiserum has been done for some strains of CIVV, and rapid identification by immunodiffusion techniques (Garnsey, 1975) and by ELISA (Davino and Garnsey, 1984).

Vector transmission has not been demonstrated. However seed transmission at a low rate has been noted. The disease can affect most citrus varieties and symptoms are primarily observed in the leaves, which show a distinct deformation and crinkle.

11.2 Field symptoms

Field symptoms for infectious variegation disease are prominent leaf curling and variegation on almost all leaves. However, there are isolates of related viruses which show no or only mild field symptoms (Garnsey *et al.*, 1984)

11.3 Indexing

Indexing can be done by mechanical transmission, ELISA or by graft transmission.

11.3.1 Mechanical transmission to herbaceous hosts

Indexing can be done to many herbaceous plants by mechanical transmission and show dramatic symptoms. Chlorotic local lesions will develop on primary leaves of Blackeye cowpea inoculated with CIVV. Chlorotic lesions also develop when inoculated to the leaves of Crotallaria (*Citrus spectabilis*). Indexing to Red Kidney bean shows bright vein chlorotic symptoms on the secondary growth leaves.

11.3.2 Indexing by graft transmission

CIVV can be indexed by graft transmission to many indicator plants. Citron, in the absence of the citrus tristeza virus is an excellent indicator under cool temperature conditions. Almost all citrus species will show typical leaf curl and a mottle reaction when infected by graft transmission. Some leaf symptoms can be readily confused with that of psorosis-A. Symptoms on mature leaves of an inoculated Madame Vinous sweet orange seedling show protuberances and bumps characteristic of infection with CIVV (Fig. 22).

These symptoms are typical and similar to those induced on mandarins or rough lemon leaves.The virus is readily transmitted from citron to citron by slicing with a knife

blade. Knife blade transmission from citron to citron is done by first slicing into an infected citron stem with one or two slices and then slicing into a citron receptor plant. This is repeated 10 or more times. The cuts are then wrapped with polyethylene tape.

11.3.3 Indexing for a mild strain by ELISA

Davino and Garnsey (1984) purified and characterized a mild strain of citrus variegation virus and used ELISA to show its field distribution. ELISA was also used to detect the presence of the virus in lemon. The time of the year for ELISA testing is important. Good results were obtained in the spring and summer, but ELISA did poorly in the fall and winter months. The use of ELISA is most effective for determining the extent of infection in an orchard or country, but should not be used as the only index in a certification program for citrus.
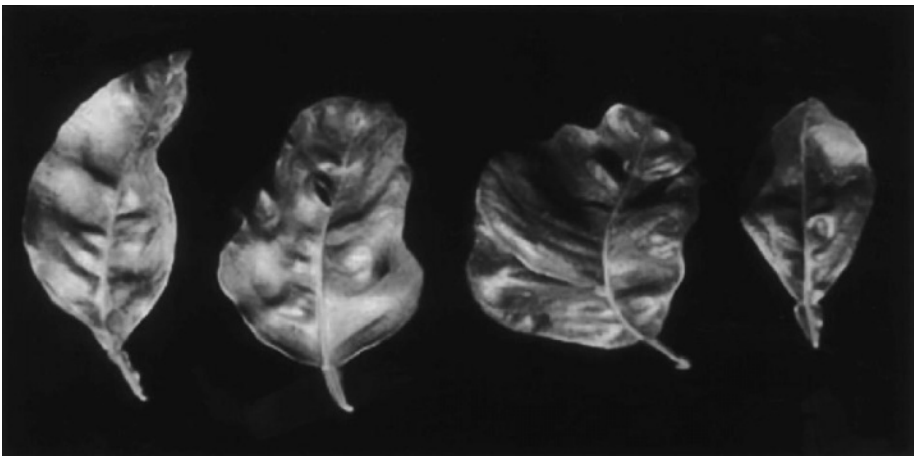
Figure 22: Symptoms of CIVV on mature leaves of Madam Vinous sweet orange leaves showing typical protuberances and bumps typical of infection with CIVV

11.4 Disinfection

Transmission can be prevented by dipping tools in commercial bleach. One part of bleach to 3-4 parts water is effective. Care should be taken to use fresh solution and use the smell test. If you cannot smell the chlorine - throw away the solution and a prepare fresh solution.

11.5 Management

Certification is the only way to assure that the CIVV will not be introduced and spread in propagative budwood. Without a certification program, various forms of the CIVV

(especially the milder strains which are less visible) can be distributed throughout a country. Detection procedures are numerous. This virus can be eliminated by shoot tip grafting (Navarro *et al.,* 1975; Roistacher *et al.*, 1976), or by Thermotherapy (Calavan *et al.*,1972b).

12. Citrus exocortis disease

12.1 Background and overview

Fawcett and Klotz (1948) described a bark shelling disease on trifoliate rootstock and named it exocortis (exo = outside and cortis = bark). Benton *et al.,* (1949;1950) described a serious bark scaling disease on trifoliate rootstock in Australia and called it "Scaly Butt". Bitters (1952) suggested that the exocortis disease of citrus may be of virus origin. Bitters et al. (1954) transmitted exocortis by inoculation and showed that nucellar lemons did not carry exocortis. He further showed that insect vectors were not involved in transmission of the disease and trifoliate rootstock was a sensitive indicator for the disease.

The classical field symptom induced by the citrus exocortis viroid (CEV) is severe bark scaling on trifoliate orange, trifoliate hybrids and Rangpur lime rootstocks (Fig 23) accompanied by various degrees of stunting of the tree.

The exocortis disease is almost certainly present in all citrus growing regions of the world. Many of the commercial citrus cultivars and rootstocks are symptomless carriers. In countries where trifoliate is the primary rootstock, bud selection over many years has avoided CEV, however other citrus viroids may be present. Sweet orange on sour orange rootstock infected with CEV can be smaller, chlorotic, and show delayed fruit color (Nauer *et al.*, 1988).

The causal organism of the exocortis disease is the citrus exocortis viroid. It is a low molecular weight RNA consisting of 371 nucleotides. Its molecules can exist as either linear or circular and the viroid can be mechanically transmitted by tools from tree to tree. It is readily mechanically transmissible from infected citron to *Gynura aurantiaca*, petunia and tomato and causes distinct and characteristic severe leaf epinasty. Nucleic acid extraction can be made from these hosts or from young symptomatic citron shoots and analyzed by sequential polyacrylamide gel electrophoresis (sPAGE). Under an electric charge, CEV will migrate through the gel as a band and can be visualized by staining with silver or ethidium bromide.

There are a number of citrus viroids of molecular weights lower than CEV which can also induce symptoms in citron. Some of these viroids induce mild bark cracking in trifoliate rootstocks which are distinct from the severe bark shelling associated with CEV. Recent studies (Roistacher *et al.*, 1993) show that individual citrus viroids can induce deep pits or finger imprints in trifoliate rootstock. These citrus viroids should be considered as independently transmitted and distinct pathogens. There are currently about 15 or more citrus viroids some of which appear closely related based on nucleotide number and nucleic acid hybridization assays. However, even closely related citrus viroids may induce different reactions in field trees. For example, the citrus cachexia viroid (-IIb) will migrate to a place on a polyacrylamide gel indicating a nucleic acid

containing approximately 300 nucleotides. It is closely related to another citrus viroid (IIa) which contains about 305 nucleotides and this viroid will not induce cachexia symptoms. However, symptoms induced in citron and other citrus cultivars and rootstocks are strikingly different. In any indexing program (citrus, grape or stone fruit) with its objective of producing disease free primary stock, it is important that all viroids be recognized and removed from propagative budwood.

Citrus viroids are distributed primarily by the introduction and propagation of infected budwood, by top working and by mechanical transmission. Mechanical trans-

Figure 23: Trifoliate rootstock showing classical bark exfoliation

mission of CEVd was first demonstrated by Garnsey and Jones (1967) who showed that contaminated tools could be disinfected by a mixture of 2% sodium hydroxide plus 2% formaldehyde. Roistacher *et al.,* (1969) demonstrated that low dilutions of sodium hypochlorite are readily available and a very effective disinfectant for all citrus viroids as well as many mechanically transmissible viruses. Garnsey (1968) reported that mechanical transmission from orange, mandarin or grapefruit is less efficient than from lemon.

However, once the viroid is present it will spread from tree to tree throughout an orchard over a period of time by hedging, pruning, clipping of fruit or collecting of budwood. CEV or s are not known to be vector or seed transmitted, and transmission by root graft, though possible, would be overshadowed by mechanical transmission.

All of the citrus viroids appear to be readily eliminated from propagative budwood by shoot tip grafting or by use of nucellar budlines. They are extremely tolerant to heat and have not been successfully eliminated from budwood by thermotherapy.

There are now a number of citrus viroids of molecular weights lower than CEV which can also induce symptoms in citron. Some of these viroids induce mild bark cracking in trifoliate, distinct from the severe bark shelling associated with CEV. Other citrus viroids can induce deep pits or finger imprints in trifoliate rootstock. These citrus viroids should be considered as independently transmitted and distinct pathogens. Currently there are over 15 viroids in the citrus viroid catalog; some of which appear closely related based on nucleotide number and nucleic acid hybridization assays. However, they may induce different reactions in field trees. For example, the citrus cachexia viroid (-IIb) contains about 305 nucleotides, is symptomless in citron but will induce the severe cachexia disease in field trees of mandarins and tangelos. Citrus viroids are distributed primarily by the introduction and propagation of infected budwood, by top working and by mechanical transmission. All of the citrus viroids appear to be readily eliminated from propagative budwood by shoot tip grafting, by use of nucellar budlines or by embryogenesis. They are extremely tolerant to heat and cannot be eliminated by thermotherapy.

12.2 Field symptoms

The classical field symptom induced by the citrus exocortis viroid (CEV) is a severe bark scaling on trifoliate or Rangpur lime rootstocks accompanied by various degrees of stunting of the tree. These symptoms are the same worldwide. Twigs and branches of both Rangpur lime and trifoliate may show chlorotic stem blotching which is symptomatic. Exocortis may severely reduce tree size on certain rootstocks. Various observations and experiments have shown that trees infected with CEV or citrus viroids are more susceptible to frost damage (Garnsey, 1982). Infection of trees with citrus viroids have been shown to delay fruit coloring (Nauer *et al.,* (1988).

12.3 Indexing for exocortis

There are two basic indexing procedures for detection of exocortis and the various citrus viroids: (i). by plants and (ii). by sPAGE.

12.3.1 Biological indexing with citron

The citron test is a very sensitive and diagnostic index for determining the presence of CEV and most of the citrus viroids. However, there are certain considerations for proper indexing. These include the use of excellent plants; warm temperatures and the citron index plants should be grown one per container. Citrus viroids are highly mechanically

transmissible and tools must be disinfected to insure that the viroids are not spread. Sodium hypochlorite is highly effective as a disinfectant, not only for citrus viroids, but for other mechanically transmissible citrus viruses (Roistacher *et al.,* 1980). Tools should be dipped in one part of commercial bleach to four parts water. Since sodium hypochlorite is highly corrosive knives and clippers should be wiped dry directly after dipping.

Clonal 861-S1 citron budded to a vigorous rootstock can detect not only CEV, but also most of the citrus viroids. Gynura is also an excellent indicator for CEV and will react strongly in this host whereas most all of the other citrus viroids do not react in Gynura. Tomato plants will also react to the citrus exocortis viroid. Inoculation to Gynura or tomato can be done by razor slash.

Different citrus viroids show different symptoms in citron (Fig. 24). Citron stems will show severe bark cracking with either CEV or with certain citrus viroids or combinations of citrus viroids. Acorn shaped fruit are also symptomatic for CEV (Bitters *et*

Figure 24 : Showing the symptoms in citrons caused by different citrus viroids ranging from very severe on the left to moderate, and mild on the right. Control plants can not be distinguished from plants infected with mild-reacting citrus viroid IIa, except by critical observation of leaves

al., 1987). Citrus viroid IIa will induce a mild petiole browning and wrinkle in citron and also an occasional mid vein bending.

However, development of these symptoms requires excellent plants free of nutrient deficiency problems and grown under warm conditions, preferably in greenhouse. The U.C. system for growing excellent plants is recommended in a certification indexing program (*see* http://ecoport.org slide show 82 http://ecoport.org.

The environmental conditions for the indicator plants are important for symptom development. The number and intensity of symptoms were found directly proportional to temperatures and warmer temperatures induced a far greater number of symptoms than cooler temperature.

12.3.2 Indexing by sPAGE

sPAGE is now the preferred method for indexing citrus viroids if the technology and skilled technicians are available (Duran Vila *et al.*, 1993). Nucleic acid extraction can be made from young citron shoots and analyzed by sPAGE. Under an electric charge, CEV will migrate through a gel and a band can be visualized by staining with silver or ethidium bromide. sPAGE technology can eliminate much time and greenhouse space in indexing and is an excellent laboratory technique. Illustrated in Fig.25 is an sPAGE gel
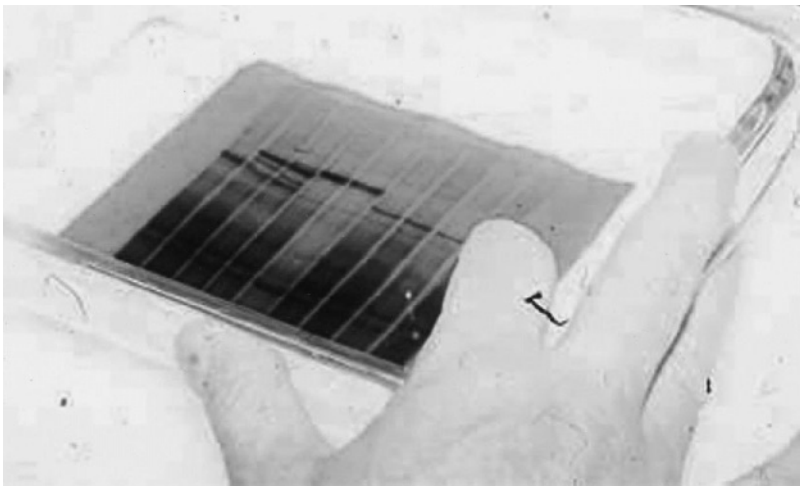
Figure 25: sPAGE, the preferred method for indexing for citrus viroid

showing four bands representing citrus viroid groups -I, -II, -III, and -IV. These bands can be eluted and slash-inoculated into citron to produce pure strains of a viroid. They may react differently in citron indicator plants and also when inoculated into trifoliate rootstocks from citron.

12.4 The relationship citrus viroid IIa and IIb

CVd IIa and IIb, only a few nucleotides apart, induce two different diseases. These two viroids can hybridize and interfere with each other and therefore may be related. –CVd-IIa is associated with mild symptoms on citron and mild bark cracking of trifoliate. CVd-IIb induces the citrus cachexia disease and shows little or no reaction in citron. These are two very different diseases. There is now evidence of a CVd-IIc. Semancik *et al.,* (1991) and Reanwarakorn and Semancik, (1999) showed that CVd-IIc induces severe

cachexia symptoms on Parson's Special mandarin. The citrus viroids are distinct and a single viroid may be responsible for a specific citrus disease (Duran-Vila *et al.*, 1988).

Distinct trunk symptoms can be caused by different citrus viroids on trifoliate rootstock and mandarin scions or rootstocks. On trifoliate rootstocks, mild bark cracking is induced by CVd-IIa; deep pits are induced by CVd-Ia; grooving is induced by CVd-IIIb and gum pocket is induced by CVd-III. Cachexia in mandarin or in mandarin hybrids is induced by CVd-IIb (Roistacher *et al.*, 1993; Marais *et al.*, 1996).The citrus viroid catalogue of 1989 shows only 12 citrus viroids, but the number is currently much greater (Table 1).

Table 1: The citrus viroid catalogue of 1989 catalog shows only 12 citrus viroids.

Group	Citrus viroids	Nucleotides
CEV	CEV (Exo)	371
I	CVd-Ia	340
	CVd-Ib	330
	CVd-Ic	317
II	CVd-IIa	302
	CVd-IIb	299
	CVd-IIc	298
	CVd-IId	297
III	CVd-IIIa	292
	CVd-IIIb	290
	CVd-IIIc	286
	CVd-IIId	280
IV	CVd-IV	275

12.5 Importance of certified budwood

In fear of the coming of the primary vector of tristeza Toxoptera citricida into the Caribbean region, the citrus industries in many countries were hurriedly shifting from the CTV susceptible sour orange to CTV tolerant rootstocks such as citranges, trifoliate, or Rangpur lime. All of these rootstocks however are susceptible to CEV. Without indexing for CTV or other citrus viroids which are symptomless in most cultivars on sour orange rootstock, exocortis became a severe problem. Also, the warmer temperatures in that region coupled with susceptible rootstocks resulted in tremendous losses to farmers. Roistacher *et al.,* (1996) showed an accumulative profit when certified exocortis-free budwood is used and propagated on an exocortis susceptible rootstock and projected over an 8 year period was almost $12,000 per ha. Losses due to exocortis were $5,683 per ha. A certification program is an absolute necessity to avoid these losses (Fig.26).

13. Citrus cachexia disease

13.1 An overview

The citrus cachexia viroid (CCaV) induces gumming symptoms in scions and rootstocks

of field trees of mandarins and mandarin hybrids, with more intensive symptoms found in tangelos and tangors. Also affected are *Citrus macrophylla*, acid limes, sweet limes, and *Fortunella* species. Many of the commercial species of citrus are symptomless *i.e.* sweet orange, grapefruit, lemon, pummelo, and such rootstocks as sour orange, trifoliate and trifoliate hybrids. The cachexia disease was named, described and first transmitted by Childs (1950). Xyloporosis, a condition affecting sweet limes, has been linked synonymously with cachexia. However, in a review by Roistacher (1988), cachexia is suggested as the preferred name, and xyloporosis reserved for the specific condition or complex associated with sweet limes as originally described by Reichert and Perlberger (1934).

The viroid nature of cachexia was proven by Semancik *et al.,* (1988a). It is a low molecular RNA consisting of about 300 nucleotides.

CUMULATIVE LOSS OVER 8 YEARS
WITH EXOCORTIS on susceptible rootstocks
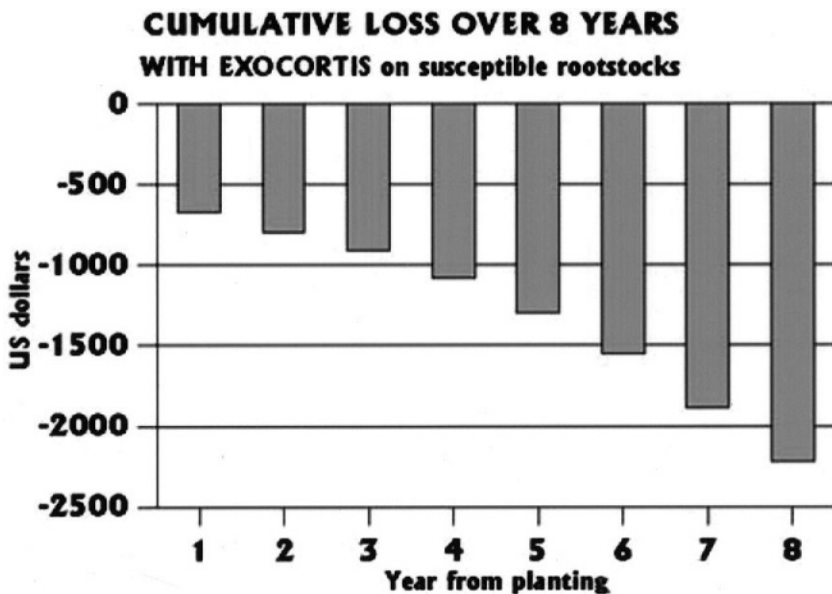
Figure26: Cumulative loss over 8 years with exocortis on susceptible rootstocks

The cachexia disease is found in most citrus growing areas of the world wherever citrus is grown was reported present in Nepal (Lama, 1996). The spread of this viroid into new citrus growing areas could present a problem because most commercial citrus cultivars are symptomless carriers and the viroid is readily transmitted by budding or by mechanical transmission.

This is especially true where mandarins, tangelos, tangors or *C. macrophylla* are grown as scions or rootstocks. Therefore, the need for indexing to determine the presence or absence of the cachexia viroid is exceptionally important if mother or foundation trees are to be free of the pathogen.

13.2 Field symptoms

CCaV induces distinct gumming in trunk and bark in many citrus species. When the bark is cut, the bark and trunk will show gum impregnation with a rough and rugose appearance. Trees may show severe to moderate stunting. Severely affected trees may show chlorosis and may decline and die. Symptoms of cachexia are similar worldwide. A severe outbreak of the disease was observed in a planting of Mapo tangelo in Sicily during a visit in 1986. The Mapo tangelo is a popular variety in Sicily and Italy. Many plants were observed declining and dying and were showing classical symptoms of gum in the bark (Terranova *et al.*,1991) Typical gumming was also found in twigs and branches. When twigs were peeled, profuse gumming was found in almost all twigs of the Mapo tangelo. These symptoms were highly diagnostic for the cachexia disease in tangelo. Leaves showed a brown stipple spotting on their underside, a symptom now observed before by the writer. Since top working is a common practice in Mediterranean countries this infection was most probably the result of this practice since cachexia-free Mapo tangelos were observed in other parts of Italy.

13.3 Biological indexing

The CCaV may be detected by using indicator plants orby sequential polyacrylamide gel electrophoresis (sPAGE). The Parson's special mandarin budded on vigorous root-stock such as rough lemon or volkamer lemon is an excellent indicator for the disease (Roistacher *et al.*, 1973). Inoculation is done by grafting buds or blind buds of the test plant below a bud of the Parson indicator and then forcing the Parson bud. After about 3 months when a shoot of the Parson indicator has grown to about one meter, the plant is cut back to about 10 cm above the bud union to again force a new single shoot. This new shoot is allowed to grow another meter. During this growth period, bud union area of the cut back area should be inspected for gumming by cutting a window into the bark. This is repeated until symptoms are seen on positive control plants inoculated with the mildest positive isolate. Small windows are cut into the bud union area of the known mild positive controls for observation of gumming and if no symptoms are observed in the mild positive control plants, the bark should be replaced and securely fastened with budding tape. This procedure may take up to one year before symptoms are seen. A complete pictorial guide to this procedure can be seen in the cachexia slide show No 70 on the http://ecoport.org website.

Different isolates of the CCaV exist within the citrus viroid (CVd) classification CVd-IIb. Some are very mild reacting and some are quite severe in their reaction to Parson's Special mandarin. The severity of their reaction can be rated from 0 to 10. A mild strain reaction will show just a slight browning at the bud union or cut back region of the indicator plant whereas with a severe reaction, the gumming in the wood may extend throughout the entire plant.

Seedlings of Parson's Special mandarin cannot be used as indicators since they are monoembryonic and are quite variable in their reaction. Also, seedlings are very slow growing and in general make poor indicators when compared to the forced clonal lines budded on a vigorous rootstock. Therefore certain clonal lines of Parson such as

#9 or #10 were selected as superior indicators and are these are available to research institutes from the USDA National Germplasm Repository at Riverside, California. Warm temperature are needed for maximum symptom expression. The Parson indicator forced at cooler temperatures may not show symptoms. The hotter the temperature, the better and quicker will be the reaction on Parson's Special mandarin (Fig. 27). Research conducted in Cuba has shown that the Clementine mandarin 11-20 is superior to the Parson's Special mandarin as an indicator for detection of CCaV (Perez *et al.*, 1993).

Figure 27: The excellent reaction of the cachexia viroid on Parson's Special mandarin

13.3.1 Indexing using sPAGE

sPAGE is the preferred indexing method where the technology and the equipment can be obtained and well trained technicians are available to do the testing (Duran-Vila *et al.*, 1991). This technique is much more rapid and definitive in showing the presence of the milder reacting forms of the CCaV which, as mentioned, may take a year or longer for symptom development. However, technical difficulties may be faced in developing countries for getting the required equipment, chemicals, and water purifiers which are

quite expensive. Thus, where sPAGE facilities are difficult to generate, biological indexing is still an option. In Spain and California, both biological and sPAGE indexes are routinely used to detect viroids in their certification programs.

The citrus viroids CVd-IIa and CVd-IIb are closely related, only a few nucleotides apart. Yet, they will induce entirely different symptoms on indicator plants and on field trees. These two viroids can hybridize and interfere with each other and therefore may be related. CVd-IIa will induce very mild symptoms on citron and mild bark cracking on trifoliate rootstock but no reaction on mandarin or mandarin hybrids. However, CVd-IIb, the cachexia viroid, will induce the citrus cachexia disease in mandarins and mandarin hybrids but will show little or no reaction in citron and will not affect trifoliate rootstocks (Semancik *et al.*, 1988b). An interference or protective action of CVd-IIa against CVd-IIb has been observed when CVd-IIa was first inoculated into Parson's Special mandarin on rough lemon rootstock and then challenged with CVd-IIb (cachexia). The protection was afforded to all four sources of the CCaV tested (Semancik *et al.*, 1991; Semancik *et al.*,1992).

13.4 Elimination from budwood

Shoot tip grafting has been found effective for elimination of CCaV. Roistacher *et al.,* (1976) eliminated CCaV from 66 out of 66 shoot tip grafted plants. Thermotherapy has consistently failed to eliminate the CCaV from citrus tissue. The use of nucellar seedlings is another approach for eliminating viroids from citrus and this, at one time was the only way to eliminate viroids. However, there are many problems associated with the nucellar as described by Roistacher (1977).

14. Gum pocket disease of citrus

This disease, which affects trifoliate orange rootstocks, is found in South Africa and has been reported in Argentina and Australia. The disease induces severe stunting and tree decline. Primary lesions are gum pockets and depressions, which develop in bark and wood of the trifoliate orange rootstock. Gum pockets are more numerous directly below the bud union. The disease was first described from South Africa as 'Gum Pocket' (Schwarz and McClean, 1969).

The disease is transmissible primarily through infected budwood and probably by mechanical transmission. In South Africa, symptoms appear 2 to 3 years after inoculation but 8 to 10 years in studies done in Argentina. The gum pocket disease has now been shown to be caused by a citrus viroid (Marais *et al.*, 1996).

14.1 Field Symptoms

Field symptoms are stunting of trees on trifoliate rootstock and distinct gum pockets in the trifoliate rootstock. The disease induces an average reduction of 22 % in the canopy of sweet orange trees on trifoliate rootstock and will induce discolorations, distortions and depressions in the trunk of the trifoliate rootstock.

14.2 Indexing

Marais *et al.,* (1996) reported that the causal agent of the gum pocket disease in trifoliate orange in South Africa is a citrus viroid in the group CVd-III. Koch's postulates were fulfilled in their experiments. Thus, gum pocket can be indexed by using sequential polyacrylamide gel electrophoresis (sPAGE). When indexed to citron, it shows the bent leaf and mild petiole browning reaction typical for the reaction on other CVd-III. sources.

14.3 Management

With the discovery of its viroid nature, gummy pitting can now be detected by sPAGE and, as with other viroids it is probable that it can be readily eliminated by shoot tip grafting. After shoot tip grafting a follow up index by sPAGE should be done to be certain that the viroid has been eliminated prior to distribution of budwood.

15. Stubborn disease of citrus

15.1 An Overview

Stubborn disease can be found in most countries that grow citrus under desert or semi arid conditions. The disease is present in the warmer areas of California and Arizona and in most of the countries of North Africa, the Near and Middle East and the Arabian peninsula. The disease has been reported in Turkey, Greece, Italy, Mexico, Spain, Sudan and Pakistan. Stubborn disease is rare in cooler climates since both the vector and organism prefer warm temperatures. Bove (1995) in his book "Virus and virus-like diseases of citrus in the near east region" observed and reported the presence of stubborn disease in all of the countries of the Near East.

The disease was first noticed about 1915 on navel orange trees near Redlands, California and named 'stubborn' because buds refused to grow properly after trees were top worked. The name "acorn disease" was also used to describe the disease because of the many acorn-shaped fruits found on stubborn-affected trees. A similar disease called "little leaf" was reported in Palestine (Reichert, 1930; Reichert and Perlberger, 1931). They illustrated a small shoot and leaf condition plus small and misshapen fruit, which strongly resembled stubborn disease. Stubborn disease has been present in Israel for many years. Fawcett *et al.,* (1944) first showed the transmissible nature of stubborn disease. A complete slide show on this disease can be found on the website http://ccoport.org as slide show 87 http://ecoport.org.

15.2 Field symptoms

Stubborn-infected trees in the field appear compressed and stunted, sometimes severely so (Fig 28). At times only a portion of the tree is affected and branches are compressed with smaller leaves. These symptoms are also present in young budded nursery trees. Leaves may show a chlorotic mottle which is also characteristic for greening-infected trees. Stunted trees remain small and rarely recover. The fruit does not color properly and the styler end retains its green color. The navel orange is most susceptible

to fruit greening. Stubborn-infected fruit are usually small, distorted and may also show an acorn shaped appearance (Fig.29). Fruit of seedy varieties may have a number of considerably smaller darker purple seeds or completely aborted seeds. This is termed "seed abortion". Also, the fruit may have an insipid taste. Grapefruit, sweet orange, tangelo, mandarin, lime and pummelo are affected. Trifoliate and trifoliate hybrids, lemons and limes are more tolerant.

15.3 *Spiroplasma citri*

A mycoplasma-like organism was discovered in the sieve tubes of stubborn-infected

Figure 28: The characteristically stunted and compressed appearance of a stubborn-infected navel orange tree. A non-infected normal tree of the same age is on the left

citrus tissue independently by Igwegbe and Calavan (1970) in California, and by Lefleche and Bove (1970) in France. Both groups of workers concluded that a mycoplasma and not a virus was the cause of the disease. Fudl Allah *et al.,* (1972) in California, and Saglio *et al.,* (1971) in France were able to culture an organism in liquid and solid medium. The organism was described and named *S. citri* by Saglio *et al.,* (1973) thereby establishing a new genus of mollicutes. Antiserum has been prepared to *S. citri* and used for detection of the organism in various assays including ELISA.

S. citri is a motile, helical mollicute with no cell wall and no peptidoglycan. The

spiral or helical morphology and motility can be seen by phase or dark field microscopy.

15.4   Vectors and vector transmission

Transmission of *S. citri* in California is primarily by the beet leafhopper Circulifer tenellus, but also by *Scaphytopius nitridus* (Kaloostian *et al.*, 1975; Kaloostian *et al.*, 1976). *S. citri* was shown to be spread from weeds or vegetable hosts to a wide variety of weeds or vegetables by leafhoppers. The weeds became infected, stunted and yellow, and when they dried up under warm or hot conditions, the vectors containing *S. citri* moved from the weed hosts to citrus. Young citrus are more susceptible than older trees. Transmission is primarily from infected weeds to citrus and poorly transmitted from infected citrus to citrus.

Ing Ming Lee *et al.*, (1973) cultured *S. citri* from the beet leafhopper N. (Circulifer)

Figure 29: Two acorn shaped navel orange fruit also showing greening (left)

tenellus. The beet leafhoppers Neoaliturus (Circulifer) tenellus and *Scaphytopius nitridus* are the primary vectors for stubborn disease in California (Kaloostian and Pierce, 1972; Ing Ming Lee *et al.*, 1973). The leafhopper *Neoaliturus haematoceps* is the primary vector in perhaps all of the countries of the Near and Middle East Region of Africa (Bove, 1995; Fos *et al.*,1986; Kersting *et al.*, 1993).

Weeds are hosts for *S. citri* and the breeding ground for the leafhopper vectors of stubborn disease. Many weeds are hosts and will harbor *S. citri* such as woody plantain (Plantago Spp.), London rocket (*Sisymbrium irio*) and mustard weed (*Brassica tournefortii*). They are the primary sources of infection of citrus (Allen, 1975; Kaloostian *et al.*, 1975; Kaloostian *et al.*, 1976; Bove *et al.*, 1979). Hence, Infection of citrus in the field is generally via infected weeds. Much stubborn infection occurs in the outdoor citrus nursery. In warm and dry climates when the weeds dry up, the leafhoppers moves from dying infected weeds to the young green citrus plants in the nursery inducing infection in the nursery trees. Older trees may be more difficult to infect in this manner but can become infected. The understanding that weeds are a primary source of infec-

tion can lead to the control of the disease by growing nursery plants under screen or in tunnels. Stubborn disease is a severe problem in Turkey, where the disease is vectored by *N. haematoceps*. The leafhopper transmits the disease from infected sesame plants which are grown as a commercial crop (Kersting *et al.*, 1993). In Syria, the disease is also vectored by *N. haematoceps* which feed on the weeds of *Salsola kali* (Russian thistle or tumbleweed). Bove (1995) indicated that this weed is a major host plant of the leafhopper but does not harbor the stubborn organism. However, he found the incidence of stubborn disease higher in the coastal regions of Syria where *S. kali* and the vector are more abundant.

15.5 Indexing

15.5.1 Graft transmission

Side grafts and young leaf piece grafts can successfully transmit stubborn disease to seedlings of Madam Vinous sweet orange indicator plants. These two inoculum sources were found superior for transmission than the use of buds or blind buds (Calavan *et al.*, 1968; 1972a). Side grafting is a good method of inoculation. Two pieces of stem are cut from the source to be indexed. They should be about the same diameter as the index seedling. The ends are cut to a sharp V and inserted into a side cut in the indicator plant. After insertion, the graft is wrapped with polyethylene tape and covered with a polyethylene bag as a sleeve. Moisture is retained by the leaves on the plant inside the bag. After a week to ten days, the polyethylene sleeve is removed and the index plant cut back to force new young growth.Leaf grafting is also an effective method of inoculation. A small piece of leaf tissue measuring about 2-3 x 10 mm is cut from the center of a young leaf. The leaf piece is inserted into the T-cut in the same manner as buds are inserted.

The first symptoms of stubborn disease in an emerging young shoot of an inoculated Madam Vinous sweet orange seedling grown under warm conditions in a greenhouse shows a slightly semi-wilted appearance. A severe reaction on the Madam Vinous indicator seedling can show extreme stunting, chlorosis, pinched leaf tip and leaf tip mottle. Stubborn disease symptoms can be confused with zinc deficiency symptoms. However, when analyzed for zinc, the levels for zinc are found to be normal in the stubborn-infected leaves.

15.5.2 Detection of *S. citri* in a culture media

S. citri can be isolated and cultured in the laboratory and diagnosed by a pH change of the phenol red indicator dye. This is a reliable method for detection. A complete description of the isolation and detection of *S. citri* is given in the handbook for detection and diagnosis of graft transmissible diseases (Roistacher, 1991).

15.5.3 Other techniques

Other techniques for detecting *S. citri* are now available using ELISA, PCR, dot-blot

hybridization and culturing (Bove, 1988). Saillard *et al.,* (1993) compared all of these techniques for detection of stubborn disease in the field and they concluded that IC-PCR to be the most promising technique for detection of low amounts of *S. citri* in cells of citrus.

15.6 Management

Control of stubborn disease can be affected by weed control in the nursery, by discarding stunted nursery trees, or most effectively, by growing citrus nursery trees under screen. As explained, the feeding of the vectors on weeds and then the vectors moving to young nursery trees in the field when the weeds dry up is the primary means of infection. Thus growing nursery plants under screen can materially reduce the incidence of stubborn disease.

16. Concave Gum Disease of Citrus

16.1 An overview

Concave gum was first recorded as a disease by Fawcett (1936). The disease is found worldwide but is heavily concentrated in the countries of the Mediterranean region but can also be found less frequently in most of the citrus growing areas of the world. Concave gum disease has been reported in Japan (Ieki and Ito, 1996).

Concave gum and blind pocket diseases are probably part of the same complex with blind pocket showing sharper and deeper concavities. Concave gum can be separated from psorosis-A on the basis of trunk and leaf symptoms on field trees, leaf symptoms on inoculated indicator seedlings, internal wood staining or gumming in branches, and by cross protection. In addition, tissue from concave gum infected trees will not react against antiserum of psorosis-A (D'Onghia *et al.*, 1998).

Concave gum induces symptoms primarily in sweet oranges, mandarins, tangors and tangelos and these can retain the pathogen and remain as symptomless hosts. Although the pathogen has not been isolated, it almost certainly is a virus and mild strain cross protection has been shown (Roistacher and Calavan, 1965). The disease is readily transmitted by grafting and by top working and possibly by root graft to adjacent trees.

The concave gum disease has not been shown to be vector or mechanically transmitted. The pathogen can be readily eliminated in budwood by thermotherapy and by shoot tip grafting *in vitro*. The disease can be quite severe and debilitating to young trees (D'Onghia *et al.*, 1992).

16.2 Field symptoms

The diagnostic symptoms of the disease in field trees include concavities in the trunk (Fig. 30), gum rings in cut section of trunk or twigs and oak leaf patterns visible on most leaves in the young emerging leaves in the spring. When a branch or twig is cut,

distinct gum rings can be seen, usually in concentric circles. Oak leaf patterns (OLP) are visible on almost all leaves of the early flush of growth during the spring months. This is especially true in areas where winter months are cold and cool temperatures prevail in the spring. OLP is a typical symptom for infection by concave gum, cristacortis and impietratura. In certain regions the disease can be quite destructive as observed by Dr. Robert Vogel, the world authority on the concave gum disease of citrus and also reported by D'Onghia *et al.,* (1992). Severe strains of the concave gum pathogen can result in the decline of trees as was observed by the author in a farm at Kudzare, Turkey.

Figure 30: Concavities associated with the concave gum diseases in the trunk of a sweet orange tree also called as blind pocket.

Concave gum disease in spread primarily by man by use of infected propagative budwood and by top working to existing infected trees, a very common practice in the Mediterranean region.

16.3 Indexing

Graft-transmission to seedling plants of mandarin, mandarin hybrids such as Dweet

tangor or sweet orange is the only current method of detection. Symptoms are mild leaf flecks and OLP visible when plants are grown under relatively cool conditions in and indexing greenhouse (Fig.31). However, in mixed infections with psorosis-A the leaf patterns can be quite strong and variable. Symptoms will disappear or will not show if temperatures in the greenhouse are too warm. The temperatures of the index facility are critical for the development of leaf symptoms. Concave gum infection rarely induces shock symptoms in the first flush of growth of sweet orange indicator plants as does psorosis-A unless there is a co-infected with both viruses. Leaf flecking is usually the

Figure 31: Oak leaf patterns then develop on the leaves of indicator plants of sweet orange, usually in the second flush of growth.

first symptom to appear 5 to 8 weeks after inoculation and OLP then develops usually in the second growth flush.

16.4 Management

The management of the concave gum disease involves its detection and elimination of the virus through a certification program. This means first, the detection of the OLP by indexing, followed by shoot tip grafting and/or thermotherapy and then followed by an index of the new treated plant. After indexing, the lack of the oak leaf patterns in the graft-inoculated indexed plant, coupled with development of OLP in the positive controls would signify that the virus has been eliminated from this treated bud source. Bud propagation and increase can then be done with the treated and certified budwood.

17. Thermotherapy for Citrus

There are three ways to eliminate graft transmissible pathogens from citrus germplasm:
i.   Growing plants from nucellar sources,
ii.   Thermotherapy, and
iii.   Shoot tip grafting.

Before 1971, growing plants from nucellar sources was the only method available to develop virus-free budlines of citrus. A seed has two types of tissue, the gametic and the nucellar, and most viruses and virus-like pathogens will not pass through the nucellus in the seed. Thus, trees developed from nucellar seedlings are usually virus-free and true to type. However, there are several problems associated with nucellar selections. For example i. It takes a long time to outgrow juvenility, ii. It take a long time for trees to come into production, iii. The quality of fruit from nucellar trees are sometimes poor, iv. Excessive thorniness, v. Upright tree growth, vi. Excessive tree vigor, vii. Tendency toward alternate bearing, viii. Unequal distribution of fruit on the tree and ix. Deterioration of fruit quality on the tree (Washington navel).

Thermotherapy can be adopted to eliminate many viruses from budwood. There are three approaches to thermotherapy of citrus. i. Hot water treatment, ii. Hot-moist air treatment and iii. Treatment in heat Chambers.

17.1 Hot water treatment

Hot water has been found to be too drastic to budwood and in many attempts for hot water therapy, the budwood was killed before the pathogen. Various scientists tried hot water treatment at 50°C for different timings and failed in eliminating the viruses.

17.1.1 Pre-conditional of budwood or plants

Pre-conditioning of budwood is necessary for successful thermotherapy and survival of budwood. When budwood was preconditioned in a warm room at approximately 28-40°C during the day and 25-30°C at night, it survived up to 5 hours at 50°C in a hot-moist air chamber. However, when the budwood was taken directly from the field for thermotherapy with no pre-conditioning, there was no survival (Roistacher and Calavan, 1972). Plants can also be pre-conditioned in a warm section of a greenhouse or by

constructing a plastic cage. The plants can be kept in these structures for 3-4 months with daytime peaks to 38-40º C. Extra heat may be applied at night with electric heaters or light bulbs regulated by a thermostat (Roistacher *et al.*, 1977b). Pre-conditioning in such a chamber eliminated the viruses of tristeza, vein enation, psorosis-A, concave gum and infectious variegation (Roistacher *et al.*, 1974).

17.2 Warm moist air method

We are indebted to Professor Lin Kong-xiang for developing the moist-hot air treatment for thermotherapy of citrus in China. By his method He and co-worker successfully eliminated the greening pathogen from citrus budwood (Lin & Lo, 1965). In the method used at Riverside, California indexing facility for moist-hot air treatment, budwood to be treated is placed inside a plastic container with a small amount of water on the bottom. The budwood is supported and held upright by placing it in a small section of plastic tubing. The entire closed container holding the budwood is then placed inside an incubator held at 50ºC. In this way, the citrus tatter leaf virus was eliminated when infected Meyer lemon budwood was kept for 3 to 22 hours at 50°C (Roistacher *et al.*, 1972). This resulted in the production of the first virus-free Meyer lemon and legislation then mandated that all Meyer lemons produced in California must come from certified material and tagged. The same technique was used at the Taizhou Agricultural School by Zhang Tian-Miao for the elimination of the citrus tatter leaf virus, which was endemic in Zhejiang Province, China.

17.3 Temperature controlled chambers

Temperature controlled chambers are the preferred method used for thermotherapy at the index facility in Riverside, California. The chambers are held at 40ºC for 16 hours a day with artificial lighting and 30ºC at night for 8 hours dark. Various chambers have been designed or modified for use in thermotherapy in different countries. Buds to be treated are first grafted to Troyer or Carrizo citranges as rootstock. After 4 to 5 weeks in the chambers, the budded plants are bent to promote forcing of the buds. Troyer citrange, trifoliate orange, and Rangpur lime were found to be highly tolerant to heat whereas rough lemon, Mexican lime, sweet orange, citron, grapefruit, and Sexton tangelo could not withstand therapeutic temperatures. After 7 to 12 weeks, plants are removed from the chamber, and the buds forced. The tissue from these heat-treated emerging shoots are then thoroughly indexed for presence or absence of viruses and other graft-transmissible pathogens. The major consideration in the use of heat treatment chambers is maintenance. The chambers must be constantly inspected. A good technician is a necessity and spare parts should be kept available. Daily inspections must be made for temperature, ballast fans, thermostat fans, lighting, and plant water needs.

17.4 Effectiveness of Thermotherapy

Concave gum, greening, impietratura, infectious variegation, psorosis-A, psorosis-B,

tatter leaf, tristeza, and vein enation were effectively eliminated by using temperature controlled chambers (Roistacher *et al.*, 1977a). *S. citri* , Dweet mottle and yellow vein viruses were difficult to eliminate by thermotherapy. Also, the citrus viroids (exocortis, cachexia and all citrus viroids) could not be eliminated by thermotherapy.

It is important to remember that lack of symptoms in plants held at warm temperatures does mean that the pathogen has been eliminated. The expression of virus symptoms can be hidden or masked at warm temperatures. However, when infected plants grown at warm temperatures are moved to a cooler room, symptoms would appear (Roistacher *et al.*, 1974). Indexing is a necessity after thermotherapy. Heat treated plants must be indexed to assure freedom from viruses and other pathogens. Indexing must include comparing the heat treated plants against the original source plant in order to be sure the pathogens have been eliminated. For a more complete slide presentation on thermotherapy, visit the http://ecoport.org website for slide show 42 at http://ecoport.org.

Acknowledgement

18. References

Ahlawat, Y.S., Varma A., Chandra K.J., Ramapanda S. and Kapur P. 1993. Serological reactivity in citrus tristeza virus strains in India. In: "Proc 12th Conf. IOCV. IOCV, Riverside", pp.108-112.

Alioto, D., Gangemi, M., Deaglio, S., Sposato, P., Noris, E., Luisoni, E. and Milne R.G. 1999. Improved detection of citrus psorosis virus using polyclonal and monoclonal antibodies. Plant Pathology 48:735-741.

Allen, R.M. 1975. *Spiroplama* organism found in naturally infected Periwinkle. Citrograph 60: 12, 428-446.

Aubert, B., Etienne, J., Cottin, R., Leclant, F., Cao Van, P., Vuillaume, C., Jaramillo, C. and Barbeau, G. 1992. Citrus tristeza disease, a new threat for the Caribbean basin. Report of a survey to Colombia, Dominican Republic, Guadeloupe, Martinique and Trinidad. DIRAD/IRFA - BP.5035 - 34032 Montepelllier, Cedex 1 - France.

Balaraman, K. and Ramakrishnan, K. 1977. A virus disease of acid lime (*Citrus aurantifolia* (Christm.) Swing.) resembling Hassaku dwarf. Mysore Journal of Agricultural Sciences 11: 2, 185-188.

Balaraman, K. and Ramakrishnan, K. 1980. Strain variation and cross protection in citrus tristeza virus on acid lime. In: "Proc. 8th Conf. IOCV, IOCV, Riverside", pp. 60-68.

Ballester Olmos, J.F., Pina, J.A., Carbonell, E.A., Moreno, P., Hermoso de Mendoza, A., Cambra, M. and Navarro, L. 1993. Biological diversity of citrus tristeza virus (CTV) isolates in Spain. Plant Pathology 42: 2, 219-229.

Bar-Joseph, M., Loebenstein, G. and Cohen, J. 1970. Partial purification of virus like particles associated with the citrus tristeza virus disease. Phytopathology 60: 75-78

Bar-Joseph, M., Loebenstein, G. and Oren, Y. 1974. Use of electron microscopy in eradication of

tristeza sources recently found in Israel. p. 83-85.

Bar-Joseph, M., Garnsey, S.M. and Gonsalves, D. 1979a The Closteroviruses A distinct group of elongated plant viruses. Adv. Virus Res.25:93-168.

Bar-Joseph, M., Garnsey, S.M., Gonsalves, D., Moscovitz, M., Purcifull, D.E. and Clark, M.F. 1979b. The use of enzyme linked immunosorbent assay for detection of citrus tristeza virus. Phytopathology 69: 190-194.

Bar-Joseph, M., Roistacher, C.N., Garnsey, S.M. and Gumpf, D.J. 1981. A review on tristeza, an ongoing threat to citriculture. Proc. Int. Soc Citriculture. Vol. 1:419-423.

Bar-Joseph, M., Roistacher, C.N. and Garnsey, S.M. 1983. The epidemiology and control of citrus tristeza disease. In: "Plant Virus Epidemiology", (eds. Plumb and Thresh), Blackwell scientific publications, Oxford. pp. 61-72.

Bar-Joseph, M. and Nitzan, Y. 1991 The spread and distribution of citrus tristeza virus isolates in sour orange seedlings. In: "Proc. 11th Conf. IOCV. IOCV, Riverside", pp. 162-165.

Benton, R.J., Bowman, M.F.T., Fraser, L. and Kelly, R.G.1949. Stunting and scaly butt of citrus associated with *Poncirus trifoliata* rootstock. "Agr. Gaz. N.S. Wales 60: 521-526, 577-582, 641-645, 654; 61: 20-22. 40."

Benton, R.J., Bowman, M.F.T., Fraser, L. and Kelly, R.G. 1950. Scaly butt and stunting of citrus. Dept. Agric. N.S.W. Sci. Bull. 70

Bitancourt, A.A. 1937. A leprose e a proxima colhieta de larangjas. O Biologico, Sao Paulo 3(2):37-40

Bitancourt, A.A. 1940a. A Doenca dos citrus no vale do paraiba. Biologico 6: 268-269.

Bitancourt, A.A. 1940b. Apodridao das radicalas dos citrus na provincia de Corrientes, Argentina. O Biologico 6:285-288, 356-364.

Bitancourt, A.A. 1944. Um teste para a identificacao precoce da tristeza dos citrus. O biologico, 10(6):169-175.

Bitters, W.P. 1952. Exocortis disease of citrus. Calif. Agr. 6: 5-6.

Bitters, W.P. and Parker, E.R. 1951. Horticultural aspects of quick decline. California Citrograph 36:222,264.

Bitters, W.P., Brusca, J.A. and Dukenshire, N.W. 1954. Effect of lemon budwood selections in transmission of exocortis. California Citrograph 39:70-71, 84-85. *See* also Citrus Leaves 34:1, 8-9, 34.

Bitters, W.P., Duran-Vila, N. and Semancik, J.S. 1987 Effect of citrus exocortis viroid on flower and fruit structure and development of Etrog citron. Plant Disease 71: 5, 397-399.

Bové, J.M. 1988. *Spiroplasma citri*: fifteen years of research. In: "Proc. 10th Conf. IOCV. IOCV, Riverside", pp. 274-284.

Bove, J.M. 1995. Virus and virus-like diseases of citrus in the Near East region. FAO, Rome.

Bove, J.M., Nhami, A., Saillard, C., Vignault, J. C., Mouches, C., Garnier, M., Moutous, G., Fos, A., Bonfils, J., Abassi, M., Kabbage, K., Hafidi, B. and Viennot Bourgin, G. 1979. Presence in Morocco of *Spiroplasma citri*, causal agent of citrus stubborn, on periwinkle (*Vinca rosea* L.) planted on the border of diseased orange orchards and probable contamination of *Cynodon dactylon* (L.) Pers. by the *spiroplasma.* Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences, D 288: 4, 399-402.

Bridges, G.D., Youtsey, C.O. and Nion, R.R. 1965. Observation indicating psorosis transmission by seed of Carrizo citrange. Proceedings of the Florida State Horticultural Society, 78: 48-50.

Bridges, G.D. and Youtsey, C.O. 1972. Natural tristeza infection of citrus species, relatives and hybrids at one Florida location from 1961-1971. Proceedings of the Florida State Horticultural Society, 85: 44-47.

Brlansky, R.H., Pelosi, R.R., Garnsey, S.M., Youtsey, C.O., Lee, R.F. Yokomi, R.K. and Sonoda, R.M. 1986 Tristeza Quick decline epidemic in South Florida. Proceedings of the Florida State Horticultural Society, 99: 66-69.

Broadbent, P., Bevington K.B. and Coote B.G. 1991. Control of stem pitting of grapefruit in Australia by mild strain protection. In: "Proc. 11th Conf. IOCV. IOCV, Riverside".pp 64-70.

Broadbent, P., Hutton, R.J. and Bevington, K.B. 1992. Guidelines for the commercial use of graft-transmissible dwarfing in Australia - potential benefits and risks. Proc. Int. Soc. Citriculture, 1992, Vol.3:697-701

Burnett, H.C. and Boring, J.M. 1960. The spread of tristeza in citrus in two Florida counties. Plant Dis. Reptr. 4: 765-766.

Calavan, E.C., Christiansen, D.W. and Roistacher, C.N. 1963. Symptoms associated with tatterleaf virus infections of Troyer citrange rootstocks. Plant Dis. Reptr. 47: 971-975.

Calavan, E.C., Olson, E.O. and Christiansen, D.W. 1972a. Transmission of the stubborn pathogen in citrus by leaf piece grafts. In: "Proc. 5th Conf. IOCV, 1969", pp. 145-153.

Calavan, E.C., Roistacher, C.N. and Nauer, E.M. 1972b. Thermotherapy of citrus for inactivation of certain viruses. Plant Diseases Reporter 56: 11, 976-980.

Calavan, E.C., Roistacher, C.N. and Christiansen, D.W. 1968. Distribution of stubborn disease virus in trees of Citrus sinesis and C.paradisi at different seasons. pp. 145-153.

Calavan, E.C., Harjung, M.K., Blue, R.L., Roistacher, C.N. and Gumpf, D.J. 1980. Natural spread of seedling yellows and sweet orange and grapefruit stem pitting tristeza viruses at the University of California, Riverside. pp. 69-75.

Cambra, M., Serra, J., Villalaba, D. and Moreno, P. 1988 Present situation of the citrus tristeza virus in the Valencian community. In: "Proc. 10th Conf. IOCV, Riverside",pp. 1-7.

Cambra, M., Camarasca, E., Gorris, M.T., Garnsey, S.M., Gumpf, D.J., and Tsai, M.C. 1993. Epitope diversity of citrus tristeza virus isolates in Spain. In: "Proc. 12th Conf. Intern. Organ. of Citrus Virologists, pp. 33-38

Chagas, C. and Rossetti, V. 1984. Transmission of leprosis by grafting. In: "Proc. 9th Conf. IOCV. IOCV, Riverside", pp. 215-17.

Chakraborty, N.K., Ahlawat, Y.S., Varma, A., Chandra, K.J., Ramapandu, S. and Kapur, S.P. 1993. Serological reactivity in citrus tristeza virus strains in India. In: "Proc. 12th Conf. IOCV. IOCV, Riverside, pp. 108-112.

Chen, G.Q., Yan, S.X. and Roistacher, C.N. 1992. First report of citrus vein enation virus in China. Plant Disease 76:1077.

Childs, J.F.L. 1950. The cachexia disease of Orlando tangelo. Plant Dis. Reptr. 34: 295-298.

Childs, J.F.L. and Johnson, R.E. 1966. Preliminary report of seed transmission of psorosis virus. Plant Dis. Rep. 50:81-83.

Clark, C.C. and da Graca J.V. 2000. Detection of citrus vein enation virus using cereal yellow dwarf virus ELISA kits. In: "Proc. 14th Conf. IOCV., IOCV, Riverside", pp. 357-359.

Cohen, M. 1972. A leaf insert graft used for virus transmission in citrus. In: "Proc. 5th Conf. IOCV, Univ. Fla. Press, Gainesville". pp. 282-284

Colariccio, A., Lovisolo, O., Boccardo, G., Chagas, C.M., d'Aquilio, M. and Rossetti, V., 2000. Preliminary purification and double stranded RNA analysis of citrus leprosis virus. In: "Proc. 14th Conf. IOCV. IOCV, Riverside", pp 159-163.

Collins, R.P. and Van Vuuren, S.P. 1990. Economics of sensitive citrus material pre-immunized with a mild isolates of citrus tristeza virus. South African CSFRI Information Bull. 220:11-12

Costa, A.S. and Grant, T.J. 1951. Studies on transmission of the tristeza virus by the vector, *Aphis citricidus*. Phytopathology 41:105-113

Costa, A.S., Grant, T.J. and Moreira, S. 1950. A possible relationship between tristeza and stem-pitting disease of grapefruit in Africa. California Citograph 35, 504, 526-528.

Costa, A.S. and Muller, G.W. 1980. Tristeza control by cross protection: a U.S.-Brazil cooperative success. Plant Disease 64: 6, 538-541.

da Graca, J.V. and S. B. Maharaj. 1991. Citrus vein enation virus, a probable luteovirus. In: "Proc.

11th Conf. IOCV. IOCV, Riverside' pp. 391-394.

Dauthy, D.Y. and Bove, J.M. 1968. Purification of crinkly leaf virus. In: "Proc. 4th Conf. IOCV. Univ. of Florida Press, Gainseville", pp. 255-263.

Davino, M. and Garnsey, S.M. 1984. Purification, characterization and serology of a mild strain of citrus variegation virus from Florida. In: "Proc. 9th Conf. IOCV. IOCV, Riverside", pp..196-203.

Derrick, K.S., Brlansky, R.H., Lee, R.F., Timmer, L.W. and Nguyen, T.K. 1988. Two components associated with the citrus ringspot virus. In: "Proc. 10th Conf. IOCV. IOCV, Riverside", pp. 340-342.

Derrick, K.S., Lee, R.F., Hewitt, B.G., Barthe, G.A. and da Graça, J.V. 1991. Characterization of citrus ringspot virus. In: "Proc. 11th Conf. IOCV. IOCV, Riverside", pp. 386-390.

Dickson, R.C., Flock, R.A. and Laird, E.F. 1956a. Citrus aphids and spread of tristeza. Calif. Citrograph 41: 324,329

Dickson, R.C., Johnson, M.M., Flock, R.A. and Laird, E.F.Jr. 1956b. Flying aphid populations in southern California citrus groves and their relation to the transmission of the tristeza virus. Phytopathology 46: 204-210

Djelouah, K. and D'Onghia, A.M. 1998. A survey for citrus ringspot virus in acitrus collection of Southern Italy. Options Mediterraneenes. In: "IAM-Bari, Mediterrannean Network on Citrus Certification: three years of activity (1995-1997), Cahiers Options Méditerranéennes, CIHEAM Paris". Serie B. 21: 107-112,

Djelouah, K., Potere, O., Boscia, D., D'Onghia, A.M. and Savino, V. 1998. Production of monoclonal antibodies to citrus psorosis-associated virus. In: "Proc. 14th Conference of IOCV, Riverside". pp. 152-158.

Dodds, J.A. 1994. Citrus tristeza virus incidence in the Central Valley: progress toward eradication. Citrograph 77:12-20.

Dodds, J.A. and Bar Joseph, M. 1983. Double-stranded RNA from plants infected with closteroviruses. Phytopathology 73: 3, 419-423.

Dodds, J.A., Jordon, R.L., Heick, J.A. and Tamaki, S.J.1984. Double stranded RNA for the indexing of citrus and Avocado viruses. In: "Proc. 9th Conference of the IOCV, Riverside" pp. 330-336.

Dodds, J.A., Jarupat, T., Lee, J.G. and Roistacher, C.N. 1987. Effects of strain, host, time of harvest, and virus concentration on double-stranded RNA analysis of citrus tristeza virus. Phytopathology 77: 3, 442-447.

D'Onghia, A.M., De Marco, P. and Savino, V. 1992. Gravi casi di concavita' gommose su navalina in Puglia. (Severe cases of concave gum on Navalina orange in Apulia.). Informatore Fitopatologico 3:39-41.

D'Onghia, A.M., Djelouah, K., Alioto, K. M., Castellano, A. and Savino, V. 1998. Elisa correlates with biological indexing for the detection citruspsorosis-associated virus. Journal of Plant Pathology, 80: 157-163

Duran-Vila, N., Pina, J.A., Ballester, F., Juarez, J., Roistacher, C.N., Rivera-Bustamente, R., and Semancik, J.S. 1988 The citrus exocortis disease. A complex of viroid-RNAs. In: "Proc. 10th Conf. IOCV. IOCV, Riverside", pp. 136-151.

Duran-Vila, N., Pina, J.A., Mollins, M.I. and Navarro, L. 1991. A new indexing method for cachexia. In: "Proc. 11th Conf. IOCV. IOCV, Riverside", pp. 219-223.

Fawcett, H.S. 1933. New symptoms of psorosis indicating a virus disease of citrus. Phytopathology 23:930 (Abstr.).

Fawcett, H.S. 1936. Citrus diseases and their control. McGraw-Hill Brook Co., Inc. New York & London. 656 pp.

Fawcett, H.S. 1938. Transmission of psorosis of citrus. Phytopathology 28:669.

Fawcett, H.S. 1947. Interrelatedness of quick decline and tristeza. California Citrograph 32:416-

418

Fawcett, H.S. and Klotz, L.J. 1939. Infectious variegation of citrus. Phytopathology 29: 911-912.

Fawcett, H.S. and Bitancourt, A.A. 1943. Comparative symptomatology of psorosis varieties on citrus in California. Phytopathology 33:837-864.

Fawcett, H.S., Perry, J.C. and Johnson, J.C. 1944. The stubborn disease of citrus. Calif. Citrograph. 29: 140-147.

Fawcett, H.S. and Klotz, L.J. 1948 Exocortis of trifoliate orange. Citrus leaves 28: 8. Also in Citrograph 33: 230.

Fawcett, H.S. and Wallace, J.M. 1947 Evidence of the virus nature of citrus quick decline. Calif. Citrograph 32:50, 88-89.

Fos, A., Bove, J.M., Lallemand, J., Saillard, C., Vignault, J.C., Ali, Y., Brun, P., Vogel, R. 1986. The leafhopper Neoaliturus haematoceps is a vector of *Spiroplasma citri* in the Mediterranean area. Annales de l'Institut Pasteur Microbiologie 137A: 1, 97-101.

Fraser, L. 1952. Seedling yellows, an unreported virus disease of citrus. Agric. Gaz. of N.S. Wales 63: 125-131.

Fraser, L. R. 1959. Woody gall, a suspected virus disease of citrus in Australia. Proc. Linn. Soc. LXXIII:9-19.

Fraser, L.R. and Broadbent, P. 1979. Virus and related diseases of citrus in New South Wales. 78 pp.

Fudl Allah, A.E.A., Calavan, E.C. and Igwegbe, E.C.K. 1972. Culture of a mycoplasmalike organism associated with stubborn disease of citrus. Phytopathology 62: 7, 729-731

Fulton, R.W. 1966. Mechanical transmission of tatterleaf virus from cowpea to citrus. Phytopathology 56: 575.

Garcia, M.L., Sanchez, de la, Torre, M.E., Costa, N. and Grau, O. 1997. Detection of Citrus Ringspot Virus and Citrus Psorosis-Associated Virus Using PCR. In: "Proc. 13th Conf. Intern. Organ. of Citrus Virologists", pp. 335-337.

Garnsey, S.M. 1968. Exocortis virus can be spread by contaminated tools. Citrus Industry, 49:13-16.

Garnsey, S.M. 1974 Mechanical transmission of a virus that produces tatterleaf symptoms in *Citrus excelsa*. In: Proc. 6th Conf. IOCV. IOCV, Riverside", pp. 137-140.

Garnsey, S.M. 1975. Purification and properties of citrus-leaf-rugose virus. Phytopathology 65: 1, 50-57.

Garnsey, S.M. 1982. Increased freeze damage associated with exocortis infection in navel oranges on Carrizo citrange rootstock. Proc. Fla. State Hort. Soc. 95:3-7.

Garnsey, S.M. and Jones, J.W. 1967. Mechanical transmission of exocortis virus with contaminated budding tools. Plant Dis. Reptr. 51:410-413.

Garnsey, S.M. and Jackson, J.L. Jr. 1975. A destructive outbreak of tristeza in central Florida. Proceedings of the Florida State Horticultural Society, 88: 65-69.

Garnsey, S.M., Baksh, N., Davino, M. and Agostini, J.P. 1984. A mild isolate of citrus variegation virus found in Florida citrus. In: "9th Conf. IOCV. IOCV, Riverside", pp. 188-195.

Garnsey, S.M., Gumpf, D.J., Roistacher, C.N., Civerolo, E.L., Lee, R.F., Yokomi, R.K. and Bar-Joseph, M. 1987. Towards a standardized evaluation of the biological properties of citrus tristeza virus. Phytophylactica 19:151-157.

Garnsey, S.M. and Timmer, L.W. 1988. Local lesion isolate of ringspot virus induces psorosis bark scaling, In: "Proc. 10th Conf. IOCV. IOCV, Riverside", pp. 334-339.

Garnsey, S.M., Permar, T.A., Cambra, M., and Henderson, C.T. 1993. Direct tissue blot immunoassay (DTBIA) for detection of citrus tristeza virus (CTV).). In: "Proc. 12th Conf. IOCV. IOCV, Riverside", pp. 39-50.

Garnsey S.M.and Hilf M.E. 2000. Putting together clues about the origins of the citrus tristeza

virus group. Phytopathology 90, No 6 (Supplement) S91

Gillings, M., Broadbent, P., Indsto, J. and Lee, R.F. 1996. Characterisation of isolates and strains of citrus tristeza clostervirus using restriction analysis of the coat protein gene amplified by the polymerase chain reaction. Jour. Virol. methods. 44:305-317.

Gottwald, T.R., Cambra, M and Moreno, P.1993. The use of serological assays to monitor spatial and temporal spread of citrus tristeza virus in symptomless trees in Eastern Spain. In: "Proc. 12th Conf. IOCV. IOCV, Riverside", pp. 51-61.

Grant, T.J. 1959 Tristeza virus strains in relation to different citrus species used as test plants. Phytopathology 49: 823-827.

Grant, T.J. and Smith, P.F. 1960. Infectious variegation of citrus found in Florida. Plant Dis. Reptr. 44: 426-429.

Grisoni, M. and Riviere, C. 1993. Analysis of epidemics of citrus tristeza virus (CTV) in young citrus groves exposed to aphid infestation under different climatic conditions in Reunion Island. Proc. 12th Conf. Intern. Organ. of Citrus Virologists, pp. 62-69.

Halma, F.F., Smoyer, K.M. and Schwalm H.W. 1944. Quick decline associated with sour rootstock. California Citrograph 29:245

Halma F.F., Smoyer, K.M. and Schwalm, H.W. 1945. Quick decline developments (map showing decline on sour orange rootstock). Citrus Leaves, March 1945:10-11

Herron, C. and Skaria, M. 1997. Isolation and partial characterization of citrus tatter leaf virus isolates from Texas. Phytopathology 87:S41

Hughes, W.A. and Lister, C.A. 1949. Lime disease in the Gold Coast. Nature 164: 889.

Ieki, H. and Ito, T. 1996. Occurrence of concave gum on Hyuganatsu (*Citrus tamurana*) in Japan. In: "Proc. 13th Conf. IOCV, IOCV, Riverside" pp.346-348.

Igwegbe, E.C.K. and Calavan, E.C. 1970. Occurrence of mycoplasma-like bodies in phloem of stubborn-infected citrus seedlings. Phytopathology 60: 1525-1526.

Iwanami, T., Omura, M. and Ieki, H. 1993. Susceptibility of several citrus relatives to Satsuma dwarf virus. In: "Proc. 12th Conf. IOCV. IOCV, Riverside", pp. 352-357.

Iwanami, T. and Ieki, H. 1997. Nucleotide sequence of the 3'-terminal region of citrus mosaic virus RNA1. . Proc. 13th Conf. Intern. Organ. of Citrus Virologists, pp. 200-206

Iwanami, T. and Kondo, Y. 2000. The nucleotide sequence of the coat protein genes of the satsuma dwarf virus and related viruses. Proc. 14th Conf. IOCV, Abst. pp.422, IOCV Riverside CA

Kaloostian, G.H. and Pierce, H.D. 1972. Note on *Scaphytopius nitridus* in California. J. Econ. Entomol. 65: 880.

Kaloostian, G.H., Oldfield, G.N., Pierce, H.D., Calavan, E.C., Granett, A.L., Rana, G.L. and Gumpf, D.J. 1975. Leafhopper - natural vector of Citrus stubborn disease ? California Agriculture 29: 2, 14-15.

Kaloostian, G.H., Oldfield, G.N., Calavan, E.C. and Blue, R.L. 1976. Leafhopper transmits disease to weed host. Citrograph 61: 11, 389-390.

Kano, T., Garnsey, S.M., Koizumi, M. and Permar, T.A. 1991. Serological diversity of citrus tristeza field isolates in Japan. In: "Proc. 11th Conf. IOCV. IOCV, Riverside", pp. 51-55.

Kersting, U., Baspinar, H., inar, A., Sengonca, and Uygun N. 1993. New findings on the epidemiology of *Spiroplasma citri* in the Eastern Mediterranean region of Turkey. In: "Proc. 12th Conf. IOCV. IOCV, Riverside", pp. 326-341.

Kitajima, E.W., Silva, D.M., Oliveira, A.R., Muller, G.W. and Costa, A.S. 1963 Thread-like particles associated with tristeza disease of citrus. Nature 201: 1011-1012.

Kishi, K. and Tanaka, S. 1964. Studies on the indicator plants for citrus viruses. Ann Phytopath. Soc. Japan 29:142-148.

Knorr, L.C. 1956. Suscepts, indicators, and filters of tristeza virus, and some differences between tristeza in Argentina and Florida. Phytopathology 46: 557-560.

Knorr, L.C. 1968. Study on the ecology of leprosis in citrus. In: "Proc. 4th Conf. IOCV. IOCV, Riverside", pp. 332-335.

Knorr, L.C. and Ducharme, E.P. 1951 This is tristeza—ravager of Argentina's citrus industry. Citrus magazine (Florida) 13:17-19.

Knorr, L.C., Malagutti, G. and Serpa, D.D. 1960. Descubrimiento de la tristeza de los citricos en Venezuela. Agron. Trop. 1:3-12.

Koizumi, M. 1984. Elimination of tatterleaf-citrange stunt virus from satsuma mandarin by shoot tip grafting following pre-heat treatment. In: "Proc. 9th Conf. IOCV. IOCV, Riverside", pp. 229-233.

Koizumi, M., Kano, T., Ieki, H. and Mae, H. 1988. China laurestine: A symptomless carrier of satsuma dwarf virus which accelerates natural transmission in the fields. In: "Proc. 10th Conf. IOCV, IOCV, Riverside", pp. 348-352.

Korkmaz, S., Çinar, A., Demirer, E. and Önelge, N. 1994. Greenhouse observations on the susceptibility. In: "Proc. of 9th Congress of the Mediterranean Phytopathology Union, Kusadasi, Turkey", pp. 305-306

Kuhara, S., Koizumi, M., Yamaguchi, A. and Yamada, S. 1981. A nation-wide campaign for certification of early satsuma 'Miyamato Wase' for citrus mosaic by means of ELISA. Proc. Int. Soc. Citriculture, 1: 441-444.

Kyriakou, A., Polycarpou, D., Efstathiou, A.and Hadjinicoli, A, 1993. Citrus tristeza virus in Cyprus. In: "Proc. 12th Conf. IOCV. IOCV, Riverside", pp. 69-72.

Laird, E.F. Jr., and L.G. Weathers. 1961. *Aphis gossypii*, A vector of vein enation virus. Plant Dis. Reptr. 45: 877.

Lama, T.K. 1996. Present status of virus and virus-like diseases of citrus in Nepal. In: "13th Proc. IOCV, IOCV, Riverside", pp 401-402.

Lastra, R., Leandro, G. and Meneses, R. 1988. El viros del la tristeza de los citricos en Costa Rica. American Phytopathology Soc. Caribbean Division, Reunion Dan Andres, Colombia. 14-17 Setiembre

LeFleche, D. and Bové, J.M. 1970. Mycoplasmas dans les agrumes attients de 'greening' de 'stubborn' ou de maladies similaires. Fruits 25(6):455-465.

Le, T.T.H., Nguyen, V.H., Huynh T.D., Lam Thi, M.N. and Huynh, V.T. 1997. Preliminary Results on the Observation of Virus and Virus-Like Diseases of Citrus in the Mekong Delta Region of Vietnam. In: "Proc. 13th Conf. Intern. Organ. of Citrus Virologists", pp. 399-400.

Lee, I.M., Calavan, E.C., Cartia, G. and Kaloostian, G.H. 1973. Stubborn disease organism cultured from leafhopper. Citrograph 59: 2, 39.

Lee, R.F., Brlansky, R.H., Garnsey, S.M. and Yokomi, R.K. 1987. Traits of citrus tristeza virus important for mild strain cross protection of citrus: the Florida approach. Phytophylactica 19:215-218.

Lee, R.F., Garnsey, S.M., Marais, L.J., Moll, J.N. and Youtsey, C.O. 1988. Distribution of citrus tristeza virus in grapefruit and sweet orange in Florida and South Africa. In: "Proc. 10th Conf. IOCV. IOCV, Riverside", pp.28-32.

Lin, K.H. and Lo, H.H. 1965. A preliminary study on the thermotherapy of yellow shoot disease of citrus. Acta Phytopathol. Sinica 4 (2): 169-175.

Lovisolo, O. 2001. Citrus Leprosis Virus: properties, diagnosis, agro ecology and phytosanitary importance. CEPP/EPPO Bulletin 31:79-89

Lovisolo, O., Colariccio, A. and Masenga, V. 2000. New experimental hosts and further information on citrus leprosis virus. In: "Proc. 14th Conf. IOCV., IOCV, Riverside", pp.164-173.

Maharaj, S.B. and Da Graca, J.V. 1988. Observation of isometric virus-like particles associated with citrus vein enation-infected citrus and the viruliferous aphid vector *Toxoptera citricidus*. Phytophylactica 20:357-360.

Maharaj, S.B. and Da Graca, J.V. 1989. Transmission of citrus vein enation virus by Toxoptera

citricidus. Phytophylactica 21:81-82

Marais, L.J. and Lee, R.F. 1986. Citrange stunt virus associated with decline of Shamouti on Swingle citrumelo rootstock in South Africa. Plant Disease 70: 9, 892.

Marais, L.J. and Breyten, J.H.J 1996. The effect of tristeza stem pitting on the Star Ruby grapefruit industry in southern Africa. Citrus Journal, 6 (3): 19-26.

Marais, L.J., Lee, R.L., Breytenbach, J.H.J., Manicom, B.Q. and Van Vuuren, S.P. 1996. Association of a viroid with gum pocket disease of trifoliate orange. Proc. 13th Conf. IOCV. IOCV. Riverside. p.236-244.

McClean, A.P.D. 1950. Virus infections of citrus in South Africa Farming in South Africa. J. Agric. Sci. 25: 289-296

McClean, A.P.D. 1954. Citrus vein enation virus. South Afr. Journ. Sci. 50: 147-151

McClean, A.P.D. 1977a. Tristeza-Virus-Complex: Influence of host species on the complex. The Citrus and Subtropical Fruit Journal, pp. 4-16.

McClean, A.P.D. 1977b. Tristeza disease of citrus trees, and sources of tristeza virus that cause the disease. Citrus and Subtropical Fruit Journal No. 523, 7-19.

McClean, A.P.D. 1977c. Tristeza virus: studies on the effectiveness of protective inoculation. Citrus-and-Subtropical-Fruit-Journal No. 524, 3-12

Mendel, K. 1956. The threat of tristeza disease in the Mediterranean basin. FAO Plant Prot. Bull. 4: 106-108

Mendt, R., Plaza, G., Boscan, R., Martinez, J. and Lastra, R. 1984. Spread of citrus tristeza virus and evaluation of tolerant rootstocks in Venezuela. In: "Proc. 9th Conf IOCV IOCV, Riverside", pp. 95-99.

Meneghini, M. 1946 "Sobre a natureza e transmissibidade de doenca ""tristeza"" dos citrus." O. Biologica 12: 115-118

Milne, R.G., Djelouahm K., Garcia, M.L., Dal, Bo E. and Grau, O. 1997. Structure of Citrus Ringspot-Psorosis-Associated Virus Particles: Implications for Diagnosis and Taxonomy. Proc. 13th Conf. Intern. Organ. of Citrus Virologists, pp. 188-196.

Miyakawa, T. 1969. Susceptibility of citrus species and other related plants to the satsuma dwarf virus. Ann. Phytopathol. Soc. Japan 35: 224-33.

Moreira, S. 1942. "Observacoes sobre a ""tristeza"" dos citrus ou ""podridao dos radicelas. Biologico 8: 269-272

Moreno, P., Guerri, J., Ballester Olmos, J.F., Albiach, R. and Martinez, M.E. 1993. Separation and interference of strains from a citrus tristeza virus isolate evidenced by biological activity and double-stranded RNA (dsRNA) analysis. Plant Pathology 42: 1, 35-41.

Muller, G.W., Costa, A.S., Kitajima, E.W.and Camargo, I.J.B. 1974. Additional evidence that Tristeza multiplies in *Passiflora* spp. In: "Proc. 6th Conf IOCV IOCV, Riverside", pp. 75-78.

Nauer, E.M., Roistacher, C.N., Calavan, E.C. and Carson, T.L. 1988.The effect of citrus exocortis viroid (CEV) and related mild citrus viroids (CV) on field performance of Washington navel orange on two rootstocks. In: Proc. 10th Conf. IOCV. IOCV, Riverside, pp.204-210..

Navarro, Luis 1993. Historia de la Naranja. Las Virosis de los agrios y su control. 24. Levante-EMV. Con la colaboracion de LaGeneralitat Valenciana Consellaria de Agricultura.

Navarro, L., Roistacher, C.N. and Murashige, T. 1975. Improvement of shoot-tip grafting *in vitro* for virus-free citrus. Journal of the American Society for Horticultural Science 100: 5, 471-479.

Navarro, L., Civerolo, E.L., Juarez, J.and Garnsey, S.M. 1991. Improving therapy methods for citrus germplasm exchange. Proc. 11th Conf. IOCV, IOCV, Riverside p 400-408

Nishio, T., Kawai, A., Takahashi, T. and Namba, S. 1989. Purification and properties of citurs tatterleaf virus. Ann. Phytopath. Soc. Japan 55:254-258

Norman, P.A. and Grant, T.J. 1953. Preliminary studies of aphid transmission of tristeza virus in

Florida. Proceedings Florida State Horticultural Society for 1953, pp. 89-92.

Omori, H. and Matsumoto, H. 1972. The cause of stem pitting and small fruit in Nasudaidai trees. In: "Proc. 5th Conf. IOCV, Univ. Fla. Press, Gainesville", pp. 143-146.

Owen-Turner J. 1990. Suspected severe stem pitting strain of tristeza virus discovered in Washington navels. Queensland Citrus Bulletin, Department of Primary Industries - Special issue.

Papasolomontos, A. and Economides, C.V. 1967. Effect of rootstocks on the incidence of impietraura diseased grapefruit fruits. Plant Dis. Reptr. 51: 684-686

Pelosi, R.R. and Powell, C.A. 1992. Nursery distribution of citrus tristeza virus in Florida. In: "Proc. Int Soc. Citriculture",2: pp. 774-775.

Permar, T.A., Garnsey, S.M., Gumpf, D.J. and Lee, R.F. 1990. A monoclonal antibody which discriminates strains of citrus tristeza virus. Phytopathology 80:224-228

Permar, T.A. and Garnsey, S.M. 1991. Comparison of biological indexing and immunological assays for identifying severe Florida isolates of citrus tristeza virus. In: "Proc. 11th Conf. IOCV. IOCV, Riverside". pp. 56-59.

Petri, L. 1931. Variegatura infettiva delle foglie di Citrus vulgaris Risso. Boll. R. Staz. Pat. Veg. NS, 11: 105-114.

Powell, N.C. 1930. Culture of the orange and allied fruit. S. Africa Central New Agency Ltd. 1930 p. 107-108.

Pujol, A.R. and Benateña, H.N. 1965. A study of psorosis in Concordia, Argentina. In: "Proc. 3rd Conf IOCV, Univ. Florida Press, Gainesville", pp. 170-179.

Reanwarakorn, K. and Semancik, J.S. 1999. Correlation of hop stunt viroid variants to cachexia and xyloporosis diseases of citrus. Phytopathology 89:568-574

Reichert, I. 1930. Diseases, new to citrus, found in Palestine. Phytopathology 20: 999-1002.

Reichert, I. and Hellinger, E. 1930. Internal decline: a new physiological disease of citrus fruits in Palestine. Hadar Vol.3:220-224.

Reichert, I. and Perlberger, J. 1931. Little leaf disease of citrus trees and its causes. Hadar 4: 193-194

Reichert, I. and Perlberger, J. 1934. Xyloporosis, the new citrus disease. Agr. Exp. Sta. Rehoboth, Palestine Bull. 12, 49pp. Also Hadar 7: 163-167, 172, 193-202

Reichert, I., Bental, A. and Yoffe, I. 1956. Transmission experiments on the tristeza and xyloporosis diseases of citrus. KVATIM 6: 69-75.

Roistacher, C.N. 1981a. A blueprint for disaster—Part 1: The History of seedling yellows disease. Citrograph 67:4-5, 24.

Roistacher, C.N. 1981b.A blueprint for disaster—Part 2: Changes in transmissibility of seedling yellows. Citrograph 67:28-32.

Roistacher, C. N. l988. The cachexia and xyloporosis disease of citrus—A review. In: "Proc. 10th Conf. IOCV. IOCV, Riverside", pp. 116-124.

Roistacher, C.N. 1991. Graft-transmissible diseases of citrus. Handbook for detection and diagnosis. FAO, Rome. 286 pp.

Roistacher, C.N. 1993. Psorosis-A Review. In: "Proc. 12th Conf. IOCV. IOCV, Riverside", pp. 139-154.

Roistacher, C.N., and Nauer, E.M. 1964. A comparison of certain sweet orange varieties as indicators for concave gum and psorosis viruses. Plant Dis. Rep. 48: 56-59.

Roistacher, C.N., and Calavan, E.C.. 1965. Cross protection studies with strains of concave gum and psorosis viruses, In: "Proc. 3rd Conf. IOCV. Univ. Florida Press, Gainesville", pp. 154-161.

Roistacher, C.N., and Blue, R.L. 1968. A psorosis-like virus causing symptoms only in Dweet tangor, In: "Proc. 4th Conf. IOCV. Univ. Florida Press, Gainesville" pp. 13-18.

Roistacher, C.N., Calavan, E.C. and Blue, R.L 1969.Citrus exocortis virus — Chemical inactivation on tools, tolerance to heat and separation of isolates. Plant Dis. Reptr. 53: 333-336.

Roistacher, C.N., and Calavan, E.C. 1972.Heat tolerance of preconditioned citrus budwood for virus inactivation. In: "Proc. 5th Conf. IOCV, Univ. Fla. Press, Gainesville,"pp. 256-261.

Roistacher, C.N., Calavan, E.C., Nauer, E.M. and Reuther, W. 1972.Virus free Meyer lemon trees. Citrograph 57: 250, 270-271.

Roistacher, C.N., Blue, R.L. and Calavan, E.C. 1973.A new test for cachexia. Citrograph 58:261-262.

Roistacher, C. N., and Calavan, E.C. l974. Survival of exocortis virus on contaminated blades. Citrograph 59:250-252.

Roistacher, C.N., Blue, R.L. Nauer, E.M. and Calavan, E.C. 1974.Suppression of tristeza virus symptoms in Mexican lime seedlings grown at warm temperatures. Plant Dis. Reptr. 58: 757-760.

Roistacher, C.N., Navarro, L. and Murashige, T. 1976. Recovery of citrus selections free of several viruses, exocortis viroid, and *Spiroplasma citri* by shoot tip grafting *in vitro,* In: "Proc. 7th Conf. IOCV. IOCV, Riverside", pp. 186-193.

Roistacher, C.N., and Kitto, S.L. 1977. Elimination of additional citrus viruses by shoot
tip grafting *in vitro.* Plant Dis. Rep. 61:594-596.

Roistacher, C. N., Calavan, E. C. and Navarro, L. 1977a.Concepts amd procedures for the importation of citrus budwood. Proc. Int. Soc. Citriculture. 1:133-136.

Roistacher, C.N., Calavan, E.C. Blue, R.L., Navarro, L. and Gonzales, R. 1977b. A new more sensitive citron indicator for detection of mild isolates of citrus exocortis viroid (CEV). Plant Dis. Reptr. 61: 135-139.

Roistacher, C.N., Nauer, E.M. and Wagner, R.C. 1980. Transmissibility of cachexia, Dweet mottle, psorosis, tatterleaf and infectious variegation viruses on knife blades and its prevention. In: "Proc. 8th Conf. IOCV. IOCV, Riverside", pp. 225-229.

Roistacher, C. N., and Bar-Joseph, M.1987. Transmission of Citrus tristeza virus (CTV) by *Aphis gossypii* and by graft transmission to and from *Passiflora* species. Phytophylactica 19: 179-182.

Roistacher, C. N., Dodds, J. A. and Bash, J. A. l987. Means of obtaining and testing strains of seedling yellows and stem pitting tristeza virus; A preliminary report. Phytophylactica 19: 199-203. (Citrus tristeza symposium, Nov. 1985 Nelspruit, S. Africa).

Roistacher, C.N., Dodds, J. A. and Bash, J. A. 1988.Cross protection against citrus tristeza seedling yellows (CTV-SY) and stem pitting (CTV-SP) viruses by protective isolates developed in greenhouse plants. In: "Proc. 10th Conf. IOCV. IOCV, Riverside", pp. 91-100.

Roistacher, C. N. and Dodds, J. A. 1993. Failure of 100 mild citrus tristeza virus isolates from California to cross protect against a challenge by severe sweet orange stem pitting isolates. In: "Proc. 12th Conf. IOCV. IOCV, Riverside", pp. 100-107.

Roistacher, C.N., Bash, J.A. and Semancik, J. S. 1993. Distinct disease Symptoms in *Poncirus trifoliata* induced by three citrus viroids from three specific groups. In: "Proc. 12th Conf. IOCV. IOCV, Riverside", pp. 173-179.

Roistacher, C.N., Canton, H. and P.S. Reddy. 1996. The economics of living with citrus diseases: Exocortis in Belize, In: "Proc. 13th Conf. IOCV., IOCV, Riverside", pp.370-375.

Ruggieri, G. 1955. Le arance impietrate. Riv Agrumic 1 (2): 65-69

Saglio, P., L'hospital, M., Leflèche, D., Dupont, G., Bové, J.M., Tully, J.G. and Frendt, E.A. 1973. *Spiroplasma citri* gen. and sp. nov.: a mycoplasma-like organism associated with 'stubborn' disease of citrus. International Journal of Systematic Bacteriology 23: 3, 191-204.

Saillard, C., Barthe, C., Renaudin, J., Bové, M. and Moreno, P. 1993. Detection of *Spiroplasma citri* by culture, ELISA, dot-blot hybridization PCR and immuno-capture PCR: an evaluation. In: "Proc. 12th Conf IOCV. IOCV, Riverside", pp. 467.

Saito, Y., Kishi K., Iwata, Y. and Tanaka, S. 1963. Purification of satsuma dwarf virus. Ann. Phytopath. Soc. Japan 28:284.

Scaramuzzi, G., Catara, A. and Cartia, G. 1968 Investigations on impietratura disease. Proc. 4th Conf IOCV, Univ. of Fla. Press, Gainesville, pp 197-200.

Schneider, H. 1954. Anatomy of bark of bud union, trunk, and roots of quick-decline-affected sweet orange trees on sour orange rootstock. Hilgardia 22:(16)567-601.

Schwarz, R.E. and McClean, A.P.D. 1969. Gum pocket, a new virus-like disease of *Poncirus trifoliata*. Plant Dis Reptr 53:337-339.

Semancik, J.S., Roistacher, C.N. and Duran-Vila, N. 1988a. A new viroid is the causal agent of citrus cachexia diseases. In: "Proc. 10th Conf IOCV. IOCV, Riverside", pp. 125-135.

Semancik, J.S., Roistacher, C.N., Rivera, Bustamante, R. and Duran Vila, N. 1988b. Citrus cachexia viroid, a new viroid of citrus: relationship to viroids of the exocortis disease complex. Journal of General Virology 69: 12, 3059-3068.

Semancik, J.S., Gumpf, D.J. and Bash, J.A. 1991. Interaction among group II viroids: A potential for protection from the cachexia disease. In: "Proc. 11th Conf IOCV IOCV, Riverside", pp.189-195.

Semancik, J.S., Gumpf, D.J. and Bash, J.A. 1992. Interference between viroids inducing exocortis and cachexia diseases of citrus. Annals of Applied Biology 121: 3, 577-583.

Skaria, M. 1993. Status of citrus tristeza virus in Texas and its potential for spread. In: "Proc. 12th Conf IOCV. IOCV, Riverside", pp. 464.

Stout, G.L. 1945. Report on statewide survey for quick decline of orange trees in California. Calif. Dept. Agr. Bull. 34: 108-115.

Stubbs, L.L. 1964. Transmission and protective inoculation with viruses of the citrus tristeza complex. Aust J Agric Res 15:752-770

Su Hong-Ji, and Tsai, Mei-Chen 1990. Distribution and detection of citrus tatterleaf virus by ELISA test with monoclonal antibodies. In "Proc. of the Asia Pacific Intern. Conf on Citriculture". (eds. Aubert, B., Tontyaporn, S. and Buangsuwon, D.), pp. 171-174.

Swingle, W.T. and Webber, H.T. 1896. The principle diseases of citrus fruits in Florida. U.S. Dept. Agr. Div. Veg. Phys. Path. Bull. 8: 42 p.

Tanaka, H. and Imada, J. 1974. *Physalis floridana*, a good production host for satsuma dwarf virus. Plant Disease Reporter 58: 603-605.

Tanaka, T. 1952. Monograph on the satsuma orange (History of the introduction of the satsuma orange into the United States). pp. 31-32.

Tanaka, S. and Kishi, K. 1963. Mechanical inoculation on leguminous plants with sap f rom Satsuma dwarf tree. Ann. Phytopathol. Soc. Jpn. 28:262-269.

Targon, L.P.N., Machado, M.A., Carvalho, S.A., Souza, A.A. and Muller G.W. 2000. Differential replication of a mild and a severe citrus tristeza virus isolate in species and varieties of citrus. In: "Proc. 14th Conf. IOCV., IOCV, Riverside", pp. 127-135.

Thornton, I.R., Emmet, R.W. and Stubbs, L.L. 1980. A further report on the grapefruit tristeza preimmunization trials at Mildura, Victoria. In: "Proc. 8th Conf. IOCV. IOCV, Riverside", pp.51-83.

Toxopeus, H. (1937) Stock-scion incompatibility in citrus and its cause. J. Pom. Hort. Sci. 14: 360-364.

Tsai, J.H., Lin, Y.H., Wang, J.J. and Lee, R.F. 2000. Recovery of orange stem pitting strains of citrus tristeza virus (CTV) following single aphid transmissions with *Toxoptera citricida* from a Florida decline isolate of CTV. Proc Fla. State Hort. Soc. 113: 75-78.

Tsuji, M., Yamato, H., Josikuri, A., Miyakawa, T. and Wakikawa K. 1989. A mother tree selection program in Hassaku citrus to control stem pitting disease (Hassaku dwarf) and its progress after 19 years. Bull. Tokushima Hort. Exp. Sta. 17:21-28.

Van Vuuren, S.P., Collins, R.P. and da Graca, J.V. 1991. The performance of exotic citrus tristeza virus isolates as preimmunizing agents for sweet orange on sour orange rootstock under natural disease pressure in South Africa. In: "Proc. 11th Conf. IOCV. IOCV, Riverside", pp.

60-63.

Van Vuuren, S,P., Collins, R.P. and da Graca, J.V. 1993. Growth and production of lime trees pre-immunized with mild citrus tristeza virus isolates. Pytophylactica 25:39-42.

Vogel, R. and Bové, J.M. 1964. Stem pitting sur bigarardier et sur oranger tarocco en Corse: Une maladie a virus. Fruits 19: 269-274.

Vogel, R. and Bové, J.M. 1976. Effect of various concave gum isolates on mandarin and sweet orange trees: Absence of correlation between reduction of growth and severity of symptom expression. In: "Proc. 7th Conf. IOCV. IOCV, Riverside", pp. 119-124.

Vogel, R. and Bové, J.M. 1980. Pollen transmission to citrus of the agent inducing cristacortis and psorosis young leaf symptoms. In: "Proc. 8th Conf. IOCV. IOCV, Riverside", pp. 188-190.

Wallace, J.M. 1945. Technique for hastening foliage symptoms of psorosis of citrus. Phytopathology 35:535-541.

Wallace, J.M. 1957. Virus-strain interference in relation to symptoms of psorosis disease of citrus. Hilgardia 27:223-245.

Wallace, J.M. 1968. Recent developments in citrus psorosis disease. In: "Proc. 4rd Conf. IOCV. Univ. Florida Press, Gainesville", pp. 1-9.

Wallace, J.M. 1978. Virus and virus like diseases. In: "The Citrus Industry, Vol. 4",(eds. Reuther, W., Calavan, E.C. and Carman, G.E.), Univ. Calif. Div. Agr. Sci., Richmond, pp. 67-184.

Wallace, J.M. and Drake, R.J. 1951. Newly discovered symptoms of quick decline and related diseases. California Citrograph 36, 136,138.

Wallace, J.M., and R.J. Drake. 1953. A virus induced vein enation in citrus. Citrus leaves 33 (2): 22,24

Wallace, J.M. and Drake, R.J. 1955. The tristeza virus in Meyer lemon. Citrus Leaves 35(1): 8-9, 23.

Wallace, J,M., Oberholzer, P.C.J., Hofmeyer, J.D.J. 1956a. Distribution of viruses of tristeza and other propagative material. Plant Dis. Reptr. 40:3-10

Wallace, J.M., Reichert, I., Bental, A. and Winocour, E. 1956b The tristeza virus in Israel. Phytopathology 46:347.

Wallace, J.M., and R.J. Drake. 1960. Woody galls on citrus associated with vein enation virus infection. Plant Dis. Reptr. 44: 580-584.

Wallace, J.M., and R.J. Drake. 1961. Induction of woody galls by wounding of citrus infected with vein enation virus. Plant Dis. Reptr. 45: 682-686.

Wallace, J.M. and Drake, R.J. 1962. Tatterleaf, a previously undescribed virus effect on citrus. Plant Dis. Reptr. 46: 211-212.

Wallace, J.M. and Drake, R.J. 1972 Studies on recovery of citrus plants from seedling yellows and the resulting protection against reinfection. In: "Proc. 5th Conf. IOCV, Univ. Fla. Press, Gainesville", pp.127-136.

Wallace, J.M. and Drake, R.J.1974. Field performance of tristeza-susceptible citrus trees carrying virus derived from plants that recovered from seedling yellows. Proceedings of the Sixth Conference of the International Organization of Citrus Virologists 67-74;

Wallace, J.M. and Drake, R.J. 1976. Progress report of studies in California on preimmunization against citrus tristeza virus in budded citrus trees. In: "Proc. 10th Conf. IOCV. IOCV, Riverside", pp.58-62.

Weathers, L.G. 1961. Response of citrus to concurrent infection with two or more unrelated viruses. In: "Proc. 2nd Conf. IOCV, Univ. of Fla. Press, Gainesville", pp. 187-196.

Webber, H.J. 1925. A comparative study of the citrus industry of South Africa. Union of South Africa Dept. of Aft. Bul. 6:1-106.

Webber, H.J. 1943. The "tristeza" disease of sour-orange rootstock. Proceedings of the American Society of Horticltural Science 43, 160-168.

Yamada, S. and Sawamura, K. 1952. Studies on the dwarf disease of satsuma orange, Citrus

unshiu Marcov. (Prelminiary report) Hort. Div. DTo.Kai-Kinki Agr. Exp. Sta. Bull. 1: 61-67.

Yamamoto, S. and Yamaguchi, A.1980. Spread of citrus mosaic through distribution of a new clone of satsuma mandarin. Proc. 10th Conf. IOCV. IOCV, Riverside. p 230-231.

Yokomi, R.K. and Damsteegt, V.D. 1991. Comparison of citrus tristeza virus transmission efficiency between *Toxoptera citricidus* and *Aphis gossypii*. Proceedings, aphid-plant inter-actions: populations to molecules. An OSU centennial event, Stillwater Oklahoma USA August 12-17, 1990. Oklahoma State University.

Yokomi, R.K., Garnsey, S.M., Lee, R.F. and Youtsey, C.O. 1991. Spread of decline-inducing isolates of citrus tristeza virus in Florida. Proc. Int. Soc. Citriculture, 1992, 2:778-780.

Zeman, V. 1931. Una enfermadad nueva en los naranjales de Corrientes. Physis 19: 410-411.

Zhang, T.M., Liang, Y. and Roistacher, C.N. 1988. Occurrence and detection of citrus tatterleaf virus (CTLV) in Huangyan, Zhejiang Province, China. Plant Disease 72:543-545.

Zhou, C., Zhao, Xueyuan and Jiang Yuanhui 1993a. Goutoucheng - A new indicator plant for citrus mosaic virus. In: "Proc. 12th Conf. IOCV. IOCV, Riverside", pp. 368-370.

Zhou Changyong, Zhao Xueyuan, Jiang Yuanhui and He Xinhua 1993b. The occurance of Sat-suma dwarf virus in China. In: "Proc. 12th Conf. IOCV. IOCV, Riverside", pp. 349-351.

Zhou Changyong, Zhou Xueyuan and Jiang Yuanhui 1996. Boat-shaped leaf symptoms of satsuma mandarin associated with citrus tristeza virus (CTV). In: "Proc 13th conf. IOCV, IOCV, Riverside", pp. 154-157.

Fungal Diseases of Fruit and Foliage of Citrus Trees[*]

L.W. Timmer[1], S.N. Mondal[1], N.A.R. Peres[2], and Alka Bhatia[1]

*[1]University of Florida, IFAS, Plant Pathology Department,
Citrus Research and Education Center, 700 Experiment Station Road,
Lake Alfred, FL, USA, 33850; [2]University of Florida, UNIEMP
Project, Instituto Biológico, São Paulo, Brazil*

Abstract : Several important diseases of the fruit and foliage of citrus trees are addressed in this chapter: Postbloom fruit drop, caused by *Colletotrichum acutatum*; Alternaria brown spot, caused by *Alternaria alternata*; scab diseases, caused by *Elsinoe fawsettii* and *E. australis*; melanose, caused by *Diaporthe citri*; and greasy spot caused by *Mycosphaerella citri*. With each disease the history, economic importance, disease cycle, epidemiology, and control are covered. Special emphasis is placed on diagnosis and identification of the diseases and pathogens. Basic methods are presented for isolation of the pathogen, culture, production of various spore forms as well as for inoculation of plants to reproduce Koch's postulates.

1. Introduction

Fungal diseases cause significant problems in the production of citrus fruits. Unlike some systemic pathogens, these fungi do not cause the decline or death of trees. Nevertheless, some reduce yield and fruit size and their effects are not always purely cosmetic. Other fungi, however, cause only external blemishes on the fruit and only need to be controlled on fruit destined for the fresh market. Fungal diseases are usually more severe in citrus areas with high rainfall and temperatures. Nevertheless, some fungi are able to infect citrus trees using only the moisture from dew and thus may be problematic even in semi-arid, winter-rainfall, citrus-producing regions.

Managing many of these diseases can be difficult. In some cases, there are cultivars that are resistant to many fungal pathogens. However, the choice of cultivars is based primarily on consumer demand and price. Thus, it may be necessary to control diseases on susceptible cultivars. Cultural practices can be manipulated to some extent to reduce the impact of these diseases. However, application of fungicides is the primary means of control for most of these diseases. Considerable research has been dedicated to evaluation of products, timing of sprays, and the development of predictive models for disease forecasting to achieve the most efficient and economical control.

In this chapter, we cover postbloom fruit drop, Alternaria brown spot, citrus scab, melanose, and greasy spot but do not address all fungal diseases. Most of those

diseases are covered elsewhere (Timmer *et al.,* 2000b). Rather, we have reviewed diseases where we have active research programs to provide readers with the fundamental information as well as the latest research findings.

2. Postbloom Fruit Drop

Postbloom fruit drop (PFD) was first described in Belize in 1979 (Fagan 1979) although the disease had been observed in that country for at least 20 years earlier. About the same time or shortly thereafter, the disease appeared in Brazil and Argentina and subsequently was reported from most of Central America, Mexico, the Caribbean islands, Florida, and elsewhere (Timmer *et al.,* 1994, 2000b). There are no reports of the disease outside of the Americas.

The disease was first attributed to *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (Fagan 1979). However, Fagan recognized two forms of the fungus, one isolated most commonly from leaves and the other from flowers. Sonoda and Pelosi (1988) described two strains of *C. gloeosporioides* from citrus trees affected by PFD, a fast-growing gray (FGG) type and a slow-growing orange (SGO) type. Agostini *et al.,* (1992) differentiated the two types morphologically and demonstrated that only the SGO strain causes PFD. The FGG and SGO strains were also differentiated by restriction fragment length polymorphisms and chromosome sizes (Liyanage *et al.,*. 1992, 1993). Using molecular characterization techniques, Brown *et al.,* (1996) demonstrated that the SGO strain was *C. acutatum* Simmonds and that the FGO strain was *C. gloeosporioides*.

The causal agent of anthracnose of Mexican or Key lime (*Citrus aurantifolia* (Christm.) Swing.) was described originally as *Gloeosporium limetticola* R.E. Clausen (Clausen 1912). Agostini *et al.* (1992) found that the causal agent of this disease closely resembled the SGO strain morphologically and that when inoculated on sweet orange (*C. sinensis* (L.) Osb.) flowers produced all of the symptoms of PFD. Brown *et al.* (1996) demonstrated that the causal agent of this disease was also *C. acutatum*. Thus, in citrus, *C. acutatum* causes PFD and lime anthracnose, whereas *C. gloeosporioides* is a common saprophyte and postharvest pathogen (Timmer *et al.,* 2000b). *Colletotrichum acutatum* does not cause postharvest anthracnose (Timmer *et al.,* 1998a). It has been suggested that PFD strains originated when lime anthracnose moved from limes to sweet oranges (Agostini *et al.,* 1992). However, molecular evidence does not support a recent origin of PFD strains from lime anthracnose types of *C. acutatum* (Peres *et al.,* unpublished).

2.1 Economic Importance

PFD has now become widespread in the humid citrus areas of the Americas (Timmer *et al.,* 1994, 2000b). It can be devastating, causing complete crop loss, in many tropical, high rainfall areas in southern Mexico, Belize, Costa Rica, and in the Caribbean. However, in the tropics, trees frequently flower again a few months later and, if the weather is drier, produce some fruit to compensate for earlier losses. Damage in subtropical areas such as Florida (USA) and São Paulo (Brazil) is more sporadic and depends on the rainfall during the bloom. In many years there is no need for control measures, but in

others, losses can be serious.

Flower infection by *C. acutatum* causes abscission of fruitlets and leave persistent calyces commonly called buttons. Yield loss is related to the number of buttons remaining on the tree (Timmer and Zitko 1995a, 1996a). However, since only a low percentage of flowers on citrus trees actually set fruit, yield loss may not be as serious as it would appear from the number of buttons on a tree. Up to 20% of the flowers can be infected without significant loss of yield (Timmer and Zitko 1992). Since the primary affect of PFD is on yield, the disease must be controlled on fruit destined for processing as well as on fruit for the fresh market.

PFD occurs on all citrus species and cultivars. However, it is most serious on citrus types with extended blooms or those that commonly flower out-of-season. In Florida, Navel and Valencia oranges are the most severely affected, whereas in Brazil,

Figure 1: Post bloom fruit drop: Lesions on petals produced by *Colletotrichum acutatum.*

Pera and Natal are the most affected sweet orange cultivars. Early orange cultivars, such as Hamlin, are more tolerant to the disease, and grapefruit (*C. paradisi* Macf.) is affected only under conditions highly favorable for the disease. Most tangerines (*C. reticulata* Blanco) and their hybrids, especially tangelos *(C. reticulata* x *C. paradisi)*, are susceptible but may not retain many of the buttons formed.

2.2 Diagnosis and Detection

PFD symptoms are relatively easy to distinguish and are diagnostic for the disease (Timmer *et al.,* 2000b). The fungus produces peach-colored to orange-brown spots on

the petals. Lesions frequently coalesce and involve the entire petal and may affect the stigma (Fig.1). Petals may become hard and dry and remain attached to the inflorescence. After infection, the calyx and the peduncles persist and the fruitlet abscises at the base. These persistent buttons are diagnostic for the disease and not produced by any other agent (Fig.2). Occasionally, the small fruitlets persist as well but never develop into a fruit. The leaves surrounding an affected inflorescence are usually small, chlorotic, and twisted suggesting involvement of a volatile hormone.

A semi-selective procedure has been developed to isolate *C. acutatum* and to differentiate it from *C. gloeosporioides* (Agostini and Timmer 1992). Addition of streptomycin and copper hydroxide to potato dextrose agar (PDA) reduces contamination. Incubation of plates for 4 days at 18°C slows the growth of *C. gloeosporioides* more than that of *C. acutatum*. One day at 27°C allows sporulation of *C. acutatum*, facilitat-

Figure 2: Post bloom fruit drop:Persistent calyces remaining after flower infection and fruit abscission. Note curled leaves with enlarged veins.

ing identification. The pathogen can be readily isolated from recently infected petals by plating directly on PDA (Fig. 3). The selective medium is needed for isolation of *C. acutatum* from leaves and other vegetative structures (Agostini and Timmer 1994).

Colletotrichum acutatum sporulates profusely on PDA in a few days at 24-27°C. For inoculation, conidia can be washed from plates, diluted to 10^5/ml, and spray inoculated onto flower clusters (Fig. 4). Flowers need to be maintained moist for about 16 h to obtain maximal infection. Symptoms usually appear in 5-6 days. Pathogenicity tests on detached flowers are not diagnostic since *C. gloeosporioides* will also produce lesions on flowers *in vitro*.

2.3 The Pathogen and the Disease Cycle

Colletotrichum acutatum produces single-celled, hyaline conidia that are oblong with a fusiform apex (Agostini *et al.,* 1992). The appressoria that are formed on vegetative tissues are pigmented and are about 6 μm long by 4-5 μm wide. *Colletotrichum acutatum* is known to form a sexual stage, *Glomerella acutata*, but that form has never been found on citrus nor has it been produced with citrus isolates (Guerber and Correll 2000).

Conidia of *C. acutatum* are produced in acervuli on the surface of symptomatic petals. During bloom, those spores are splash-dispersed to healthy flowers where they germinate and penetrate without forming appressoria. The cycle continues as long as
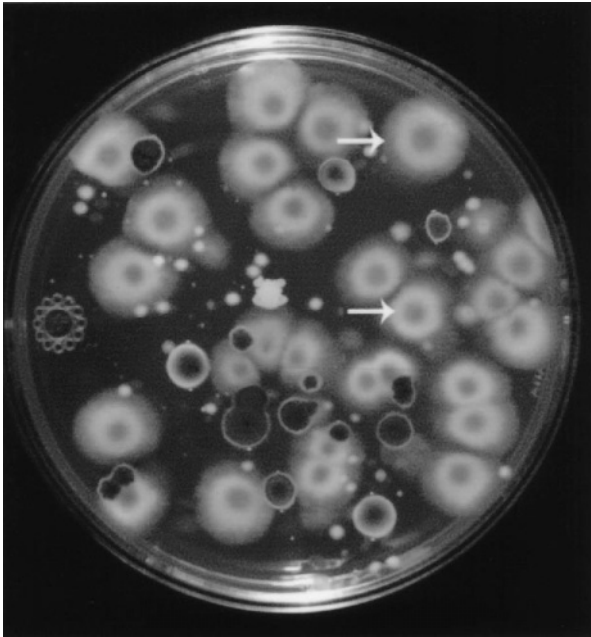
Figure 3: Fungal structures : Colonies of *Colletotrichum acutatum*, white with gray centers, on the selective medium.

petal tissue is available (Denham 1979; Timmer 1999; Timmer and Brown 2000). Conidia that are splash-dispersed to vegetative tissues germinate to form appressoria. An infection peg penetrates the surface and forms a latent infection, and the fungus may persist for long periods in this state (Agostini *et al.,* 1992; Zulfiqar *et al.,* 1996). Once the tree flowers again, nutrients washed from the petals stimulate the germination of appressoria, which then form a few conidia on primary hyphae (Zulfiqar *et al.,* 1996; Timmer and Brown 2000). These conidia are splash-dispersed to new flowers to re-initiate the disease cycle. In contrast to other species of *Colletotrichum* or *C. acutatum*

on other hosts, this pathogen does not readily colonize vegetative tissues and appears to reproduce solely on petal tissues. In contrast, the lime anthracnose strain infects and reproduces on young leaves, twigs, and fruit, but only affects limes.

2.4 Epidemiology

PFD is favored by high rainfall during the bloom period (Denham and Waller 1981). Conidia are first splash-dispersed from infected leaves to initiate infection on isolated flower clusters. The pathogen then can be splash-dispersed from those flowers through the tree and to nearby trees (Agostini *et al.,* 1993). Even if rain disperses the spores, flowers must remain moist for at least 8-10 h after a rainfall to attain even low levels of infection (Timmer and Zitko 1993). Dews alone provide enough moisture for infection
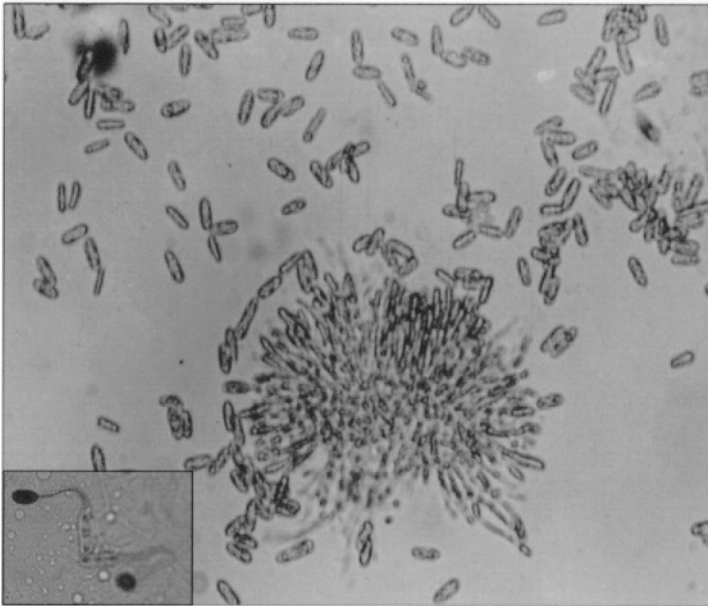
Figure 4: Fungal structures: Acervulus with conidia of *Colletotrichum acutatum*. In set:
        Conidium germinating to form a pigmented appressorium.

to occur but do not disperse spores through the tree. Thus, under most conditions, commercially important outbreaks seldom occur as a result of dew in the absence of rainfall. Occasionally, in tropical areas, where there may be large quantities of inoculum on vegetative tissues, rain is not needed to disperse spores.

The optimum temperature for growth of the fungus and symptom development is high, about 24-27°C, but *C. acutatum* grows well down to 15°C (Agostini *et al.,* 1992). However, the amount of infection that develops in a bloom period is a result of the interaction of the effect of temperature on flower development and on the disease. High temperatures speed flower development and shorten the bloom period, thus lessening

the chance that sufficient rainfall events will occur to cause damage. In contrast, low temperatures delay flower development and increase the chance that PFD will develop. Alternating high and low temperatures are most favorable for PFD.

Inoculum availability is also a key factor in the epidemiology of the disease. Multiple blooms in a single year increase the probability that high populations of *C. acutatum* will be present on leaves. Likewise, the presence of declining trees in a grove, that often flower off-season, tend to maintain high levels of inoculum. High levels of infection in one year make the chance of infection the next more likely, but the severity of PFD is more dependent on weather conditions during bloom (Timmer and Zitko 1993, 1995a). Epidemics do not usually develop quickly from the low levels of inoculum found on vegetative tissues. However, infected flowers can produce up to 10 million spores per petal, and the disease can become explosive even when a low percentage of the flowers are affected.

Some short-distance dispersal of PFD probably occurs by bees or other insects that visit flowers, by birds, or by equipment moving through groves. Grove-to-grove spread may occur on diseased flower petals that are carried on equipment, picking sacks, or even the clothing of workers. Bee hives moved from grove to grove may be responsible for some long distance spread. It is unclear how PFD became so wide-spread throughout the citrus areas of the Americas in a period of perhaps 20 years.

2.5 Disease Management

Some modifications of cultural practices are useful in reducing the severity of PFD. Use of drip or microsprinkler irrigation rather than overhead irrigation avoids wetting the canopy and minimizes disease occurrence in dry periods. Removal of declining trees prior to bloom avoids buildup of inoculum on off-season bloom. In some areas, irrigation of trees in the dry season can induce a bloom during a period less favorable to PFD.

In areas where PFD is prevalent, 1-3 applications of fungicides may be necessary during bloom. Several studies have demonstrated that benomyl is highly effective for control of PFD (Fagan 1984; Timmer and Zitko 1992, 1996a; Peres *et al.,* 2002c,b). The manufacture of benomyl has been discontinued recently, but other benzimidazole fungicides such as carbendazim and thiophanate methyl are registered for use on citrus in many areas and should provide good control. There has been some concern that the pathogen may develop resistance to benzimidazoles. However, recent studies have found no highly resistant isolates of *C. acutatum* in São Paulo (Brazil) and Florida (USA), even where benomyl was used frequently (Peres *et al.,* 2003). Other fungicides such as folpan, strobilurims, and ferbam are also effective for PFD control (Peres *et al.,* 2000b). Mixtures or rotation of products may be necessary to petals do not appreciably reduce disease or delay the epidemic. However, if applications are delayed until many flowers are affected, the disease becomes difficult to control.

The PFD model to time spray applications was developed and validated in Florida (Timmer and Zitko 1993, 1996a). Predictions were based on total rainfall and leaf wetness in the last 5 days, and the number of flowers per tree currently affected by PFD. The system was relatively effective under Florida conditions. More recently, Peres *et al.* (2002a) developed a computer-assisted fungicide application decision system (PFD-

FAD). This system considers not only the inoculum present in the grove and weather conditions during the bloom, but also disease history, varietal susceptibility, and the stage of bloom. PFD-FAD has been found to be effective in Brazil and should be widely applicable in tropical as well as subtropical areas.

3. *Alternaria* Brown Spot of Citrus

Alternaria brown spot (ABS) is a serious disease of tangerines and their hybrids. The disease was first described on Emperor mandarin in Australia in 1903 (Cobb 1903) and later appeared in Florida (Whiteside 1976), South Africa (Schutte *et al.,* 1992), Israel (Solel 1991), Turkey (Canihos *et al.,* 1997), Colombia (Castro *et al.,* 1994), and Spain (Vicent 2000) as well as Brazil and Argentina (Peres *et al.,* unpublished).

Dancy tangerines and their hybrids (Minneola tangelo, Orlando tangelo, Sunburst tangerine, Nova, and Lee tangerine) are all very susceptible to ABS. Some mandarin hybrids of unknown origin, for example, Murcotts, are also affected. Grapefruit can be affected but the disease is not a commercial problem (Timmer *et al.,* 2000b). Sweet oranges, true lemons (*C. limon* (L.) Burm.f.), and most other citrus species are not affected by brown spot. The symptoms of the disease are mostly attributable to a host-specific toxin that affects membranes and kills cells (Kohmoto *et al.,* 1979).

A similar disease called Alternaria leaf spot, affects rough lemon (*Citrus jambhiri* Lush.) and Rangpur lime (*C. limonia* Osb.) (Ruehle 1937) and is associated with a different host-specific toxin (Kohmoto *et al.,* 1979). That toxin affects the respiratory system rather than membrane. Alternaria leaf spot only affects the above two species of citrus. Another disease caused by *Alternaria* affects Mexican limes in Mexico and is called *mancha foliar de los cítricos* (Stapleton and Garza-Lopez 1988; Palm and Civerolo 1994). A host-specific toxin has not been associated with this disease. Black rot of fruit, a postharvest disorder, is also caused by *Alternaria* spp. (Timmer *et al.,* 2000b). Alternaria leaf spot of rough lemon is of little economic importance as it only affects plants in nurseries and seed blocks (Timmer *et al.,* 2000b). Only Alternaria brown spot will be considered extensively in this section.

There is still some confusion as to the taxonomy of the causal organism of this disease. *Alternaria* species are difficult to classify because there is no known sexual stage of the fungus. The pathogen was originally known as *Alternaria citri* Ellis & Pierce (Pegg 1966) and was later renamed to *A. alternata* Fr. (Keissler) pv. *citri* (Solel 1991). Recently, Simmons (1999) described ten new "morphospecies" of *Alternaria* from a worldwide collection of *Alternaria* isolates from citrus, basing his classification on the characteristics of spore chain formation and spore morphology. But molecular data from mitochondrial and beta-tubulin gene sequencing seems to indicate strongly that all these morphospecies are basically just one species and are referred to as *A. alternata* (Su *et al.,* 2001; Peever *et al.,* unpublished).

The causal agent of *mancha foliar de los citricos* has been described as *A. limicola*. That species is clearly distinguishable from other Alternarias in citrus morphologically and by molecular techniques (Palm and Civerolo 1994; Peever *et al.,* unpublished).

3.1 Economic Importance

ABS is a serious disease of susceptible mandarins and their hybrids. The disease causes blemishes on fruit that greatly diminish the value of the fruit for the fresh market. Since all of the susceptible cultivars are grown almost exclusively for the fresh market, control of the disease is essential. In addition, the disease causes fruit drop, especially if infected shortly after bloom. ABS also affects leaves and twigs producing defoliation and twig dieback. Yield losses can be substantial if conditions are favorable for disease development (Timmer and Zitko 1997).

The disease is very serious in humid areas such as Florida (USA), Colombia, and Brazil and may not be controllable in high rainfall areas. However, even in winter rainfall areas where dew is the only moisture available during most of the growing season, *Alternaria* may produce significant fruit blemishes. The disease is also important in many semi-arid growing regions in Israel, South Africa, Turkey, and Australia.

3.2 Diagnosis and Detection

The two main trademarks of an ABS infection are veinal necrosis on leaves and pock marks on mature fruit. Small circular brown-black lesions may appear on fruit and leaves as early as 24 h after infection. As the lesions develop, they are usually surrounded by a yellowing of the host tissue on the periphery of the lesion. As the lesions mature on the leaves, necrosis begins to occur as a result of fungal toxin production. The toxin is transported in the leaf veins and a characteristic necrosis along the leaf veins is indicative of ABS (Fig.5). This veinal necrosis is especially pronounced in leaves of very susceptible varieties of tangerines. The leaves eventually fall from the tree and extensive defoliation can occur. Young shoot and twig infection results in leaf distortion and twig dieback.

On fruit, the lesions become more sunken as they mature. The lesion eventually becomes walled off by a corky layer and is elevated above the surface of the fruit. The corky area falls off leaving a sunken pock mark on the fruit surface (Fig.6). The lesions and the pock marks they may leave are very undesirable on fruit intended for the fresh fruit market. Young infected fruitlets often absciss as may mature fruit that has a severe ABS infection.

The fungus can be isolated by plating small pieces of tissue from the edges of lesions, on PDA, amended with 10µg/ml of benomyl. Benomyl inhibits the growth of other ubiquitous faster growing fungi, such as *Colletotrichum* spp., that may also have colonized the host. The colony morphology of *A. alternata* on PDA can be confused with other dark-spored fungi. To confirm the identification, the fungus can be induced to sporulate by placing chopped up pieces of the colony onto PDA on nutritionally poor media such as cornmeal agar or a combination of mostly technical agar with 5% potato dextrose broth. After about 3-5 days of incubation at 25°C and alternating 12 h of light and dark, chains of dark multi-celled spores can be seen arising from the chopped up pieces of agar when observed under a dissecting microscope. These chains of spores confirm presence of *Alternaria* as other similar-looking fungi do not produce spores in a catenulate fashion (Fig.7).

3.3 Pathogen and the Disease Cycle

Alternaria alternata is a typical small-spored species of *Alternaria*. Conidia are multicellular, pigmented, tapered, with beaks of short, but varying length. Conidia vary greatly in size but are typically 25 to 40 μm in length and 15 to 22 μm in width. Spores are produced in branched or unbranched chains. Branching patterns have been used to separate species of *Alternaria* on citrus (Simmons 1999), but these criteria do not
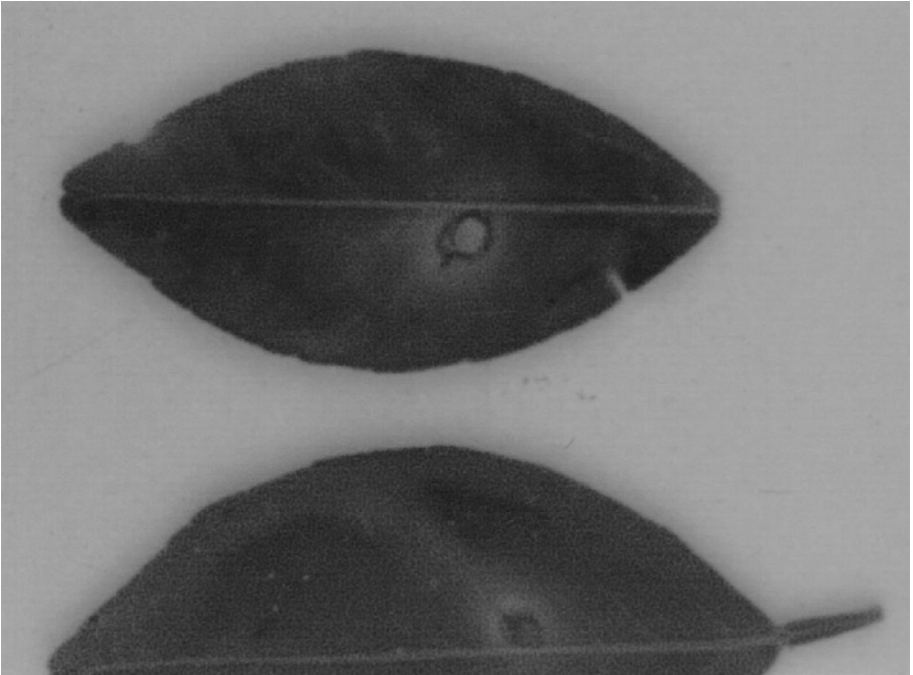
Figure 5: Foliar symptoms: *Alternaria* brown spot on Sunburst tangerine leaves. Note the chlorotic haloes

correspond well with molecular data (Peever *et al.,* 2003).

    Alternaria alternata does not have a sexual stage and hence, the disease cycle is relatively simple. Conidia produced on the host surface are wind-dispersed. These germinate and infect the plant producing lesions on fruit, leaves, and twigs. Once the lesions mature, conidia are formed once again on the surface of symptomatic tissue (Timmer 1999).

3.4 Epidemiology

The fungus survives primarily on mature leaves and twigs in the citrus tree. The spores of the fungus are hardy and can survive long periods of unfavorable conditions. Conidia are produced on lesions on mature leaves when the relative humidity is high. Rainfall events or changes in relative humidity trigger the fungus to release spores (Timmer *et al.,* 1998b). The spores are mostly wind-dispersed. Infection of fruit and leaves occurs when a spore lands on the plant surface and germinates. Symptoms can appear as early as 24 h (Canihos *et al.,* 1999). A single spore can produce many germ tubes. Young leaves, twigs, and fruitlets are very susceptible to infection. Optimum conditions for infection are warm temperatures (20-29°C) and 8-10 h of continuous leaf wetness (Canihos
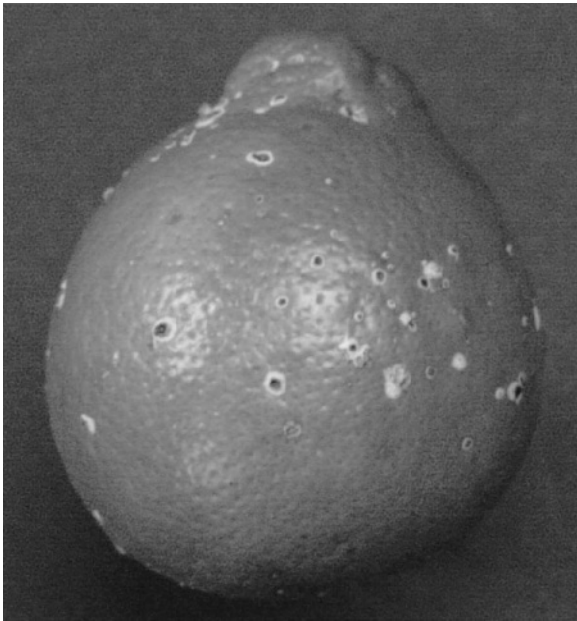
Figure 6: Fruit symptoms: Raised, corky lesions of *Alternaria* brown spot on a Minneola tangelo fruit.

et al., 1999; Timmer *et al.,* 2000a). ABS is polycyclic in nature as the fungus grows on the infected plant tissue and produces successive crops of spores throughout the growing season. Leaves are susceptible to infection until they are fully expanded and mature. Fruit usually becomes resistant to infection by about mid-summer in most areas (Timmer *et al.,* 2000b).

3.5 Disease Management

A combination of cultural and chemical control methods provide the best means to

control ABS (Timmer and Peever 1997). Efforts should be made to maintain disease-free nursery stock in the nursery to ensure that young trees, when planted out in the field, are disease-free (Timmer 2000b). This can be most easily accomplished by growing susceptible varieties in the greenhouse with subterranean irrigation to maintain leaves dry at all times. In the field, wider row and tree spacings provide better air circulation and more rapid drying of foliage. Use of overhead irrigation is not recommended. Excessive use of nitrogenous fertilizers and water may promote vigorous vegetative
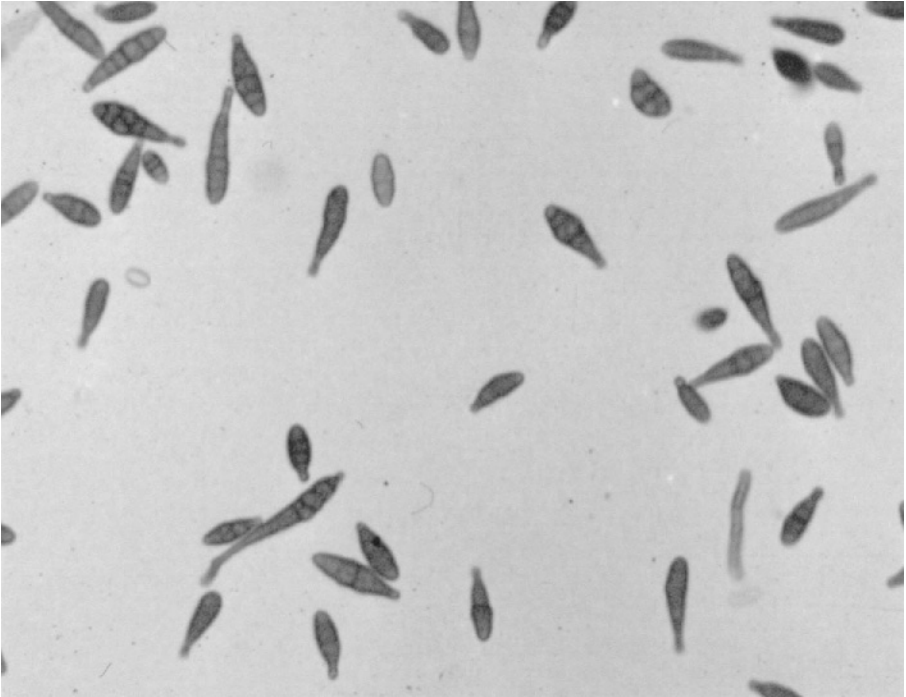
Figure 7: Fungal structures : Conidia of *Alternaria alternata*. "Reproduced from the Compendium of Citrus Diseases with permissions from APS Press, Inc."

growth that is favorable for disease development (Timmer, 1999). Planting susceptible cultivars in low-lying areas prone to fogs and poor air circulation is not recommended.

Fungicide applications are central to the control of this disease and especially to obtain blemish-free fruit. Copper fungicides, iprodione, and strobilurin fungicides work well to control this disease (Timmer and Zitko 1997). Frequency of sprays can range from as few as 3 to as many as 10 or more per growing season, depending on susceptibility of the cultivar, the disease history of the grove, and the weather conditions.

Yields of marketable fresh fruit can be very low in moderate to highly infested areas in Florida.

In humid areas, the first spray is applied when the spring growth flush is one-fourth expanded. Another spray may be required when the leaves mature. The next spray is applied at petal fall, and every 10-21 days thereafter until mid-summer (Timmer, 2002). Applications of copper fungicides may cause stippling and blackening of scars on fruit, especially when the weather is hot and dry. Many fungi have developed resistance to strobilurin fungicides when they are used frequently. The detailed relationships between environmental factors and ABS severity on citrus in Florida have been studied and have resulted in the development of the Alter-Rater model for timing fungicide sprays (Timmer *et al,*. 2000a). The model is based on the occurrence of rainfall events, the duration of leaf wetness, and the average daily temperature. It has been evaluated in the field over 2 years and has proved quite successful in reducing disease severity, often with fewer sprays than used in the common grower schedule (Bhatia and Timmer, 2003).

4. Citrus Scab Diseases

Several scab diseases of citrus have been described (Timmer 2000b). Citrus scab, formerly referred to as sour orange scab, is caused by *Elsinoe fawcettii* Bitancourt & Jenkins whose asexual stage is *Sphaceloma fawcettii* Jenkins (Bitancourt and Jenkins 1936; Timmer 2000b). In Florida, Whiteside (1978) described two biotypes of citrus scab based on host range. One type attacks all of the citrus species and cultivars that are normally susceptible to scab, whereas the other does not infect sour orange (*Citrus aurantium* L.), Temple tangor (*C. reticulata* x *C. sinensis*), or sweet orange fruit. Later, Timmer *et al.* (1996) designated these as the Florida Broad Host Range (FBHR) and Florida Narrow Host Range (FNHR) pathotypes (Fig.8). Two additional pathotypes of *E. fawcettii* were described by the same authors the "Tryon's" and the "Lemon" pathotypes. Sweet orange scab is caused by *Elsinoe australis* Bitancourt & Jenkins as the asexual stage (Bitancourt and Jenkins 1937; Timmer 2000b). This scab species affects only fruit and not any vegetative tissues of sweet orange, mandarins, and possibly other species of citrus. Hyun *et al.* (2001) described a new form of citrus scab on natsudaidai (*C. natsudaidai* Hayata) in Korea. Like sweet orange scab, it only affects fruit, but can be distinguished from *E. australis* by molecular techniques.Jenkins (1936) described a distinct form of scab from Australia called Tryon's scab caused by *Sphaceloma fawcettii* var. *scabiosa* (McAlp & Tryon) Jenkins. That variety was separated from citrus scab primarily on the basis of conidial size. However, Timmer *et al.* (1996) found no differences in spore size among Australian isolates of the scab fungus and were unable to separate it from other *E. fawcettii* isolates using molecular techniques or morphology. This variety is now considered a pathotype of *E. fawcettii* (Tan *et al.,* 1996; Timmer *et al.,* 1996).

4.1 Economic Importance

Scab diseases occur in most citrus growing areas of the world. Citrus scab is very

widespread and occurs nearly everywhere. However, not all pathotypes occur in all areas. The 'Lemon' and 'Tryon's' pathotypes predominate in Australia, but others may occur (Timmer and Broadbent 1995; Tan *et al.,* 1996; Timmer *et al.,* 1996). The FBHR and FNHR pathotypes occur in Argentina, but scab does not seem to be a commercial problem on grapefruit as it is in Florida. Many more pathotypes of citrus scab may occur locally and await further investigation and description.

Sweet orange scab occurs primarily in southern South America. It is widespread in Brazil, Argentina, and Paraguay (Timmer, 2000b). Although scab occurs on

Figure 8: Fruit symptoms: Raised pustules of citrus scab on a Temple tangor.

sweet orange in Uruguay, Diaz *et al.* (1992) recovered only the FBHR pathotype which also is capable of infecting sweet orange. Scab has also been described on sweet orange in India (Palanisivami *et al.,* 1993). However, it is unclear as to whether this is attributable to *E. australis* or a sweet orange-infecting pathotype of *E. fawcettii.*

Scab primarily affects the external appearance of the fruit. Thus, it is a serious problem in the production of fruit for the fresh market. However, this disease is seldom a problem in winter rainfall areas where much of the fruit for the fresh market is

produced. If severe, scab can reduce fruit size, but it is seldom that the disease warrants control on fruit for processing.

4.2 Diagnosis and Detection

Scab diseases of citrus are relatively easily distinguished from other diseases on the basis of symptoms alone. On many species and cultivars, scab produces raised, warty pustules on the leaves and fruit (Fig.9). Newly formed lesions are slightly raised and pink to light brown, but they turn dark brown to gray with age. Lesions of citrus scab on grapefruit are raised when fruit is young, but are quite flat and may resemble windscar on mature fruit. Likewise, lesions of sweet orange scab on oranges and tangerines and those of the Australian pathotypes on lemons are quite flat. However, it is impossible to
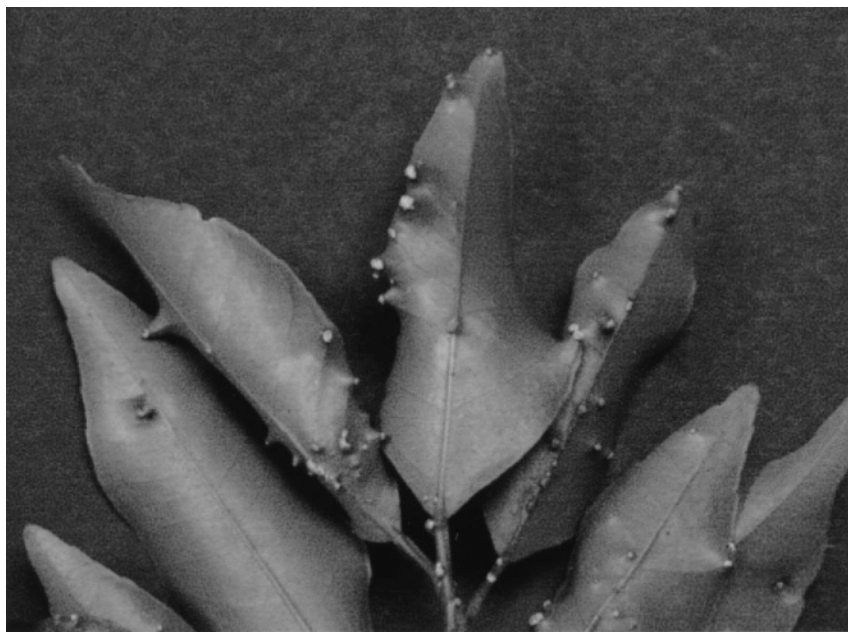
Figure 9: Foliar symptoms: Raised pustules of citrus scab on Temple tangor.

identify the type of scab based on symptoms alone. Pustules of citrus scab may form sharply pointed raised areas or more rounded pustules similar to those on fruit. Leaf infection often results in considerable leaf distortion.

One means to confirm identifications is to isolate the causal fungus. *Elsinoe* spp. are very slow-growing and form characteristic, raised colonies on agar (Fig.10). A procedure for isolation has been described along with a semi-selective medium (Whiteside 1986). Once the fungus has been obtained in pure culture, conidia can be produced by crushing pieces of the colony in Fries medium, incubating for 2 days, then washing the resultant microcolonies, and incubating them an additional 24 h in sterile

lake water (Whiteside 1975a). Currently, the only means to determine whether an isolate is *E. fawcettii* or *E. australis* is to produce conidia and inoculate leaves of rough lemon and sweet orange fruit. All known pathotypes of *E. fawcettii* infect rough lemon leaves, but *E. australis* does not infect leaves of any citrus species. Lack of infection of rough lemon and production of symptoms on sweet orange fruit confirms *E. australis*.

At times, it may be desirable to determine the pathotype of *E. fawcettii*. The differential host range to identify pathotypes is given in Table 1. Cleopatra mandarin is rarely, if ever, infected under field conditions, but it is affected by some pathotypes if inoculated experimentally and is useful as a differential host.

4.3 The Pathogen and the Disease Cycle

Elsinoe spp. produce small, hyaline conidia that are rather non-descript and of little
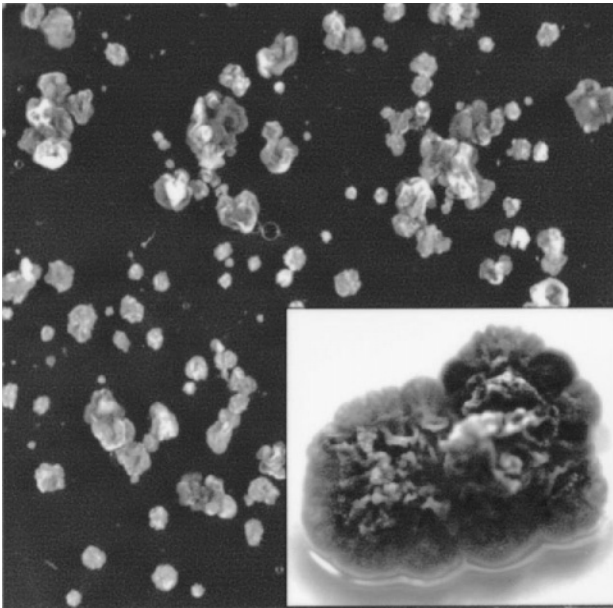
Figure 10: Fungal structures : Colonies of *Elsinoe fawcettii* on the selective medium. In set : Three-week-old colony on potato dextrose agar."Reproduced from the Compen dium of Citrus Diseases with permissions from APS Press, Inc."

taxonomic value. Conidia are produced on acervuli on the surface of lesions on leaves or fruit. Brief periods of wetting stimulate formation of conidia and infection occurs very rapidly (Whiteside 1975a; Timmer 1999). After infection, the symptoms appear in 5-7 days and acervuli form shortly thereafter. Larger, spindle-shaped conidia form on lesions produced by *E. fawcettii* after dews, but have never been reported with *E. australis*.

The sexual stages of both species of *Elsinoe* have been reported in Brazil

(Bitancourt and Jenkins 1936, 1937), but are not considered important in the disease cycle. Only a single specimen of the teleomorph of *E. fawcettii* exists and it may be an immature stage of *E. australis.* It was found on Satsuma mandarin (*C. unshiu* (Macf.) Marc.) which is susceptible to *E. fawcettii* (Timmer *et al.,* 1996). Since *E. australis* only forms conidia on fruit, the source of inoculum for early maturing cultivars is problematic. Harvesting of fruit should remove most of the inoculum prior to formation of young fruit. Nevertheless, scab occurs on early and late-maturing cultivars. Thus, sexual stage of *E. australis* may be more important the disease cycle than has been reported.

4.4 Epidemiology

Conidia of *Elsinoe* spp. are dispersed primarily by rain-splash dispersed. Scab does not spread very rapidly and most of the inoculum appears to originate within the same tree. The spindle-shaped conidia of *E. fawcettii* are capable of spreading greater distances and can be wind-borne (Whiteside 1975a). Spread of citrus scab is extremely

Table 1: Differential host range for differentiation of pathotypes of *Elsinoe fawcettii* and differentiation of *E. australis.*

Species	Pathotype	Rough Lemon	Sour orange	Cleopatra mandarin	Grape fruit	Sweet orange fruit
E. fawcettii	FBHR[z]	+	+	+	+	+
E. fawcettii	FNHR[z]	+	—	+	+	—
E. fawcettii	Tryon's	+	—	+	—	—
E. fawcettii	Lemon	+	—	—	—	—
E. australis	—	—	—	—	—	+

slow, and healthy trees adjacent to infected trees may remain disease-free for several years. Most of the spread of citrus scab probably occurs on nursery trees. Budwood and/or rootstock seedlings may carry the disease and the close proximity of trees in nursery promotes spread. Overhead irrigation often contributes to the spread of spores within the nursery.

The epidemiology of sweet orange scab is largely unknown. Conidia produced on acervuli on fruit are dispersed by water-splash to other fruit within trees or to adjacent trees (Timmer 2000b). Since there is apparently no leaf infection, we presume that the nursery spread that occurs with citrus scab does not occur with sweet orange scab. This situation again suggests a potential important role for airborne ascospores in the epidemiology of sweet orange scab.

4.5 Disease Management

Since citrus scab spreads so slowly in the field, there are opportunities to avoid the disease completely. If buds are selected from disease-free sources and propagated on

seedlings free of scab, they can be maintained in a healthy condition. If scab susceptible species and cultivars are grown in the greenhouse with sub-irrigation, the foliage remains dry and scab-free. If nursery trees are planted out at any reasonable distance from sources of inoculum, they should remain healthy indefinitely.

Use of undertree irrigation in groves can also minimize scab severity. Since the wetting periods required for scab are so short, even brief irrigations increase disease. If disease was severe the previous season, inoculum can be reduced by hedging and topping or selective hand-pruning of affected shoots. Harvest of affected fruit prior to bloom can also decrease inoculum for upcoming season.

Once citrus scab is established in a grove, there is little alternative to the use of fungicides in humid areas. Leaves and fruit are susceptible to citrus scab only when they are very young. Leaves become resistant when they are half-expanded and fruit is no longer susceptible 6-8 weeks after petal fall. Citrus scab in Florida can usually be controlled by three fungicide applications. The first is made when the spring flush is about $1/4^{th}$ expanded to protect new leaves and avoid the build-up of inoculum on leaves before bloom. The second spray is made at petal fall and the third about 3 weeks later.

Less is known about timing of sprays for control of sweet orange scab. However, one application at petal fall and one 3 weeks later appear to provide good control (Prates *et al.,* 1995).

Many fungicides are effective for control of scab diseases (Timmer and Zitko 1997, 1999; Zitko and Timmer 1997, 1998; Timmer and Bushong 2000; Agostini *et al.,* 2002). Copper fungicides are widely used because they are inexpensive, but they are not highly effective. Dithiocarbamates such as ferbam and ziram, triazoles such as fenbuconazole and difenoconazole, dithianon, benzimidazoles such as benomyl and thiophanate methyl as well as all of the newer strobilurin fungicides are all highly effective for scab control. Many of these fungicides are able to reduce sporulation on scab lesions and have post-infectional activity (Bushong and Timmer 2000). The wide range of products available allows the rotation of products to avoid resistance. Products can be selected that also control other diseases at appropriate times.

5. Melanose

Melanose was first noted in Florida by Swingle and Webber (1896), but the cause was not determined until the work of Fawcett (1912) and Floyd and Stevens (1912). *Phomopsis citri* H. Fawcett non (Sacc.) Traverso & Spessa was found to produce melanose lesions, but the fungus could not be reisolated from the symptoms produced. Wolf (1926) found the sexual stage of the fungus on decaying citrus twigs and named it *Diaporthe citri* F.A. Wolf. Ruehle and Kuntz (1940) demonstrated that both conidia of *P. citri* and ascospores of *D. citri* were capable of producing symptoms of melanose.

Diaporthe citri causes a postharvest decay, stem-end rot, as well as melanose (Fawcett 1912). The fungus forms quiescent infections on the calyx and infects the fruit after harvest (Timmer *et al.,* 2000b). This postharvest disease will not be addressed further in this chapter.

5.1 Economic Importance

Melanose occurs in most citrus-growing regions of the world, but is only important where fruit is grown for the fresh market in humid areas. Melanose is a factor in areas with significant rainfall in the 3-4 months following petal fall (Timmer *et al.,* 2000b). The disease is of little consequence on fruit grown for processing in any area. Since the fungus reproduces on dead twigs, it is usually only important in groves older than 10 years that have significant dead wood.

All species and cultivars of citrus are affected by melanose, but grapefruit and lemons are the most susceptible. Monetary losses can be great and Timmer and Zitko (1996b) estimated that a 10% reduction in packout of grapefruit resulted in losses of 866 $US per hectare.

5.2 Diagnosis and Detection
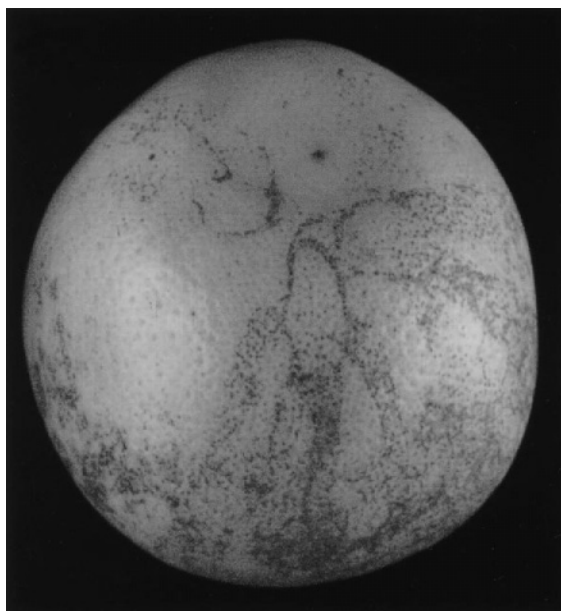
Figure 11: Fruit symptoms: Melanose on grapefruit. Note tear-stained pattern produced by water dispersal of spores on fruit.

Symptoms of melanose appear on young leaves, fruit, and twigs about 1 week after infection. Initially, lesions are small, brown, and sunken, but a periderm develops rapidly beneath infected cells and produces raised, reddish-brown lesions (Timmer *et al.,* 2000b). Symptoms consist of scattered, reddish-brown, raised spots that may form a pattern of water droplets or tear-stain where spores have been washed down the fruit (Fig.11). If infection is severe on young fruit, lesions coalesce to cover extensive areas and crack to form the "mudcake" melanose symptoms. Symptoms on leaves and twigs

are similar but on leaves, lesions are often surrounded by a chlorotic halo (Fig.12). Severe infection of leaves may cause distortion and twisting of the lamina, and even leaf drop. Badly affected twigs may die back.

Melanose symptoms on fruit may be confused with rust mite damage, but the latter produces confluent russetted areas that are not raised (Timmer *et al.,* 2000b). Melanose infection that occurs late in the susceptible period produces flatter, gray-black lesions which can easily be confused with scab pustules on grapefruit. Stippling produced by application of copper fungicides can also be confused with melanose lesions (Timmer *et al.,* 2000b). Copper phytotoxicity produces lesions that are black rather than reddish-brown and tend to be more superficial. The pattern of lesions is the most useful characteristic for differentiation of the two. Melanose symptoms usually occur in patterns of water droplets and are most severe on interior fruit. Copper damage
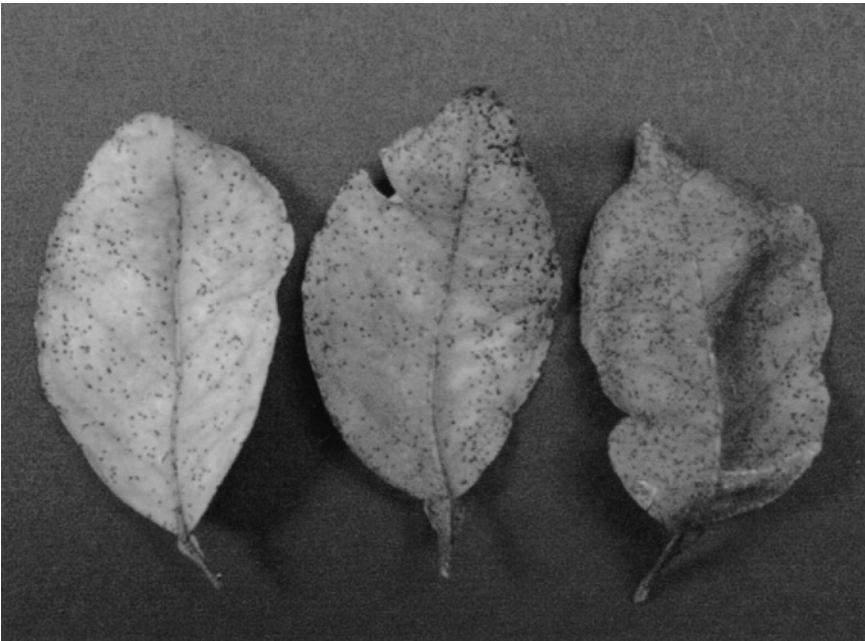
Figure 12: Foliar symptoms: Melanose lesions on grapefruit leaves

usually occurs in a spray pattern on the exterior surface of outside fruit.

Diaporthe citri is not readily isolated from symptomatic fruit, leaves, or twigs, but it is possible to isolate from recently infected tissues. This pathogen can be easily isolated from fruit affected by stem-end rot by plating interior portions of decayed tissues on any of the common media. The fungus grows relatively slowly and forms a fan-shaped colony that is chalky white in color.

The pathogen can also be isolated by incubating dead twigs, collected in the grove, with pycnidia in a humid chamber for 24 h. Tendrils or spore masses exuding from

the pycnidia can be picked from the surface aseptically and transferred to culture media. Pycnidia can be produced in the laboratory by collecting twigs with melanose symptoms, drying them thoroughly, soaking them 3-4 h per day, 3 days per week, for 8-10 weeks (Mondal and Timmer unpublished). Once pycnidia are mature, twigs are incubated for 24 h in a humid chamber. Conidia can be transferred to cultural media for isolation or to a microspore slide for identification using characteristics of the alpha and beta conidia.

Perithecia usually form on twigs and small branches that have been dead for long periods of time. Perithecia of *D. citri* have been produced in culture by combining different isolates of *P. citri* (Yamato 1976).

Conidia for inoculation can be produced on sterilized twigs in tubes (Whiteside 1977a), on twigs embedded in agar plates, or directly on PDA. *Phomopsis citri* will produce pycnidia on PDA if recently isolated cultures are used or if cultures are initiated by streaking conidia directly on plates. Alternatively, surface sterilized citrus twigs can be embedded in PDA and inoculated with *P. citri*. Pycnidial production usually requires 30-45 days at 25°C.

To conduct inoculations, conidia exuding from pycnidia are collected with a sterile needle, suspended in sterile, distilled water, and washed 2-3 times to remove the mucilage that inhibits germination (Whiteside 1977a). Conidial concentration is adjusted to 10^5/ml and orange juice is added to a final concentration of 1% to provide nutrients for germination. Leaves are sprayed with the conidial suspension and maintained moist for at least 24 h. Fruit inoculations are best conducted in the field on young, melanose-free trees about 1-2 months after petal fall. Fruit are sprayed with the conidial suspension and wrapped with moist cotton. Alternatively, pycnidia-bearing twigs can be placed above the fruit and the fruit observed for infection after a rainfall event or irrigation (Whiteside 1977a). Fruit should not be covered with plastic bags, since the ethylene generated naturally often causes fruit abscission. The laboratory inoculation procedure described by Whiteside (1977a) can also be used, but appropriate controls and standards must be used to interpret results correctly. Symptoms usually develop in about 1 week with all procedures.

5.3 Pathogen and the Disease Cycle

The pycnidia of *P. citri* measure 200-450 μm and produce alpha and beta conidia (Wolf 1926; Yamato 1971). Alpha conidia are 2.5-4 x 5-9 μm, hyaline and non-septate. Beta conidia, also called stylospores, are 0.7-1.5 μm wide x 20-30 μm long, filiform and hooked. Beta conidia do not germinate on cultural media and are produced mostly in older pycnidia. Perithecia of *D. citri* are 125-160 μm in diameter with tapering beaks 200-800 μm long and develop singly or in groups. Ascospores are 3.2-4.5 x 11.5-14.2μm, hyaline, two-celled and each cell contains two oil droplets.

Pycnidia are produced on twigs that have recently died (Fig.13). Once twigs are substantially decayed, pycnidia no longer produce spores. Virtually all of the inoculum comes from within the same tree. Spores are never produced on affected leaves or fruit. The fungus persists in living twigs and colonizes the twigs and grows saprophytically when they die. Thus, the main cycle is from twigs to twigs and the infection of fruit,

which is of the most economic importance, is of no significance in reproduction of the fungus (Timmer 1999). Perithecia and ascospores, although produced commonly in citrus groves, represent a relatively small portion of the inoculum. However, ascospores are airborne and serve to spread the pathogen to newly established groves (Fig. 14).

5.4 Epidemiology

Conidia of *P. citri* are water-splash dispersed from dead twigs to susceptible young leaves, twigs, and fruit. Leaves and twigs are susceptible to infection until they are mature. Fruit are susceptible as long as they are growing rapidly. That period may be as short as 3 months where temperatures are high such as in Florida (Whiteside 1980), slightly longer in Texas (Timmer and Fucik 1976; Timmer *et al.,* 1979), and up to 5
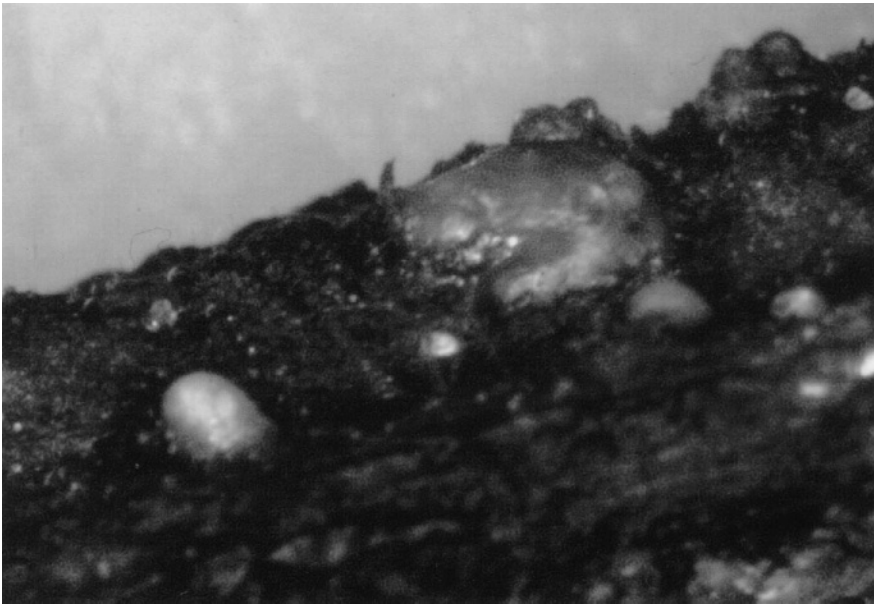
Figure 13: Fungal structures: Dead citrus twig with pycnidia of *Phomopsis citri*. Conidia oozing from pycnidia.

months in Japan (Timmer *et al.,* 2000b) where temperatures during the growing season are much lower.

Long periods of continuous wetting are required for severe damage from melanose to occur. Minimal amounts of infection occur with 4-8 h of leaf wetness at optimal temperatures, but 24 h of wetness is often required to maximize the amount of infection (Fawcett 1921; Homma and Yamada 1969; Kuramoto and Yamada 1975; Ushiyama 1976; Agostini *et al.,* 2003). Optimum temperatures for infection and disease development are 24-28°C.

When temperatures are outside of the optimal range, much longer wetting peri-

ods are needed to maximize infection under field conditions, little melanose results from single rain events, but infection may be severe when rains continue for 2-3 days (Timmer and Fucik 1976; Timmer *et al.,* 1979; Whiteside 1980). Most infection in Florida and Texas occurs in early summer when extensive rains sometimes occur before the fruit is large enough to be resistant. Melanose is less severe in humid, tropical areas, possibly because dead wood decays before many pycnidia are formed. Melanose is most severe where large numbers of twigs have been killed by freeze damage or trees are declining for any number of causes.

5.5 Disease Management

Since most of the inoculum for melanose is produced on dead twigs, pruning of dead
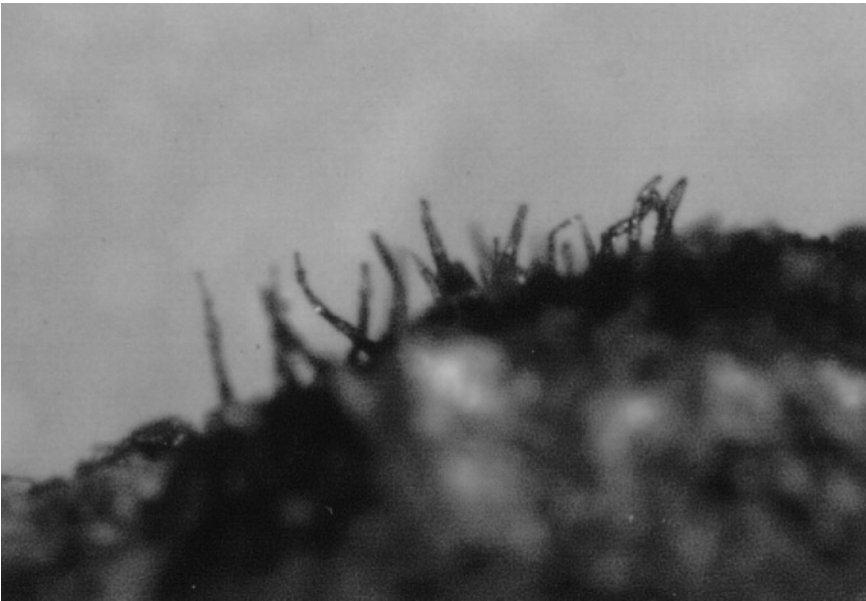
Figure 14: Fungal structures: Perithecia of *Diaporthe citri* on a dead citrus twig; note long beaks.

wood would seem to be a practical means to reduce disease severity. Stevens (1915) substantially reduced diseased severity by careful pruning to remove small twigs and moderately reduced severity by commercial pruning. However, the fact that most of the inoculum is produced on recently killed, small twigs makes hand pruning a very expensive control measure. Mechanical topping can remove much dead wood from the tree and may be a useful measure, especially in old grapefruit or lemon groves. Often fruit in old groves is so severely affected that it is impractical to try to control the disease. Fruit for the fresh market is often produced most economically on trees less than 15 years old.

In practice, fungicide sprays offer the most practical means to control the disease. Copper fungicides are still the most effective and most economical for melanose control (Timmer 1974; Whiteside 1975b; Timmer 2002). Strobilurins and some other products control the disease but often do not have long residual effects or are expensive.

Copper products have some negative aspects – metallic copper can accumulate in soil to phytotoxic levels if used over many years (Alva and Graham 1991); sprays applied during hot weather or in combination with acidic products or oils can cause stippling damage on fruit (Timmer *et al.,* 2000b; Timmer 2002), and they may have detrimental effects on non-target organisms. Copper fungicides are strictly protective and have no effect on inoculum, are not redistributed, and are not systemic. To achieve maximum effect, copper products are best applied frequently at low rates (Timmer and Zitko 1996b). The best timing of sprays must be adjusted for the local climatic conditions. Other products may be substituted for copper fungicides where the risk of phytotoxicity is high or non-target effects are likely (Timmer 2002).

6. Greasy Spot

Greasy spot was first observed in Florida by Fawcett (1915), who attributed it to an unknown pathogen or to poor nutrition. Greasy spot became a serious concern for growers in the 1940s because of the severe defoliation it caused. For many years, the problem was thought to be caused by rust mite (*Phyllocoptruta oleivora* (Ashmead) because sulfur and other miticides reduced disease severity (Thompson 1948). Thompson *et al.,* (1955) failed to reproduce greasy spot symptoms by infestation of potted grapefruit seedlings with rust mite. Tanaka and Yamada (1952) in Japan isolated a fungus from greasy spot lesions, demonstrated that it was pathogenic to citrus, and identified it as a *Mycosphaerella* sp. with a *Cercospora* sp. as the asexual stage. Fisher (1961) described the disease in Florida as a fungal disease and demonstrated that it was controlled by copper fungicides. Fisher (1961) found a *Cercospora*-like fungus associated with the disease and described a new species, *Cercospora citri-grisea*. Whiteside (1970a) found the sexual stage of the fungus in decomposing leaf litter and described the greasy spot pathogen as *Mycosphaerella citri*. The asexual stage was re-described as a *Stenella* sp. (Whiteside 1972a). Mondal and Timmer (unpublished) found that *M. citri* is a heterothallic fungus and that two mating types are required for sexual reproduction.

Other diseases similar to greasy spot have been described in various citrus areas and are mostly caused by other species of *Mycosphaerella* (Timmer *et al.,* 2000b). Yamada (1956) described the fungus in Japan as *M. horii* Hara. However, Ieki (1986) described the causal agent of greasy spot in Okinawa as *M. citri*. A disease called pseudo greasy spot has been described in Japan and attributed to a yeast, *Sporobolomyces* sp. (Koizumi 1986).

Greasy spot diseases also occur in Australia (Wellings 1981) and in southern South America (Timmer *et al.,* 2000b), but the etiology of these diseases has not been thoroughly investigated.

6.1 Distribution and Economic Importance

Greasy spot is widespread in the Caribbean Basin including Florida, Texas, Mexico, Central America, northern South America, and all of the islands (Timmer *et al.,* 2000b). It causes serious defoliation accompanied by yield loss (Whiteside 1977b) in all areas where summer rainfall is high. In addition to defoliation, *Mycosphaerella* causes rind blotch on fruit. Rind blotch produces external blemishes especially on grapefruit and reduces its acceptance for the fresh market (Whiteside 1970b, 1972b). Greasy spot occurs in Argentina (Timmer *et al.,* 2000b) but is not a serious economic problem. This disease probably occurs in other countries in southern South America, but there are no confirmed reports. In most areas it is of little economic importance. Greasy spot also occurs in Australia, but most citrus is grown in arid or semi-arid areas and the disease is of minor importance.

Yield loss to greasy spot in Florida can be up to 45% on grapefruit and 25% on sweet orange (Whiteside 1977b). Generally, greasy spot is most severe on lemons, grapefruit, and early oranges. Valencia oranges and most tangerines are tolerant to the disease (Timmer *et al.,* 2000b). Rind blotch is a very important problem on fresh grapefruit. If the disease is moderate to severe, the fruit is not acceptable for the fresh market. Rind blotch is not usually a significant problem on other types of citrus.

6.2 Diagnosis and Detection

6.2.1 Symptoms

Greasy spot can usually be identified by characteristic lesions on the underside of the leaf (Timmer *et al.,* 2000b). Spots are tan to light brown turning black with age and have irregular margins and an oily appearance. Chlorotic areas usually appear on the upper surface of an infected leaf. Lesions on lemons and grapefruit are generally light in color, flatter, and surrounded by yellow halos (Fig.15). On sweet oranges and tangerines, lesions are dark brown to black, more raised and less chlorotic.

On fruit, *M. citri* invades stomates producing necrosis of a few cells beneath the stomata (Whiteside 1970b, 1972b). The rind blotch symptom is composed of hundreds of minute, necrotic, black specks. Chlorophyll is retained in infected areas giving the fruit a greenish cast encompassing the numerous black dots. Since stomates do not occur over oil glands, no necrotic dots occur there. The symptom is sometimes difficult to distinguish from damage caused by rust mite and may occasionally be confused with small melanose pustules (Fig.16).

6.2.2 Isolation of the Pathogen

Mycosphaerella citri is a slow-growing fungus that is difficult to isolate, but isolation of the fungus provides one of the best means to confirm its presence in an area (Whiteside 1981). To isolate from lesions, a leaf piece with lesions, about 3-4 cm long by 2-3 cm wide, is first dipped in 70% ethanol for 3-5 sec, then in 10% bleach for 10-15 min, and finally rinsed thoroughly with sterile, distilled water. The leaf piece is wrapped around

the index finger of one hand to hold it firmly in place. A sharp scalpel is used to aseptically remove the epidermis and a few cell layers exposing the infected, discolored mesophyll. Tiny bits of mesophyll are removed with a fine needle and placed on corn meal agar plates. Small dark brownish-green colonies form after 5-7 days at 24-27°C. Typical, elongate conidia, 6-50 x 2.0-3.5 μm, are produced on recently isolated *M. citri* colonies in about 1 week, but are seldom produced on colonies that have been subcultured. Both conidia and hyphae are rough-walled.

        To isolate from fruit, areas of the rind with numerous black specks are surface sterilized by wiping with 95% ethanol and flaming. Using a stereoscopic microscope, a thin layer of cells is removed aseptically with a sterile scalpel. Again tiny specks are removed with a sterile needle, transferred to agar, and allowed to grow as described above
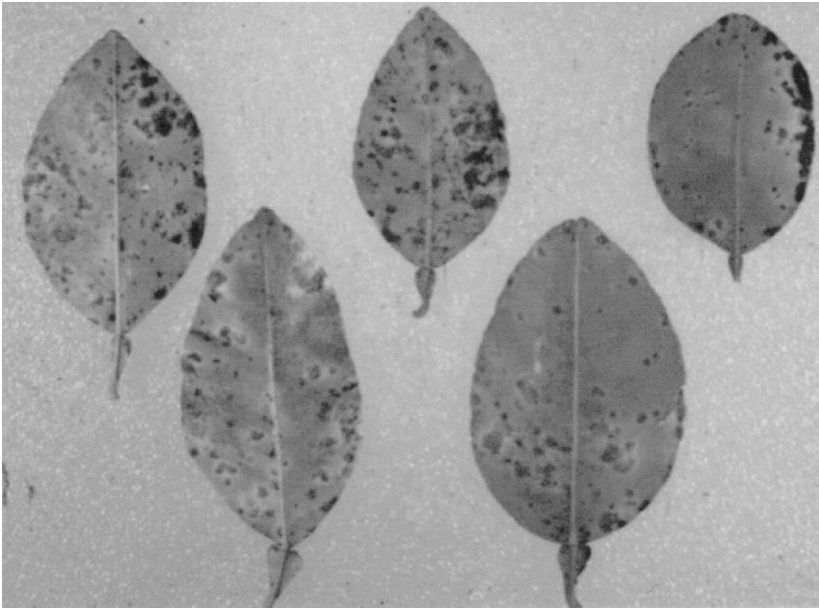
Figure 15: Foliar sypmtoms: Greasy spot lesions on grapefruit leaves.

6.2.3 Fungal Structures

The disease and the fungus can be identified from structures produced on decomposing leaves on the grove floor (Whiteside 1970a, 1981). Densely aggregrated pseudothecia, about 58-90 μm in diameter, are produced on partially decomposed leaves under the tree (Fig.17). To search for pseudothecia, leaves should be selected that are pliable and beginning to break up but which are not yet skeletonized. The aggregated groups appear as grayish spots from 3-6 mm in diameter. Spermagonia are also produced on decomposing leaves, but spermatia do not germinate in cultures. Presumably

they serve to fertilize female structures in the production of pseudothecia.

Asci and ascospores can be observed by crushing pseudothecia in a drop of stain on a microscope slide (Whiteside 1970a, 1981; Mondal and Timmer 2002). Ascospores are hyaline, tapered with single septum and no constriction at the septum and vary from about 2-3 x 6-11 µm (Fig.18). Ascospores can be collected from decaying leaves with mature pseudothecia by first soaking leaves for 30 min. Then, the leaves are attached to a Petri dish lid with the underside down using petroleum jelly. The ascospores can be ejected into a drop of liquid on a microscope slide for identification or onto agar media for isolation of single spores.

Pseudothecia can also be produced from leaves with symptoms (Whiteside 1981). Leaves are thoroughly dried and then subjected to alternate wetting and drying cycles for 3-4 weeks (Mondal and Timmer 2002). The leaves are observed for presence

Figure 16: Fruit symptoms: Greasy spot rind blotch on grapefruit

of pseudothecia as above.

6.2.4 Inoculation and Reproduction of Symptoms

In some cases, it may be necessary to inoculate plants to confirm the identity of cultures. The fungus is grown in liquid Fries solution for 10-14 days. The mycelium is chopped in a blender for 10 sec and then filtered through cheesecloth (Whiteside 1974). Inoculum is sprayed onto the underside of the leaves and the plants kept moist for 24 h. Symptoms develop slowly and require 4-6 weeks on lemon, 6 weeks on grapefruit, and 12 weeks on oranges, even at favorable temperatures.

6.3 Biology and the Disease Cycle

As mentioned previously, pseudothecia are produced on decomposing leaves on the grove floor. Pseudothecia are 58-90 μm in diameter and contain numerous asci that are 25-35 x 5.0-5.5 μm (Whiteside 1970a). Ascospores are small (2-3 x 6-11 μm) and are forcibly ejected into the air when leaves are wetted. Spores deposited on the underside of leaves germinate and the mycelium grows epiphytically. Germinated spores will survive several days of dry conditions and will be able to resume growth when moisture or high humidity returns. Honeydew from insects or other nutrients favor the development of the epiphytic mycelium.

Mycosphaerella citri grows epiphytically for some time before it penetrates through the stomata (Whiteside 1970a; Mondal and Timmer 2003a). The fungus may
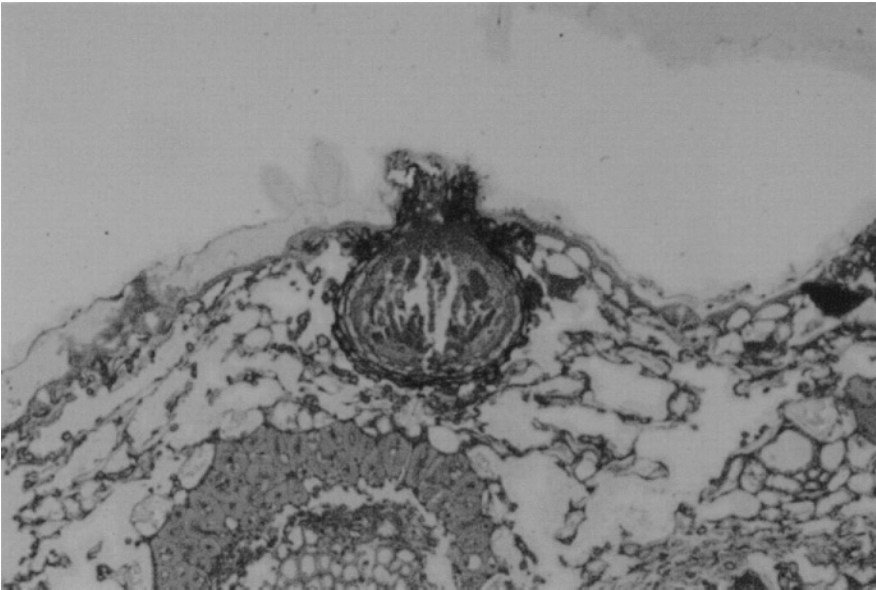
Figure 17: Fungal structures: Light micrograph of a pseudothecium of *Mycosphaerella citri* on a dead grapefruit leaf

have to grow for several days to several weeks before it is able to penetrate the leaf depending on how favorable the conditions are. Epiphytic mycelium can be observed by clearing and staining leaf discs (Skaria and Tao 1996). When invading hyphae reach the rim of the stomatal opening, the tip swells to form an appressorium. A penetration peg infects through the stomata and grows very slowly and requires 6-8 weeks to colonize the mesophyll. Symptoms may not appear for several weeks after the mesophyll first becomes colonized under field conditions.

Some time after the symptoms develop the leaf will abscise prematurely. The fungus grows saprophytically in the dead leaf and eventually forms pseudothecia again. Under laboratory conditions, maximum production of pseudothecia occurs when

leaves are wet 2 h per day, 3 days per week, or are wet 10-30 min, 4-5 times per week (Mondal and Timmer 2002). The optimum temperature for pseudothecia is 28°C. Leaves that are maintained continuously dry never produce pseudothecia and those maintained continuously wet are rotted by other organisms before pseudothecia are formed.

6.4 Epidemiology

Most of the inoculum is ascospores produced on the grove floor in decomposing leaf litter (Whiteside 1974; Mondal *et al.,* 2003a). As pointed out above, ascospores are produced following periodic wetting and drying of fallen leaves. Peaks in ascospore production usually occur several weeks after a major leaf drop depending on rain events and temperature. In Florida, the major leaf drop period is in January to early March (Whiteside 1974; Timmer *et al.,* 1995, 2000c). Formerly, when groves were irrigated
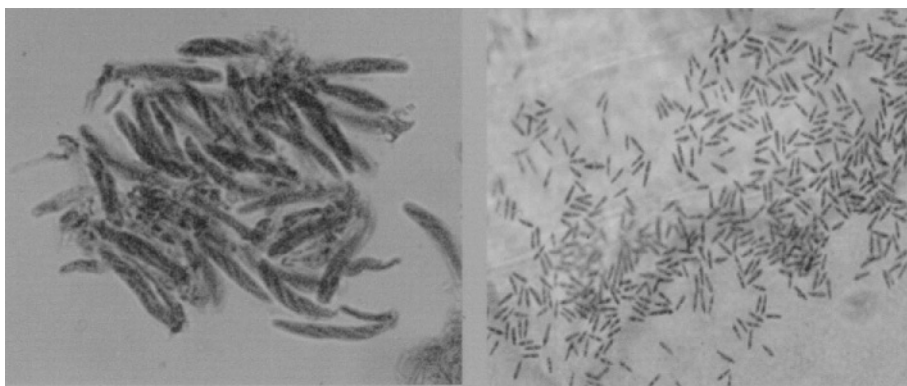
Figure 18: Fungal structures : Asci (left) and ascospores (right) of *Mycosphaerella citri.*

infrequently, if at all, ascospore peaks occurred after the rainy season began in June (Whiteside 1974). Currently, with frequent microsprinkler irrigation, peak releases occur in April-May due to frequent wetting and drying (Timmer *et al.,* 1995, 2000c; Mondal *et al.,* 2003b). Peak ascospore releases in Costa Rica occur in June following the beginning of the rainy season (Hidalgo *et al.,* 1997). In Texas where rains begin later, peak spore release is in July-September (Timmer *et al.,* 1980).

Since infection is dependent on the development of the epiphytic mycelium, conditions must be favorable for growth to obtain much infection. In Florida, such growth develops best when temperatures are higher than 22°C and there is at least 6 h of leaf wetness each night (Whiteside 1974). In Florida, very little epiphytic growth develops during the spring months when conditions are dry, even though peak ascospore production occurs at that time (Mondal and Timmer 2003a). Thus, most infection of the spring growth flush that occurs in March actually does not occur until June

or July. The fungus then develops in the mesophyll and symptoms first appear in November. Growth flushes that occur in summer are infected much more rapidly, but symptoms do not appear until mid-to-late winter (Mondal and Timmer 2003a). Leaves do not usually fall until the weather begins to warm in the spring. Infection in Florida can occur at any time of the year and thus disease cycles overlap throughout the year. The occurrence of continuous overlapping cycles is probably even more common in more tropical areas.

Conidia production by *M. citri* is usually low. Asexual spores are produced most commonly on the epiphytic growth on mature leaves in late summer in Florida (Whiteside 1970a). Thus conidia may be a significant source of inoculum for summer flushes when ascospore numbers are low.

Greasy spot is endemic in most areas where it occurs. *Mycosphaerella citri* is capable of long-distance spread since it produces air-borne ascospores and conidia. We have recovered ascospores as high as 7.5 m above the grove and as far as 90 m from the grove (Mondal *et al.,* 2003b). Thus, the potential for long distance spread exists. Nevertheless, most of the inoculum for infection of a tree comes from the same or adjacent trees.

Much less is known about fruit infection. Mycelium also grows superficially on fruit and infection is presumed to occur in much the same manner as on leaves.

6.5 Disease Management

Fortunately, greasy spot is relatively easy to control. *Mycosphaerella citri* is highly sensitive to a wide range of products such as copper fungicides, sterol-biosynthesis inhibitors, benzimidazoles, and strobilurins, as well as petroleum oils (Timmer *et al.,* 2000c). Applications of standard fungicides protect leaves from development of the epiphytic growth if applied early enough. Even if they are applied after the epiphytic growth is well-established, even protectant fungicides will kill the fungus and prevent infection. The mechanism of action of petroleum oils is not well-established, but oils probably kill epiphytic mycelium as well as induce some resistance in the host to fungal growth in the mesophyll (Whiteside 1973a). Other products such as micronutrients and even miticides (Timmer and Zitko 1995b; Whiteside 1983) will kill epiphytic mycelium and reduce greasy spot severity.

In Florida, timing of sprays to control greasy spot on the spring flush is relatively straight-forward. Sprays applied anytime from late April to June provide relatively good control on those leaves (Timmer *et al.,* 1995, 2000c; Whiteside 1972c). A single application of fenbuconazole and probably other fungicides prevent development of greasy spot for 15-18 months (Mondal and Timmer 2003a). Control on summer flushes is more difficult. First, flushes occur sporadically making timing more difficult. Secondly, infection occurs rapidly and sprays must be timed more precisely. To protect flushes in summer, sprays need to be applied within one month after they are fully expanded. In tropical areas, little information is available on the timing of applications. However, based on work in Florida, all major growth flushes probably need to be sprayed within one month after full leaf expansion to achieve a higher level of control.

Cultural methods can conceivably be used to help reduce inoculum and the

severity of greasy spot. In Florida, the peak of ascospore production has been shifted from the summer rainy season to the dry springtime by frequent microsprinkler irrigation in the spring (Mondal and Timmer 2002; Mondal *et al.,* 2003b). This should reduce the amount of infection and greasy spot severity, but controlled comparisons have not been made.

It may also be possible to reduce inoculum in the leaf litter. Whiteside (1973b) evaluated fungicide applications to the grove floor for that purpose. He found that benomyl was the only fungicide that reduced ascospore production. Theoretically, any method that destroys or covers leaf litter should reduce inoculum levels. Thus, tillage of groves or mulching with organic material should eliminate large numbers of spores. Frequent wetting of leaves causes them to be colonized by other fungi, preventing pseudothecial production (Mondal and Timmer 2002). More frequent irrigation may help reduce inoculum. Even weed cover beneath the trees might prevent dispersal of ascospores. Recently, we have found that application of lime or urea prevents pseudothecial development and reduces inoculum (Mondal and Timmer 2003b). These cultural manipulations may provide additional management tools for inoculum reduction.

However, it is uncertain whether reducing inoculum will reduce disease severity. Since greasy spot severity is related to the amount of epiphytic mycelium rather than to spore numbers, it is possible that reducing spore numbers may not reduce disease. If conditions are favorable, just a few spores could produce copious amounts of epiphytic mycelium. Thus, the benefits of management tools to reduce inoculum remain to be seen.

Timing of fungicide applications for control of rind blotch has not been thoroughly investigated. Presumably the infection pattern would follow that occurring on spring flush leaves. Most fungicides that control greasy spot on leaves also control the disease on fruit. Whiteside (1982) reported that petroleum oils were relatively ineffective for rind blotch control. The heavier 470 petroleum oils were recently found to be nearly as effective as standard fungicides (Bhatia *et al.,* 2002).

7. References

Agostini, J.P. and Timmer, L.W. 1992. Selective isolation procedures for differentiation of two strains of *Colletotrichum gloeosporioides* from citrus. Plant Disease, 76:1176-1178.

Agostini, J.P., Timmer, L.W., and Mitchell, D.J. 1992. Morphological and pathological characteristics of strains of *Colletotrichum gloeosporioides* from citrus. Phytopathology, 82:1377-1382.

Agostini, J.P., Gottwald, T.R., and Timmer, L.W. 1993. Temporal and spatial dynamics of postbloom fruit drop of citrus in Florida. Phytopathology, 83:485-490.

Agostini, J.P. and Timmer, L.W. 1994. Population dynamics and survival of strains of *Colletotrichum gloeosporioides* on citrus in Florida. Phytopathology, 84:420-425.

Agostini, J.P., Tesoriero, A.J. and Timmer, L.W. 2002. Evaluation of products for the control of citrus scab on Temple tangors, 2001. Fungicide and Nematicide Tests (online) Report 57: M10.DOI.10.1094/FN57. The American Phytopathological Society, St. Paul, MN.

Agostini, J.P., Bushong, P.M., Bhatia, A., and Timmer, L.W. 2003. Effect of environmental factors on the severity of citrus scab and melanose. Plant Disease (submitted).

Alva, A.K. and Graham, J.H. 1991. The role of copper in citriculture. Advances in Agronomy, 1:145-170.

Bhatia, A., Tesoriero, A.J., and Timmer, L.W. 2002. Evaluation of fungicides for control of greasy spot on grapefruit, 2000-2001. Fungicide and Nematicide Tests (online). Rep. 57:M02.DOI.1094/FN57. American Phytopathology Society, St. Paul, MN.

Bhatia, A. and Timmer, L.W. 2003. Evaluation of the Alter-Rater model for timing of fungicide applications for control of Alternaria brown spot of citrus. Plant Disease, 87:(accepted).

Bitancourt, A.A. and Jenkins, A.E. 1936. *Elsinoe fawcettii,* the perfect stage of the citrus scab fungus. Phytopathology, 26:393-396.

Bitancourt, A.A. and Jenkins, A.E. 1937. Sweet orange fruit scab caused by *Elsinoe australis*. Journal of Agricultural Research Center, 54:1-18.

Brown, A.E., Sreenivasaprasad, S., and Timmer, L.W. 1996. Molecular characterization of Slow-Growing Orange and Key Lime Anthracnose strains of *Colletotrichum* from citrus as *C. acutatum.* Phytopathology, 86:523-527.

Bushong, P.M. and Timmer, L.W. 2000. Postinfection control of citrus scab and melanose with benomyl, fenbuconazole and azoxystrobin. Plant Disease, 84:1246-1249.

Canihos, Y. Erkilic, A. and Timmer, L.W. 1997. First report of Alternaria brown spot of Minneola tangelo in Turkey. Plant Disease, 81:1214.

Canihos, Y., Peever, T.L. and Timmer, L.W. 1999. Temperature, leaf wetness, and isolate effects on infection of Minneola tangelo leaves by *Alternaria* sp. Plant Disease, 83:429-433.

Castro, B.L., Leguizamon, J.E. and Lopez, J.A. 1994. La mancha foliar de los cítricos en la zona cafetera. Avances Técnios 198. Cenicafe, Chinchiná, Caldas, Colombia.

Clausen, R.E. 1912. A new fungus concerned in wither tip varieties of *Citrus medica.* Phytopathology 2:217-236.

Cobb, N.A. 1903. Letters on the diseases of plants – *Alternaria* of the citrus tribe. Agricultural Gazette New South Wales, 14:955-986.

Denham, T.G. 1979. Citrus production and premature fruit drop disease in Belize. PANS, 25:30-36.

Denham, T.G. and Waller, J.M. 1981. Some epidemiological aspects of postbloom fruit drop disease (*Colletotrichum gloeosporioides*) in citrus. Annual of Applied Biology, 98:65-67.

Diaz, L.E., Gimenez, G., Zefferino, E. and Cardeiras, J.T. 1992. Relevamiento de especies y biotipos de sarnas de los citrus en Uruguay. Fitopatologia Brasileira, 17:165 (Abstr.).

Fagan, H.J. 1979. Postbloom fruit drop: a new disease of citrus associated with a form of *Colletotrichum gloeosporioides.* Annual of Applied Biology, 91:13-20.

Fagan, H.J. 1984. Postbloom fruit drop of citrus in Belize: II. Disease control by aerial/ground spraying. Turrialba, 34:179-186.

Fawcett, H.S. 1912. The causes of stem-end rot citrus fruits (*Phomopsis citri* n. sp.). Phytopathology, 2:109-113.

Fawcett, H.S. 1915. Citrus diseases of Florida and Cuba compared with those of California. California Agricultural Experiment Station Bulletin 262.

Fawcett, H.S. 1921. Temperature relation of growth in certain parasitic fungi. University of California Publications Agricultural Sciences, Series 4, p. 183-232.

Fisher, F.E. 1961. Greasy spot and tar spot of citrus in Florida. Phytopathology, 51:297-303.

Floyd, B.F. and Stevens, H.E. 1912. Melanose and stem-end rot. Florida Agricultural Experiment Station Bulletin, 111:1-16.

Guerber, J.C. and Correll, J.C. 2000. Characterization of *Glomerella acuta*, the teleomorph of *Colletotrichum acutatum*. Mycologia, 93:216-229

Hidalgo, H., Sutton, T.B., and Arauz, F. 1997. Epidemiology and control of citrus greasy spot on Valencia orange in the humid tropics of Costa Rica. Plant Disease, 81:1015-1022.

Homma, Y. and Yamada, S.L. 1969. Factors influencing infection and development of citrus

melanose caused by *Diaprothe citri* (Fawc.) Wolf. Bulletin Horticultural Research Station Japan Series, B9:85-96.

Hyun, J.W., Timmer, L.W., Lee, S.C., Yun, S.H., Ko, S.W. and Kim, K.S. 2001. Pathological characterization and molecular analyses of *Elsinoe* isolates causing scab diseases of citrus in Jeju Island in Korea. Plant Disease, 84:1013-1017.

Ieki, H. 1986. The causal fungus of citrus greasy spot in Okinawa district of Japan. Annals of the Phytopathology Society of Japan, 52:484-487.

Jenkins, A.E. 1936. Australian citrus scab caused by *Sphaceloma fawcettii scabiosa.* Phytopathology, 26:195-197.

Kohmoto, K., Scheffer, R.P., and Whiteside, J.O. 1979. Host selective toxins from *Alternaria citri*. Phytopathology 69:667-671.

Koizumi, M. 1986. *Sporobolomyces roseus*, a causal agent of citrus pseudo greasy spot (Nise-Ohan-Byo) and the infection process of disease. Annals of the Phytopathology Society of Japan 52:758-765.

Kuramoto, T. and Yamada, S.L. 1975. The influence of environmental factors an citrus melanose infection due to *Diaporthe citri*. Bulletin Fruit Tree Research Station, B2:75-86.

Liyanage, H.D., McMillan, R.T., and Kistler, H.C. 1992. Two genetically distinct populations of *Colletotrichum gloeosporioides* from citrus. Phytopathology, 82:1371-1376.

Liyanage, H.D., Köller, W., McMillan, R.T., and Kistler, H.C. 1993. Variation in cutinase from two populations of *Colletotrichum gloeosporioides* from citrus. Phytopathology, 83:113-116.

Mondal, S.N. and Timmer, L.W. 2002. Environmental factors affecting pseudothecial development and ascospore production of *Mycosphaerella citri*, the cause of citrus greasy spot. Phytopathology, 92:1267-1275.

Mondal, S.N. and Timmer, L.W. 2003a. The relationship of epiphytic mycelial growth of *Mycosphaerella citri* to greasy spot development on citrus and to disease control of fenbuconazole. Plant Disease, 87:186-192.

Mondal, S.N. and Timmer, L.W. 2003b. The effect of urea, $CaCO_3$ and dolomite on pseudothecial development and ascospore production of *Mycosphaerella citri,* the cause of citrus greasy spot. Plant Disease, (Accepted).

Mondal, S.N., Bhatia, A., and Timmer, L.W. 2003a. How to promote greasy spot without really trying. Citrus Industry, 89(1):15-16.

Mondal, S.N., Gottwald, T.R., and Timmer, L.W. 2003b. Environmental factors affecting the release and dispersal of ascospores of *Mycosphaerella citri*. Phytopathology, (Submitted).

Palanisivami, A., Naryanaswany, T. and Jeyarajan, R. 1993. Sweet orange (*Citrus sinensis* L.) a new host for scab caused by *Sphaceloma australis*. Indian Journal of Mycology and Plant Pathology, 23:217-218.

Palm, M.E. and Civerolo, E. 1994. Isolation, pathogenicity, and partial host range of *Alternaria limicola*, causal agent of *mancha moliar de los citricos* in Mexico. Plant Disease, 78:879-883.

Peever, T.L., Su, G., Carpenter-Boggs, L., and Timmer, L.W. 2003. Molecular systematics of citrus-associated *Alternaria* spp. Mycologia, (submitted).

Pegg, K.G. 1966. Studies of a strain of *Alternaria citri* Pierce, the causal organism of brown spot of Emperor mandarin. Queensland Journal of Agriculture and Animal Science, 23:14-18.

Peres, N.A.R., Kim, S., Beck, H.W., Souza, N.L., and Timmer, L.W. 2002a. A fungicide application decisión (FAD) support system postbloom fruti drop of citrus (PFD). On line. Plant Health Progress doi: 10.1094, PHP-2002-0731-01-RV.

Peres, N.A.R., Souza, N.L., and Timmer, L.W. 2002b. Postbloom fruit drop in Brazil and Florida: occurrence and control by fungicides. Proceedings of International Society of Citriculture (in press).

Peres, N.A.R., Souza, N.L., Zitko, S., and Timmer, L.W. 2002c. Activity of benomyl for control of postbloom fruit drop of citrus caused by *Colletotrichum acutatum*. Plant Disease, 86:620-

624.

Peres, N.A.R., Souza, N.L., Peever, T.L., and Timmer, L.W. 2003. Benomyl sensivity of isolates of *Colletotrichum acutatum* and *C. gloeosporioides* from citrus. Plant Disease, 87:(submitted)

Prates, H.S., Rodrigues, J.C.V., and Nogueira, N.L. 1995. Eficiencia de diferentes fungicidas no controle da verrugose da laranja doce. Fitopatol. Bras., 205:296.

Ruehle, G.D. 1937. A strain of *Alternaria citri* Ellis & Pierce causing a leaf spot of rough lemon in Florida. Phytopathology, 27:863-865.

Ruehle, G.D. and Kuntz, W.A. 1940. Melanose of citrus and its commercial control. Florida Agricultural Experiment Station Bulletin 349, University of Florida, Gainesville.

Schutte, G.C., Lesar, K.H., Pelser, P. du T., and Swart, S.H. 1992. The use of tebuconazole for the control of *Alternaria alternata* on 'Minneola' tangelos and its potential to control postharvest decay when applied as a pre-harvest spray. Proceedings of the International Society of Citriculture, 7:1070-1079.

Simmons, E.G. 1999. Alternaria themes and variations (226-235): Classification of citrus pathogens. Mycotaxon, 70:263-323.

Skaria, M. and Tao, Z. 1996. A leaf disk clearing and staining technique to quantify ascospores of *Mycosphaerella citri* young citrus leaves. Subtropical Plant Science, 48:16-18.

Solel, Z. 1991. Alternaria brown spot on Minneola tangelo in Israel. Plant Pathology, 40:145-147.

Sonoda, R.M. and Pelosi, R.R. 1988. Outbreak of citrus postbloom fruit drop caused by *Colletotrichum gloeosporioides* from lesions on citrus blossoms in the Indian River of Florida. Proceedings of Florida State Horticulture Society, 101:36-38.

Stapleton, J.J. and Garza-Lopez, J.G. 1988. Epidemiology of a citrus leaf spot disease in Colima, Mexico. Phytopathaology, 78:440-443.

Stevens, H.E. 1915. Pruning for melanose. Proceedings of the Florida State Horticultural Society, 28:122-123.

Su, G., Peever, T.L. and Timmer, L.W. 2001. Molecular systematics of citrus-associated *Alternaria* spp. Phytopathology, 91(Suppl):S190.

Swingle, W.T. and Webber, H.J. 1896. The principal diseases of citrus fruits in Florida. U.S. Department of Agriculture, Division of Vegetable Physiology and Pathology, Bulletin 9, p. 1-42.

Tan, M.K., Timmer, L.W., Broadbent, P., Priest, M. and Cain, P. 1996. Differentiation by molecular analysis of *Elsinoe* spp. causing scab diseases of citrus and its epidemiological implications. Phytopathology, 86:1039-1044.

Tanaka, S. and Yamada, S. 1952. Studies on the greasy spot (black melanose) of citrus. I. Confirmation of the causal fungus and its taxonomic study. (English summary) Horticulture Division, National Tokai-kinki Agricultural Experiment Station, Bulletin No., 1:1-15.

Thompson, W.L. 1948. Greasy spot on citrus leaves. Citrus Industry 29(4):20-22, 26.

Thompson, W.L., Johnson, R.B., and Sites, J.W. 1955. Greasy spot control. Citrus Industry 36(6):5-10, 14.

Timmer, L.W. 1974. Evaluation of fungicides and application times for control of melanose on Texas grapefruit. Plant Disease Reporter, 58:504-506.

Timmer, L.W. 1999. Diseases of fruit and foliage. In: "Citrus Health Management" (eds. Timmer, L.W. and Duncan, L.W.). APS Press Inc. St. Paul. p 107-115.

Timmer, L.W. (ed.) 2002. Florida Citrus Pest Management Guide. University of Florida, Institute of Food Agricultural Science, Cooperative Extension Service Publication SP-43, 150 pp.

Timmer, L.W. and Broadbent, P. 1995. Citrus scab diseases – an international perspective. Citrus Industry, April 1995.

Timmer, L.W. and Fucik, J.E. 1976. The relationship of rainfall distribution, fruit growth, and fungicide application to the incidence of melanose on grapefruit in Texas. Plant Disease

Report, 60:565-568.

Timmer, L.W., Reeve, R.J., and Fucik, J.E. 1979. The effect of rainfall, fruit growth, and fungicide application on melanose severity on Texas grapefruit, 1976-1978. Journal Rio Grande Valley Horticultural Society, 33:49-53.

Timmer, L.W., Reeve, R.J., and Davis R.M. 1980. Epidemiology and control of citrus greasy spot on grapefruit in Texas. Phytopathology, 70:863-867.

Timmer, L.W. and Zitko, S.E. 1992. Timing of fungicide applications for control of postbloom fruit drop citrus in Florida. Plant Disease, 76:820-823.

Timmer, L.W. and Zitko, S.E. 1993. Relationships of environmental factors and inoculum levels to the incidence of postbloom fruit drop of citrus. Plant Disease, 77:501-504.

Timmer, L.W., Agostini, J.P., Zitko, S.E., and Zulfiqar, M. 1994. Postbloom fruit drop, an increasingly prevalent disease of citrus in the Americas. Plant Disease, 78:329-334.

Timmer, L.W. and Zitko, S.E. 1995a. Early season indicators of postbloom fruit drop of citrus and the relationship of disease and fruit production. Plant Disease, 79:1017-1020.

Timmer, L.W. and Zitko, S.E. 1995b. Evaluation of nutritional products and fungicides for control of citrus greasy spot. Proceeding of the Florida State. Horticulture Society, 108:83-87.

Timmer, L.W., Gottwald, T.R., McGovern, R.J., and Zitko, S.E. 1995. Time of ascospore release and infection by *Mycosphaerella citri* in central and southwest Florida. Proceedings of the Florida State Horticulture Society, 108:374-377.

Timmer, L.W. and Zitko, S.E. 1996a. Evaluation of model for prediction of postbloom fruit drop of citrus. Plant Disease, 80:380-383.

Timmer, L.W. and Zitko, S.E. 1996b. Evaluation of copper fungicides and rates of metallic copper for control of melanose on grapefruit in Florida. Plant Disease, 80:166-169.

Timmer, L.W., Priest, M., Broadbent, P. and Tan, M.K. 1996. Morphological and pathological characterization of species of *Elsinoe* causing scab diseases of citrus. Phytopathology, 86:1032-1038.

Timmer, L.W., and Peever, T.L. 1997. Managing Alternaria brown spot. Citrus Ind. 78(6):24-25.

Timmer, L.W. and Zitko, S.E. 1997. Evaluation of fungicides for control of Alternaria brown spot and citrus scab. Proceedings of the Florida State Horticulture Society, 110:71-76.

Timmer, L.W., Brown, G.E., and Zitko, S.E. 1998a. The role of *Colletotrichum* spp. in postharvest anthracnose of citrus and survival of *C. acutatum* on fruit. Plant Disease, 82:415-418.

Timmer, L.W., Solel, Z., Gottwald, T.R., Ibáñez, A.M., and Zitko, S.E. 1998b. Environmental factors affecting production, release, and field production of conidia of *Alternaria alternata*, the cause of brown spot of citrus. Phytopathology, 78:1218-1223.

Timmer, L.W. and Zitko, S.E. 1999. Evaluation of fungicides for control of citrus scab on Duncan grapefruit, 1998. Fungicide Nematicide Tests, 54:553.

Timmer, L.W. and Brown, G.E. 2000. Biology and control of anthracnose diseases of citrus. In: Host Specificity, Pathology, and Host-Pathogen Interactions of *Colletotrichum* (eds. Prusky, D., Freeman, S., and Dickman, M.B.) APS Press Inc., St. Paul, p. 300-316.

Timmer, L.W. and. Bushong, P.M. 2000. Evaluation of fungicides for control of citrus scab on Duncan grapefruit, 1999. Fungicide and Nematicide Tests, 55:567.

Timmer, L.W., Darhower, H.M., Zitko, S.E., Peever, T.L., Ibáñez, A.M. and Bushong, P.M. 2000a. Environmental factors affecting the severity of Alternaria brown spot of citrus and their potential use in timing fungicide applications. Plant Disease, 84:638-643.

Timmer, L.W., Garnsey, S.M., and Graham, J.H. (eds.) 2000b. Compendium of Citrus Diseases, 2nd ed. APS Press Inc., St. Paul, 92 pp.

Timmer, L.W., Roberts, P.D., Darhower, H.M.. Bushong, P.M., Stover, E.W., Peever, T.L., and Ibáñez, A.M. 2000c. Epidemiology and control of citrus greasy spot in different citrus-growing areas in Florida. Plant Disease, 84:1294-1298.

Ushiyama, K. 1976. Studies on citrus melanose disease of satsumas. IV Observations on the germination and penetration of spores of the causal fungus *Diaporthe citri* and on affected tissues. Bulletin Kanagawa Horticultural Experiment Station, 23:11-18.

Vicent, A., Armengol, J., Sales, R., and Garcia-Jiménez. 2000. First report of Alternaria brown spot of citrus in Spain. Plant Disease, 84:1044.

Wellings, C.R. 1981. Pathogenicity of fungi associated with citrus greasy spot in New South Wales. Transactions of the British Mycological Society, 76:495-499.

Whiteside, J.O. 1970a. Etiology and epidemiology of citrus greasy spot. Phytopathology, 6:1409-1414.

Whiteside, J.O. 1970b. Symptomatology of orange fruit infected by the citrus greasy spot fungus. Phytopathology, 60:1859-1860.

Whiteside, J.O. 1972a. Histopathology of the citrus greasy spot fungus and identification of the causal fungus. Phytopathology, 62:260-263.

Whiteside, J.O. 1972b. Blemishes on citrus rind caused *Mycosphaerella citri*. Plant Disease Report, 56:671-674.

Whiteside, J.O. 1972c. Effectiveness of spray materials against citrus greasy spot in relation to time of application and infection periods. Proceedings of the Florida State Horticulture Society, 84:56-63.

Whiteside, J.O. 1973a. Action of oil in the control of citrus greasy spot. Phytopathology, 63:262-266.

Whiteside, J.O. 1973b. The possibilities of using ground sprays to control greasy spot. Proceedings of the Florida State Horticulture Society, 86:19-23.

Whiteside, J.O. 1974. Environmental factors affecting infection of citrus leaves by *Mycosphaerella citri*. Phytopathology, 64:115-120.

Whiteside, J.O. 1975a. Biological characteristics of *Elsinoe fawcetti* pertaining to the epidemiology of sour orange scab. Phytopathology, 65:1170-1175.

Whiteside, J.O. 1975b. Evaluation of fungicide for the control of melanose on grapefruit in Florida. Plant Disease Reporter, 59:656-660.

Whiteside, J.O. 1976. A newly recorded Alternaria-induced brown spot disease on Dancy tangerines in Florida. Plant Disease Reporter, 60:326-329.

Whiteside, J.O. 1977a. Site of action of fungicides in the control of melanose. Phytopathology, 67:1067-1072.

Whiteside, J.O. 1977b. Behavior and control of greasy spot in Florida Citrus groves. Proceedings of the International Society of Citriculture, 3:981-986.

Whiteside, J.O. 1978. Pathogenicity of two biotypes of *Elsinoe fawcetti* to sweet orange and some other citrus cultivars. Phytopathology, 68:1128-1131.

Whiteside, J.O. 1980. Timing of fungicides spray treatments for citrus melanose control. Proceedings of the Florida State Horticultural Society, 93:21-24.

Whiteside, J.O. 1981. Diagnosis of citrus greasy spot based on experiences with this disease in Florida. Proceedings of the International Society of Citriculture, 1:336-340.

Whiteside, J.O. 1982. Timing of single-spray treatments for optimal control of greasy spot on grapefruit leaves and fruit. Plant Disease, 66:687-690.

Whiteside, J.O. 1983. Fungicidal effects of some acaricides on *Mycosphaerella citri*. Plant Disease, 67:864-866.

Whiteside, J.O. 1986. Semiselective media for the isolation of *Elsinoe fawcettii* from scab pustules. Plant Disease, 70:204-206.

Wolf, F.A. 1926. The perfect stage of the fungus which causes melanose of citrus. Journal of Agricultural Research, 33:621-625.

Yamada, S. 1956. Studies on the greasy spot (black melanose) of citrus. II. Morphological characteristics of the causal fungus (*Mycosphaerella horii* Hara). Horticulture Division,

National Tokai-kinki Agricultural Experiment Station, Okitsu Japan, Bulletin, 3:49-62.

Yamato, H. 1971. The perfect stage of citrus melanose fungus in Japan. Annals Phytopathology Society of Japan, 37:355-356.

Yamato, H. 1976. A species of *Diaporthe* pathogenic to citrus. Annals Phytophatololy Society of Japan, 42: 56-59.

Zitko, S.E. and Timmer, L.W. 1997. Evaluation of fungicides for control of citrus scab and Alternaria brown spot, 1995. Fungicide and Nematicide Tests, 52:414.

Zitko, S.E. and Timmer, L.W. 1998. Evaluation of fungicides for control of citrus scab and melanose on grapefruit, 1996. Fungicide Nematicide Tests, 53:490.

Zulfiqar, M., Brlansky, R.H., and Timmer, L.W. 1996. Infection of flower and vegetative tissues of citrus by *Colletotrichum acutatum* and *C. gloeosporioides*. Mycologia, 88:121-128.

Citrus Huanglongbing : Review, Present status and Future Strategies

J.V. da Graça[1] and L. Korsten[2]

[1]Texas A and M University-Kingsville, Citrus Center, 312 N. International Blvd., Weslaco TX 78596, USA ; [2]Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, 0002 South Africa

Abstract: Citrus huanglongbing (HLB), formerly known as greening, is a highly destructive disease of citrus, especially on sweet orange and mandarin varieties. A range of primary and secondary leaf symptoms are associated with HLB, making field diagnosis difficult, unless the typical lop-sided greened fruit are present. To date, the causal organism has not been cultured on artificial media and a diagnostic polymerase chain reaction technique has been developed to confirm the presence of the pathogen. The disease is caused by two closely related phloem-limited bacterial species, "*Candidatus* Liberibacter asiaticus" and "*Candidatus* L. africanus". The former, which causes the more severe Asian form, is found in Asia from southern Japan in the east through southeast and south Asia to eastern Iran in the west, as well as Saudi Arabia, Mauritius and Reunion. It is transmitted by the psylla, *Diaphorina citri*. The milder, yet still serious, African form is less heat tolerant, and is transmitted by another psylla species*, Trioza erytreae*. This form is found in Yemen, throughout eastern and southern Africa, as well as in Mauritius and Reunion. Of concern is the fact that both psylla species are capable of transmitting both bacterial species under experimental conditions. In Reunion, propagating healthy trees and releasing hyperparasites for vector control achieved almost complete control. Elsewhere, HLB is best controlled through integrated disease management involving the use of healthy nursery material, removal of infected trees or branches, and integrated vector control. The recent arrival of the vectors in citrus producing areas previously regarded as HLB-free highlights the potential threat of one of the most serious diseases of citrus, thus emphasizing the need for effective quarantine services.

1. Introduction

Citrus huanglongbing (yellow shoot disease) was first noted by farmers in southern China in the late nineteenth century as a problem of unknown cause (Zhao, 1981). By the 1920's similar diseases were recorded in Taiwan, known there as likubin (drooping disease) (Ôtake, 1990), Philippines (mottle leaf disease) (Lee, 1921) and India (citrus die-back) (Raychaudhuri *et al.,* 1974). In the late 1920's, a similar malady of citrus was observed in South Africa, called yellow branch or greening, depending on the production region (van der Merwe and Andersen, 1937). In Indonesia, the disease was first recorded in the 1940's and was described as citrus phloem degeneration (Aubert *et al.*, 1985). During the 1960's, the connection was made between the diseases described under these different names (McClean and Schwarz, 1970).

For many years, the disease was known outside China as "greening", probably because the name was more commonly used in South Africa where extensive research was done from the 1950's. Although the disease was first described in English in 1919 by Reinking as a yellowing and leaf mottle of citrus occurring in China, no reference was made to earlier descriptions from China (da Graça, 1991). According to the international nomenclature rules, the first official description of the disease should have priority over subsequent names used. Therefore, because of the earlier recognition of the disease in China and the pioneering work done by K.H. Lin (Lin Kungxiang), citrus pathologists at the 13th conference of the International Organization of Citrus Virologists in China adopted "huanglongbing" (HLB) as the official name (Moreno *et al.*, 1996). According to Zhao (1981), "huanglong" means the yellowing of some new shoots in the green canopy, and "bing" means disease. Specifically, "huanglong" means "yellow dragon" because as symptoms progress they appear "draped" over the tree almost like a "yellow dragon" (CAB International, 2000).

The identity of the causal organism followed a similar treacherous path with the causal agent being first attributed to poor drainage (Raychaudhuri *et al.*, 1974), nematode damage (Ôtake, 1990) and later mineral deficiency or toxicity (Hector, 1944, van der Merwe and Andersen, 1937). The demonstrations of graft- and insect-transmissibility suggested that the causal organism was a virus (McClean and Oberholzer, 1965). In 1970, Laflèche and Bové, reported mycoplasma-type bodies in sieve tubes of sweet orange infected with HLB. Soon thereafter, Moll and Martin (1973) observed bacterium-like organisms in HLB-infected citrus plants similar to that observed in the insect vector *Trioza erytreae* (Del Guercio). The effective suppression of symptoms and the disappearance of the organisms after penicillin G treatment of HLB-infected trees proved the Gram negative nature of the unculturable bacterium (Bové *et al.*, 1980). Based on 16S rDNA comparative studies, the phloem-limited bacterium was identified as belonging to the alpha subdivision of the *Proteobacteriaceae* (Jagoueix *et al.*, 1994).

Two distinct forms of greening are recognised based on the wider geographical spread of the more severe, lower elevated (360 m) and higher temperature (30-35° C) disease with its psylla vector *Diaphorina citri* (Kuwayama) (Capoor *et al.*, 1967). The more restricted, less severe, temperature sensitive (27° C) African form of HLB is normally found at higher elevations (900 m above sea level) and is transmitted by *T. erytreae*. Comparative 16S rDNA studies (Jagoueix *et al.*, 1994), led to the proposed classification of the causal agent "*Candidatus*" with the generic name Liberobacter (meaning, bacteria of the phloem) (Jagoueix *et al.*, 1996). Initially, the proposed names were "*Candidatus* Liberobacter africanum" and "*Candidatus* L. asiaticum" (Jagoueix *et al.*, 1994), but were soon changed to "*Candidatus* Liberibacter africanus" and "*Candidatus* L. asiaticus", to comply with the rules of the *International Code of Nomenclature of Bacteria* (Garnier *et al.*, 2000b).

Da Graça (1991) published an extensive review of greening, but this was just prior to any significant molecular understanding of the causal organism, while Garnier and Bové (1993) covered the initial DNA studies in their review. Since then, much has been elucidated in terms of the identity of the causal agent and its control. This review attempts to give an overall picture of the disease and the causal organism up to the

present, and to discuss present and possible future management strategies.

2. Geographical distribution and economic impact

Da Graça (1991) lists 24 countries and territories in east, south-east, south and western Asia and in eastern and southern Africa, where HLB had been reported. Since then, its presence has been confirmed in four additional south-east Asian nations, namely Vietnam (Garnier and Bové, 1996), Myanmar, Laos and Cambodia (Garnier and Bové, 2000).

For other major citrus production regions such as North and South America, Australia and the Mediterranean countries, HLB remains a major threat if introduced. Globally, HLB has been regarded as one of the most important threats to commercial and sustainable citrus production. For instance, HLB has resulted in the destruction of 30 million trees in Indonesia (Tirtawidjaja, 1980). On the Indonesian island of Bali four million trees were eradicated during 1986-88, although these trees were replaced with mandarins in 1991, 40% were infected by 1993, and 90 % by 1996 (Aubert, 1993). In the early 1960's, nearly 25,000 ha were planted to citrus, but 10 years later five million (*i.e.*, 60% of the plantings) were lost to HLB. In Thailand, many trees are dying and going out of production five to six years after planting. Such losses are significant, since profits are only attainable 10 years after planting resulting in losses of over US$8,000/ha (Roistacher, 1996). In Bali, four million trees were eradicated during 1986-88 and replaced with mandarins in 1991. However, by 1993, 40% of these trees were infected and in 1996 more than 90% showed HLB symptoms (Aubert, 1993). In south-western Saudi Arabia, all sweet orange and mandarin trees had declined by 1986 leaving only limes (Aubert, 1993, Bové, 1986).

Crop losses of 30-100% have been reported in South Africa during the 1932-1936 and 1939-1946 periods (Oberholzer *et al.,* 1965, Schwarz, 1967). By 1958, the disease affected 100 000 sweet orange trees (Oberholzer *et al.,* 1965) and by the mid 1970's, it was estimated that four of the eleven million trees planted in South Africa (36%) were affected with HLB (Buitendag and von Broembsen, 1993). By then, major citrus production areas, which represented 20% of the industry, were eliminated, making it the most serious disease in South Africa. Of even greater concern was that areas previously regarded as HLB-free were showing tree symptoms (Green and Catling, 1971). By the mid 1990's the disease were reported in the Cape which, with its Mediterranean type climate, was regarded as "not likely" to get HLB. The use of a national quarantine barrier (McClean *et al.*, 1969) and restriction on sales of citrus trees from the northern regions were the disease is endemic to the coastal areas where psylla are endemic (McClean *et al.*, 1969) and not controlled, proved ineffective.

Although losses resulting from HLB has been more extensively documented in Asia than in Africa, it is estimated that globally more than 60 million trees had been destroyed by the disease by the early 1990's (Aubert, 1993).

3. Disease symptoms

Depending on the age of a tree and time and stage of infection, the first symptoms of

HLB usually start with the appearance of a yellow shoot. If infection occurs soon after propagation, yellowing progresses over the entire canopy. However, if infection occurs at a later stage of growth, the symptoms and the causal organism remain confined to the sector initially infected. If a sector of a tree is affected, then only those parts will show typical symptoms (Fig. 1), while the rest of the tree exhibits normal growth and produces normal healthy fruit of good quality (Oberholzer *et al.,* 1965).

A range of symptoms can be observed on infected trees and branches, which include heavy leaf and fruit drop, followed by out of season flushing and blossoming (Catling, 1969, Martinez, 1972, Oberholzer *et al*., 1965). Severely infected trees often appear stunted, usually are sparsely foliated and can die back. Chronically infected trees are sparsely foliated and show extensive twig die-back symptoms. Infected trees produce reduced crops of low quality fruit (Oberholzer *et al.,* 1965).

Figure 1: Citrus tree with HLB infected segment showing appearance of a dragon draped over the tree.

Initial foliar symptoms of African HLB are vein yellowing and a variegated type of chlorosis (blotchy mottle), which appear on fully mature leaves (Schneider, 1968, Manicom and van Vuuren, 1980). Secondary symptoms include small, upright leaves ("rabbit's ears") with a variety of chlorotic patterns resembling those induced by zinc, iron, manganese, calcium, sulphur and/or boron deficiencies (Oberholzer *et al.,* 1965, Schneider, 1968, McClean and Schwarz, 1970). Many of the latter may be almost entirely devoid of chlorophyll, except for occasional circular green spots ("green islands") distributed at random on the leaves (Fig. 2) (Oberholzer *et al.,* 1965).

The Asian form of the disease induces similar symptoms, but with more exten-

sive yellowing, die-back and decline (Martinez and Wallace, 1968, Zhao, 1981), and in some cases death of small trees (1-2 years) (Lin, 1963). It is also more tolerant to heat, and thus is found in lower lying, hotter areas. In South Africa, leaf symptoms are more pronounced in the cool areas, compared to the lower lying hotter areas, and are more pronounced in winter (Schwarz, 1968b). African HLB can also be eliminated by exposure to extended periods of heat (Labuschagne and Kotzé, 1984). Both forms of greening have only been found in Reunion and Mauritius, usually separated by the temperature preferences, although both forms were detected in some trees using molecular probes (Garnier *et al.*, 1996).

The most reliable diagnostic symptom of HLB represent the fruits which when infected, are small, lopsided with a curved columella and seed, if present, is mostly aborted. A bitter, salty taste is also characteristic of affected fruit. With infected trees there is a continuous and premature shedding of greened fruit while those remaining on

Figure 2: Leaf mottle symptoms of HLB with green islands.

the tree do not color properly (McClean and Schwarz, 1970), hence the former name "greening disease".

Symptoms can be exacerbated by the presence of other pathogens. Co-infection with *Citrus tristeza virus* (CTV) is common, and there are reports from several Asian countries that such trees have more severe symptoms (Martinez, 1972, Bhagabati and Nariani, 1980, Huang *et al.*, 1980). Of interest is that some isolates of CTV apparently protect trees from HLB infection (van Vuuren *et al.*, 2000). Blotchy mottle, the most characteristic leaf symptom, can be confused with other diseases such as stubborn (*Spiroplasma citri*), severe forms of CTV, Phytophthora root rot and water logging

(Calavan, 1968, McClean and Oberholzer, 1965, Schneider, 1968). Due to the non specific nature of leaf symptoms, HLB can often be confused with mineral deficiency or other stress related leaf symptoms (Korsten *et al.,* 1993). Symptoms of zinc deficiency are also associated with the early stages of citrus blight (Brlansky, 2000).

Root systems are usually poorly developed in severely affected trees, with relatively few fibrous roots (Oberholzer *et al.,* 1965), possibly due to root starvation. New root growth is suppressed and the roots often start decaying from the rootlets (Zhao, 1981).

4. Transmission

HLB was first transmitted experimentally by grafting (Chen, 1943), thereby establishing the causal agent as a pathogen. Natural spread was demonstrated by exposing healthy seedlings in an infected citrus orchard (Schwarz, 1964), and the vector in Africa was identified shortly thereafter as the citrus psylla, *T. erytreae* (McClean and Oberholzer, 1965). The vector of the disease in Asia was then identified as another species of psylla, *D. citri* (Tirtawidjaja *et al.*, 1965, Salibe and Cortez, 1966, Martinez and Wallace, 1967, Capoor *et al.*, 1967).

T. erytreae exists in Africa from the Red Sea coast through east and central Africa to South Africa, as well as in Cameroon. It is also found in Yemen, Madagascar, Mauritius, and, before bio-eradication, Reunion. More recently it has also been described in Madeira Island (Jagouiex *et al.,* 1996). It is sensitive to excessive heat, and thrives in cooler, higher areas 500 m and more above sea level. *D. citri*, on the other hand, is found in hotter, lower lying areas throughout south and south-east Asia, as far west as eastern Iran and Saudi Arabia, in Reunion, Mauritius, St Helena, Guadeloupe, Brazil, Florida (Knapp *et al.*, 1998) and, most recently, Venezuela (Cermeli *et al.*, 2000), Texas (French *et al*., 2001), and Mexico (D.Thomas, pers.comm. 2002). Samples have also been collected in Argentina, Bahamas, Cuba, Dominican Republic and Puerto Rico, and there are unconfirmed reports from Costa Rica and Honduras (S.Halbert, pers.comm). The heat preferences of the two species correspond to that of the two forms of HLB, although it has been shown experimentally that both species can transmit both forms (Massonie *et al.*, 1976, Lallemand *et al.*, 1986). Recently *D. citri* was reported from northern Irian Jaya (West Papua) province in Indonesia near Papua New Guinea (PNG) (Davis *et al*., 2000). During a survey of northern Australia, PNG and adjacent regions by the Australian Quarantine and Inspection Service (AQIS), it was found that the eradication campaign near Sorong failed and that HLB established more than 1000 km to the east (Davis *et al*., 2000). This raised concerns of movement of planting material or ornamental hosts of *D. citri* that can result in further spread of the disease. Thus far PNG and north Queensland remain psylla and HLB-free.

Under experimental conditions, HLB can be transmitted by some species of dodder (Raychaudhuri *et al*., 1974, Garnier and Bové, 1983, Ke *et al*., 1988).

5. Causal organism

The demonstrations that greening is a graft- and insect-transmissible disease led to the

conclusion that a virus was responsible (McClean and Oberholzer, 1965, da Graça, 1991). In 1970, Laflèche and Bové reported mycoplasma-type bodies in sieve tubes of sweet orange infected with HLB. The observation of cellular organisms in the phloem of HLB-infected citrus and their absence in healthy material indicated that a procaryotic organism was responsible (Laflèche and Bové, 1970). Similar organisms were observed in psylla (Chen *et al.*, 1973, Moll and Martin, 1973). On the basis of electron microscope studies it was suggested that the organism was a true bacterium, belonging to the Gracilicute division (Garnier and Bové, 1978). All attempts to isolate and culture the organism on artificial medium and proof Koch's postulates have been unsuccessful (Garnier and Bové, 1993). The development of monoclonal antibodies (MA) using extracts of infected plants (Martin-Gros *et al.*, 1987) enabled researchers to show that there is considerable serological diversity (Garnier *et al.*, 1987, 1991). One MA (MA 1A5) was able to recognize all non-Chinese Asian strains, but not the African strain (Gao *et al.*, 1993).

The next step in the study of the HLB bacterium was the use of DNA probes. DNA digests from infected plants were inserted into a bacteriophage; one of the inserts, In 2.6, hybridizes with all Asian strains tested, but not the African form, at high stringency (Villechanoux *et al.*, 1992). This insert contains genes for conserved ribosomal proteins (Villechanoux *et al.*, 1993). In 1994, Jagoueix *et al.,* proposed, on the basis of the sequence of the 16S rDNA and the ß operon, that the bacterium responsible for HLB is a member of the subdivision of the *Proteobacteriacea*, Subsequently the Asian species was designated "*Candidatus* Liberobacter asiaticum", and the African species "*Candidatus* L. africanum". These names have since been corrected to "*Candidatus* Liberibacter asiaticus" and "*Candidatus* L. africanus" (Garnier *et al*., 2000b).

6. Host range

All species of citrus appear to be susceptible, irrespective of the rootstock used (Aubert, 1993, da Graça, 1991). However, symptoms are often severe on sweet orange, mandarins and their hybrids; moderate on grapefruit, lemon and sour orange; while lime, pummelo and trifoliate orange are regarded as being more "tolerant" (Manicom and van Vuuren, 1990). Both species of liberibacter have been transmitted to periwinkle (*Catharanthus roseus*) via dodder inducing marked foliar yellowing (Garnier and Bové, 1983, Ke *et al.*, 1988), the dodder itself also appears to support HLB multiplication (Ghosh *et al.*, 1977).

The psylla species which transmit HLB from citrus to citrus, feed on many other rutaceous species. *D. citri* has a preference for *Murraya* spp. (Chakraborty *et al.*, 1976), and it has been suggested that *T. erytreae*'s original hosts include *Vepris undulata*, *Clausena anisata* and *Zanthoxylum capense* (Moran, 1978). Su *et al.,* (1995) has reported the detection of Asian HLB by DNA-hybridization in *Severinia buxifolia* and *Limonia acidissima*, and African HLB was detected in *Toddalia lanceolata* (= *Vepris undulata*) (Korsten *et al.*, 1996). The Cape chestnut (*Calodendrum capense*), an ornamental rutaceous tree in South Africa, has been shown to be infected with HLB (Garnier *et al.*, 2000a), subsequently this organism was shown to be a subspecies of the African

form of greening (Garnier *et al.*, 2000b). This third Liberibacter was classified as 'Candidatus Liberibacter africanus subsp. capensis' (Garnier *et al*., 2000b). Phylogenetic analysis demonstrated that the bacterium belongs to the genus 'Candidatus Liberibacter' and further 16S rDNA sequencing together with serological studies, classified the *C. capense* Liberibacter as being more closely related to 'Candidatus L. africanus' than to 'Candidatus L. asiaticus' (Garnier *et al*., 2000b).

7. Detection

Field diagnosis of HLB is difficult because of the non-specific nature of foliar symptoms. Since it is easy to confuse HLB leaf symptoms with nutrient deficiencies, other diseases or stress related factors, positive confirmation with fruit symptoms is often required in the field. Prior to the more recent positive identification of the causal agent with molecular techniques, the only other method for confirmation was inoculation of biological indicators such as sweet orange, Orlando tangelo (Schwarz, 1968a) or Ponkan mandarin (Matsumoto *et al.*, 1968). Following the identification of a fluorescent phenolic compound, gentiosyl-ß-glucoside from fruit albedo or bark extracts, a diagnostic technique for confirmation of HLB was developed (Schwarz, 1968b, van Vuuren and da Graça, 1977). However, this method soon proved non-specific since stressed trees contained the same marker. In addition other similar diseases such as stubborn diseased also contained the same marker (Schwarz, 1970). Alternative detection techniques were subsequently developed for rapid identification of the disease. These included, immunofluorescence microscopy (Korsten *et al*., 1993, 1996), ELISA using monoclonal antibodies (MAs) (Garnier *et al*., 1987, 1991, Gao *et al*., 1993, Korsten *et al*., 1993, 1996) and DNA hybridization (Korsten *et al*., 1996) or PCR (Jagoueix *et al*., 1996, Korsten *et al*., 1996).

The development of monoclonal antibodies allowed for more rapid and sensitive detection, but the specificities of the MAs and the strain diversity of the bacterium made their use for general detection impractical (Villechanoux *et al.,* 1992, 1993, Korsten *et al.*, 1993). At the time the use of DNA probes proved more suitable for detection of HLB in infected citrus material (Korsten *et al*., 1993, Su *et al.,* 1992, Villechanoux, *et al*., 1992). Two DNA probes, ln-2.6 (Villechanoux *et al*., 1992) and AS-1.7 (Planet *et al.*, 1995) containing genes for ribosomal proteins (ß operon), were developed for L. asiaticus and L. africanus respectively. The necessity of using two different probes for detection of L. africanum and L. asiaticus, and the fact that DNA extraction for dot-blot hybridisation is very time consuming, led to the development of additional detection procedures (Jagoueix *et al.*, 1996). The polymerase chain reaction (PCR), first described in the mid-1980s has since become a powerful technique for the selective amplification of DNA or RNA sequences. In the detection of HLB it is necessary to identify the causal agent of the disease unambiguously, rapidly and at a level of infection that is not visually apparent. Ribosomal genes are particularly appropriate targets for PCR-directed identification, as the genes occur in high copy numbers, are highly conserved and are flanked by spacer regions that contain comparatively variable sequences. DNA sequence data on ribosomal genes can be obtained by PCR with broad-range primers (universal primers) that anneal to the highly

conserved ribosomal gene sequences and amplify across regions that contain nucleotide variation. Planet *et al.,* (1995) developed a PCR technique whereby a fragment of the *rplKAJL-rpoBC* operon (b operon) (Jagoueix *et al*., 1994) of the Asian Liberibacter strain from Poona (India) and the African Liberibacter was amplified. This section of the Liberibacter genome represents a conserved region of the 16SrDNA of the Liberibacter spp. Three primers have been developed and are currently commercially used during PCR detection of HLB (Jagoueix 1996, Hocquellet, *et al.*, 1999). Currently the PCR technique is ISO 17025 accredited and is being used for commercial detection of HLB in suspect plant material by Plant Pathology Laboratories, University of Pretoria, South Africa.

8. Control and management

The evidence that a procaryote was the causal organism led to research on the use of tree injections with antibiotics to eliminate the bacteria. Tetracycline hydrochloride had some beneficial effects (Schwarz and van Vuuren, 1970, van Vuuren, 1977, van Vuuren *et al.*, 1977), but proved to be phytotoxic (van Vuuren, 1977), and attention turned to a more soluble less toxic derivative N-pyrrolidinomethyl tetracycline (Buitendag and Bronkhorst, 1983). Due to the potential for re-infection, and high costs, attention turned to vector control (Buitendag and von Broembsen, 1993). Several insecticides against psylla are available, and the development of a trunk application technique has proved effective (Buitendag, 1988). To assist in determining the optimum timing of insecticides, Samways *et al.,* (1986) proposed the placing of sticky yellow traps in orchards which could detect a population threshold; this method has not been widely adopted and scouting is often used.

Several parasitic wasp species attack citrus psylla. *D. citri* is a host for *Tamarixia radiata*, and *T. erytreae* is attacked by *Tetrastichus dryi*. However, the efficacy of these parasites is limited by the existence of hyperparasitic wasps. Only in the Indian Ocean islands of Reunion and Mauritius was success achieved where *T. radiata* and *T. dryi* were introduced without the hyperparasites (Aubert *et al.*, 1984, Quilici, 1988). In Reunion, the use of these parasites, combined with the establishment of disease-free foundation blocks and nurseries, resulted in a dramatic reduction in the incidence of HLB - in 1995, 20 years after the launching of this strategy, only 0.5% of trees surveyed had symptoms (Aubert *et al.*, 1996). *T. radiata*, and another wasp species, *Diaphorencyrtus aligarhensis*, are currently being evaluated in Florida for potential use there (Hoy and Nguyen, 2000). In addition, *D. citri* appears to be an excellent food source for several ladybeetle species (Michaud *et al*., 2002).

The only way to grow citrus productively in countries where the disease has become endemic such as in South Africa and Asia, is by managing the disease using sound integrated pest management strategies (Aubert, 1990,1993, Aubert and Quilici, 1984, Aubert and Xia, 1990, Buitendag, 1991, Buitendag and von Broembsen, 1993, Samways, 1990). In South Africa, Buitendag and von Broembsen (1993) recommend a strategy of providing growers with disease-free nursery trees, focussing on reducing the inoculum by removing infected trees or branches, and following an effective psylla control program. In China, there are reports of successful management by eradicating

infected trees and non-citrus psylla hosts, planting HLB-free trees, and controlling psylla populations (Ke and Xu, 1990, Xu *et al.*, 1991). Bové *et al.,* (2000) conducted a program in Indonesia, and showed that if citrus is eradicated before replanting, only HLB-free budwood is used for replanting and the control *D. citri* using insecticide sprays is effective, rehabilitation of a citrus industry could be possible. Eradication of alternate hosts in close proximity (5 km) to nurseries or commercial plantings of citrus, have been suggested and shown to be effective in Asia where *Murraya* spp are the principal alternate hosts (Aubert 1990, 1993, Aubert and Xia 1990). However, this approach is impractical in the African continent as natural forest species are hosts to both the HLB organism and the psylla vector.

In cases where the disease is not present, effective quarantine measures are essential to prevent the introduction of the HLB organism or the vector. Since numerous alternative hosts have been reported, it is essential to include them in any preventative quarantine strategy. Risk assessment studies have become essential for Sanitary and Phytosanitary issues in international trade. In addition perceived risks in terms of biological warfare, has become a reality and HLB has been listed as a national biological threat for the USA even before the September 11th, 2001 terrorist attacks.

Despite the fact that *D. citri* has been present in Brazil for several decades, HLB has not been detected there. Furthermore, *D. citri* was recently discovered in Florida (Knapp *et al.*, 1998) and Texas (French *et al*., 2001), and *T. erytreae* in Madeira Island (Jagoueix *et al.,* 1996). This constitutes a major risk for the respective citrus industries if the pathogen should ever be introduced. Preventative measures require a thorough risk assessment study and effective quarantine regulations. The possibility furthermore exist that the vector could be introduced "naturally" or through alternate hosts such as *Murraya* spp., which has recently been identified by the plant quarantine regulatory authorities in the USDA APHIS/PPQ data base records show 40 interceptions of live *D. citri* at ports between 1985 and 1998. One interception contained 46 live *D. citri* from India. The majority of these interceptions were on *Murraya* spp., especially *M. koenigii* (Halbert, 1998). This poses a potential threat for the local industry since the adult *D. citri* can transmit the disease since it can persist in the vector for up to three months, thus highlighting the importance of an effective risk management plan.

9. Conclusion and future prospects

HLB can be considered one of the classic diseases in the history of plant pathology. Its checkered history, riddled with incorrect assumptions, cultural and social differences and economic impact, provides valuable insight into human-insect-plant disease ecology. The global spread of the disease in its two forms with different vectors provides a challenge to countries that are currently HLB-free, such as Australia, Mediterranean countries and the Americas, to retain their status through effective quarantine. For countries that have the less severe African form, prevention of acquiring the more severe Asian form is crucial. The spread of the vectors across international boundaries is especially difficult, C. N. Roistacher (pers.comm.), in reference to tristeza and its aphid vectors, commented that laws can be enacted forbidding moving pathogens and infected plants across borders, but "someone forgot to tell the insects".

The successful biocontrol program in Reunion has raised hopes that the vectors can be controlled in other areas (Hoy and Nguyen, 2000), provided no hyperparasites are present. The recent reports of native predators feeding off the recently introduced psylla in Florida (Michaud *et al*., 2002) are encouraging, even though predators were not found to be effective in controlling HLB in South Africa (van den Berg and Deacon, 1987). Another possibility is the introduction of resistance genes. De Lange *et al.,* (1985) began a breeding program in South Africa, but thus far no commercial varieties have appeared. Recently, the gene for bovine lysozyme, an enzyme with anti-bacterial properties, was cloned and introduced into citrus where it was expressed (Yang *et al*., 2001). It may prove to play a future role in controlling bacterial diseases such as HLB and canker in the future.

Risk assessment models used to predict the likelihood of disease introductions into disease-free areas or countries, and Geographical Information Systems, which can provide predictive global maps of how diseases can spread, provide valuable tools to study plant diseases. So far the HLB organism has defied culture in artificial media, but future research groups will periodically re-attempt culturing as new technologies become available. There is still much to be learned about this disease, its vectors, the causal organism, and the control or management of the disease.

10. References

Aubert, B. 1990. Integrated activities for the control of huanglongbing-greening and its vector *Diaphorina citri* Kuwayama in Asia. Proceedings of the 4th Asia Pacific International Conference on Citriculture, Thailand. pp 133-44.

Aubert, B. 1993. Citrus greening disease, a serious limiting factor for citriculture in Asia and Africa. Proceedings of the 4th Congress of the International Society of Citrus Nurserymen, South Africa. pp 134-142.

Aubert, B., Garnier, M., Guillaumin, D., Herbagyandodo, B., Setiobudi, L. and Nurhadi, F. 1985. Greening, a serious threat for the citrus production of the Indonesian archipelago. Future prospects of integrated control. Fruits 40: 549-563.

Aubert, B., Grisoni, M., Villemin, M. and Rossolin, G. 1996. A case study of huanglongbing (greening) control in Reunion. In: "Proceedings of the 13th Conference of the International Organization of Citrus Virologists" (eds. da Graça, J. V., Moreno P. and Yokomi, R. K.), IOCV, Riverside, pp. 276-278.

Aubert, B. and Quilici, S. 1984. Biological control of the African and Asian citrus psyllids (Homoptera: Psylloidea), through eulophid and encyrtid parasites (Hymenoptera: Chalcidoidea) in Reunion Island. In: "Proceedings of the 9th Conference of International Organization of Citrus Virologists. (eds. Garnsey, S. M., Timmer, L. W. and Dodds, J. A.), IOCV, Riverside, pp.100-108.

Aubert, B., Sabine, A., Geslin, P. and Picard, L. 1984. Epidemiology of the greening disease in Reunion Island before and after the biological control of the African and Asian citrus psyllas. Proceedings of the International Society of Citriculture 1: 440-442.

Aubert, B. and Xia, Y.H. 1990. Monitoring flight activity of *Diaphorina citri* on citrus and *Murraya* canopies. Proceedings of the 4th Asia Pacific International Conference on Citriculture, Thailand. pp. 181-87.

Bhagabati, K. N. and Nariana, T. 1980. Interaction of greening and tristeza pathogens in Kagzi lime (*Citrus aurantifolia* (Christm.) Swing.) and their effect on growth and development of

disease symptoms. Indian Phytopathology, 33: 292-295.

Bové, J. M. 1986. Greening in the Arabian peninsula: Toward new techniques for its detection and control. FAO Plant Protection Bulletin, 34: 7-14.

Bové, J. M., Erti Dwiastuti, M., Trivartno, A., Supriyanto, A., Nasli, E., Becu, P. and Garnier, M. 2000. Incidence of huanglongbing and citrus rehabilitation in North Bali, Indonesia. In: "Proceedings of the 14th Conference of the International Organization of Citrus Virologists" (eds. da Graça, J. V., Lee, R. F. and Yokomi, R. K.). IOCV, Riverside, pp 200-206.

Bové, J. M., Bonnet, P., Garnier, M. and Aubert, B. 1980. Penicillin and tetracycline treatment of greening disease-affected citrus plants in the glasshouse, and the bacterial nature of the procaryote associated with greening. In: "Proceedings of the 8th Conference of the International Organization of Citrus Virologists" (eds. Calavan, E. C., Garnsey, S. M. and Timmer, L. W.), IOCV, Riverside, pp 91-102.

Brlansky, R. H. 2000. Blight. In: "Compendium of Citrus Diseases" 2nd Ed. (eds. Timmer, L. W., Garnsey, S. M. and Graham, J. H.). APS Press, St Paul, pp 65-66.

Buitendag, C. H. 1988. Current trends in the control of greening disease in citrus orchards. Citrus and Subtropical Fruit Journal, 640: 6-7, 10.

Buitendag C. H. 1991. The current status and the control of greening disease of citrus in the Republic of South Africa. Citrus Journal, 1(1): 35-40.

Buitendag, C. H. and Bronkhorst, G. J. 1983. Micro-injection of citrus trees with N-pyrrolidinomethyl tetracycline (PMT) for the control of greening disease. Citrus and Subtropical Fruit Journal, 592: 8-10.

Buitendag, C. H. and von Broembsen, L. A. 1993. Living with citrus greening in South Africa. In: "Proceedings of the 12th Conference of the International Organization of Citrus Virologists" (eds. Moreno, P. , da Graça, J.V. and Timmer, L.W.) IOCV, Riverside, pp 269-273.

CAB International. 2000. Crop Protection Compendium. CAB International, Wallingford, UK.

Calavan, E. C. 1968. A review of stubborn and greening diseases of citrus. In: "Proceedings of the 4th Conference of the International Organization of Citrus Virologists" (ed. Childs, J. F. L.), University of Florida Press, Gainesville, pp 105-117.

Capoor, S. P., Rao, S. P. and Viswanath, S. M. 1967. Diaphorina citri Kuway., a vector of the greening disease of citrus in India. Indian Journal of Agricultural Science, 37: 572-576.

Catling, H. D. 1969. The control of citrus psylla Trioza erytreae (Del Guercio)(Homoptera: Psyllidae). South African Citrus Journal, 420: 9-16.

Cermeli, M., Morales, P. and Godoy, F. 2000. Presencia del psílido asiático de los cítricos Diaphorina citri Kuwayama (Hemiptera:Psyllidae) en Venezuela. Boletín de Entomología Venezolana 15: 235-243.

Chakraborty, N. K., Pandey, P. K., Chatterjee, S. N. and Singh, A. B. 1976. Host preference in Diaphorina citri Kuwayama, vector of greening disease in India. Indian Journal of Entomology, 38: 196-197.

Chen, M.H., Miyakawa, T. and Matsui, C. 1973. Citrus likubin pathogens in the salivary glands of Diaphorina citri. Phytopathology, 63: 194-195.

Chen, Q. 1943. A report of a study on yellow shoot of citrus in Chaoshan. New Agriculture Quarterly Bulletin, 3: 142-175.

Da Graça, J. V. 1991. Citrus greening disease. Annual Review of Phytopathology, 29: 109-136.

Davis, R. I., Jacobson, S. C., Rahamma, S. and Gunua, T. G. 2000. Surveillance for citrus huanglongbing (greening) disease in New Guinea and north Queensland. Australasian Plant Pathology, 29: 226.

De Lange, J. H., Vincent, A. P. and Nel, M. 1985. Breeding for resistance to greening disease in citrus. Citrus and Subtropical Fruit Journal, 614: 6-9.

French, J. V., Kahlke, C. J. and da Graça, J. V. 2001. First record of the Asian citrus psylla,

Diaphorina citri Kuwayama (Homoptera:Psyllidae) in Texas. Subtropical Plant Science, 53: 4-8.

Gao, S., Garnier, M. and Bové, J. M. 1993. Production of monoclonal antibodies recognizing most strains of the greening BLO by *in vitro* immunization with an antigenic protein purified from the BLO. Proceedings of the 12th Conference of the International Organization of Citrus Virologists (eds. Moreno, P., da Graça, J. V. and Timmer, L. W.). IOCV, Riverside, pp 244-249.

Garnier, M. and Bové, J. M. 1978. The organism associated with citrus greening disease is probably a member of the Schizomycetes. Zentralblatt für Bakteriologie Parasitenkunde Infektionskrankheiten und Hygiene Reiha, A 241: 221-222.

Garnier, M. and Bové, J. M. 1983. Transmission of the organism associated with the citrus greening disease from sweet orange to periwinkle by dodder. Phytopathology, 73: 1358-1363.

Garnier, M. and Bové, J. M. 1993. Citrus greening disease and the greening bacterium. In: "Proceedings of the 12th Conference of the International Organization of Citrus Virologists" (eds. Moreno, P., da Graça, J. V. and Timmer, L. W.) IOCV, Riverside, pp 212-219.

Garnier, M. and Bové, J. M. 1996. Distribution of the huanglongbing (greening) liberobacter species in fifteen African and Asian countries. In: "Proceedings of the 13th Conference of the International Organization of Citrus Virologists" (eds. da Graça, J. V., Moreno, P. and Yokomi, R. K.) IOCV, Riverside, pp 388-391.

Garnier, M. and Bové, J. M. 2000. Huanglongbing in Cambodia, Laos and Myanmar. In: "Proceedings of the 14th Conference of the International Organization of Citrus Virologists" (eds. da Graça, J. V., Lee, R. F. and Yokomi, R. K.) IOCV, Riverside, pp 378-380.

Garnier, M., Bové, J. M., Jagoueix-Eveillard, S., Cronje, C. P .R., Sanders, G. M., Korsten, L. and Le Roux, H. F. 2000a. Presence of A*Candidatus* Liberibacter africanus@ in the Western Cape province of South Africa. In: "Proceedings of the 14th Conference of the International Organization of Citrus Virologists" (eds. da Graça, J. V., Lee, R. F. and Yokomi, R. K.). IOCV, Riverside, pp 369-372.

Garnier, M., Gao, S. J., He, Y. L., Villechanoux, S., Gandar, J. and Bové, J. M. 1991. Study of the greening organism (GO) with monoclonal antibodies: Serological identification, morphology and serotypes and purification of the GO. In: "Proceedings of the 11th Conference of the International Organization of Citrus Virologists" (eds. Brlansky, R. H., Lee, R. F. and Timmer, L. W.). IOCV, Riverside, pp 428-435.

Garnier, M., Jagoueix-Eveillard, S., Cronje, C. P. R., Le Roux, H. F. and Bové, J. M. 2000b. Genomic characterization of a liberibacter present in an ornamental Rutaceous tree, *Calodendrum capense*, in the Western Cape province of South Africa. International Journal of Systematic and Evolutionary Microbiology 50: 2119-2125.

Garnier, M., Jagoueix, S., Toorawa, P., Grisoni, M., Mallesard, R., Dookun, A., Saumtally, S., Autrey, J. C. and Bové, J. M. 1996. Both huanglongbing (greening) Liberobacter species are present in Mauritius and Reunion. In: "Proceedings of the 14th Conference of the International Organization of Citrus Virologists" (eds. da Graça, J. V., Moreno, P. and Yokomi, R. K.). IOCV, Riverside, pp 392-394.

Garnier, M., Martin-Gros, G. and Bové, J. M. 1987. Monoclonal antibodies against the bacterial-like organism associated with citrus greening disease. Annales de l=Institut Pasteur/Microbiologie 138: 639-650.

Ghosh, S. K., Giannotti, J. and Louis, C. 1977. Multiplication intense des procaryotes associés aux maladies de type Agreening@ des agrumes dans les cellules criblées de Cuscutes. Annales des Phytopathologie, 9: 525-530.

Green, G. C. and Catling, H. D. 1971. Weather induced mortality of the citrus psylla, *Trioza erytreae* (Del Guercio) (Homoptera:Psyllidae), a vector of greening virus in some citrus

production areas of Southern Africa. Agricultural Meteorology, 8: 305-317.

Halbert, S. 1998. Asian citrus psyllids and greening disease of citrus. Pest alert: A literature review. Entomology LR-ACP/CG-1 Florida Department of Agriculture and Consumer Services.

Hector, J. M. 1944. Conference on "greening" disease of citrus. Citrus Grower, 120: 3-7.

Huang, C.H., Chen, M.J. and Chiu, R.J. 1980. Separation of a mycoplasma-like organism from the likubin complex in citrus. Plant Disease, 64: 564-566.

Hocquellet, A., Toorawa, P., Bové, J. M. and Garnier, M. 1999. Detection and identification of the two "*Candidatus* Liberobacter" species associated with citrus huanglongbing by PCR amplification of ribosomal protein gene of the ß operon. Molecular and Cellular Probes, 13: 373-379.

Hoy, M. A. and Nguyen, R. 2000. Classical biological control of Asian citrus psylla. Citrus Industry, 81 (12): 48-50.

Jagoueix, S., Bové, J. M. and Garnier, M. 1994. The phloem-limited bacterium of greening disease of citrus is a member of a subdivision of the Protobacteria. Current Microbiology, 44: 379-386.

Jagoueix, S., Bové, J. M. and Garnier, M. 1996. PCR detection of the two 'Candidatus' liberobacter species associated with greening disease of citrus. Molecular and Cellular Probes, 10: 43-50.

Ke, C. and Xu, C.F. 1990. Successful integrated management of huanglongbing disease in several farms of Guangdong and Fujian by combining early eradication with targeted insecticide spraying. In: "Proceedings of the 4th Asia-Pacific Conference on Citrus Rehabilitation" (eds. Aubert, B., Tontyporn, S. and Buangsuwon, D.). FAO-UNDP, Chiang-Mai, Thailand, pp 145-148.

Ke, S., Li, K. B., Ke, C. and Tsai, J. H. 1988. Transmission of the huanglongbing agent from citrus to periwinkle by dodder. In: "Proceedings of the 10th Conference of the International Organization of Citrus Virologists" (eds. Timmer, L. W., Garnsey, S. M. and Navarro, L.). IOCV, Riverside, pp 258-264.

Knapp, J., Halbert, S., Lee, R., Hoy, M., Clark, R. and Kessinger, M. 1998. The Asian citrus psyllid and citrus greening disease. Citrus Industry, 79 (10): 28-29.

Korsten, L., Sanders, G. M., Su, H. J., Garnier, M., Bové, J. M. and Kotzé, J. M. 1993. Detection of citrus greening-infected citrus in South Africa using a DNA probe and monoclonal antibodies. In: "Proceedings of the 12th Conference of the International Organization of Citrus Virologists" (eds. Moreno, P., da Graça, J. V. and Timmer, L. W.). IOCV, Riverside, pp 224-232.

Korsten, L., Jagoueix, S., Bové, J. M. and Garnier, M. 1996. Huanglongbing (greening) detection in South Africa. In: "Proceedings of the 13th Conference of the International Organization of Citrus Virologists" (eds. da Graça, J .V., Moreno, P. and Yokomi, R. K.). IOCV, Riverside, pp 395-398.

Labuschagne, N. and Kotzé, J. M. 1984. Effect of temperature on expression of greening disease symptoms and possible disease inactivation of the pathogen in Eureka lemon. Phytophylactica, 20: 177-178.

Laflèche, D. AND Bové, J. M. 1970. Mycoplasma type structures in orange leaves with greening disease. Comptes Rendus des Séances de l'Académie des Sciences, Paris, Série, D 270: 1915-1917.

Lallemand, J., Fos, A. and Bové, J. M. 1986. Transmission de la bacterie associé à la forme africaine de la maladie du Agreening@ par le psylle asiatique *Diaphorina citri* Kuwayama. Fruits, 41: 341-343.

Lee, H. A. 1921. The relation of stocks to mottled leaf of citrus leaves. Philippines Journal of Science, 18: 85-95.

Lin, K.-H. 1963. Further studies on citrus yellow shoot. Acta Phytophylactica Sinica, 2: 243-

251.

Manicom, B. Q. and van Vuuren, S. P. 1990. Symptoms of greening disease with special emphasis on African greening. In: "Proceedings of the 4th International Asia-Pacific Conference on Citrus Rehabilitation, (eds. Aubert, B., Tontyporn, S. and Buangsuwon, D.). FAO-UNDP, Chiang-Mai, Thailand, pp127-131.

Martin-Gros, G., Iskra, M. L., Garnier, M., Gandar, J. and Bové, J. M. 1987. Production of monoclonal antibodies against phloem limited prokaryotes of plants: A general procedure using extracts from infected periwinkles as immunogen. Annales de l'Institut Pasteur/ Microbiologie, 138: 625-637.

Martinez, A. L. 1972. Combined effects of greening and seedling yellows pathogens in citrus. In: "Proceedings of the 5th Conference of the International Organization of Citrus Virologists" (ed. Price, W.C.). University of Florida Press, Gainesville, pp 25-27.

Martinez, A. L. and Wallace, J. M. 1967. Citrus leaf mottle-yellows disease in the Philippines and transmission of the causal virus by a psyllid, *Diaphorina citri*. Plant Disease Reporter, 51: 692-695.

Martinez, A. L. and Wallace, J. M. 1968. Studies on leaf mottle-yellows disease of citrus in the Philippines. In: "Proceedings of the 4th Conference of the International Organization of Citrus Virologists' (ed. Childs, J. F. L.). University of Florida Press, Gainesville, pp 167-176.

Massonie, G., Garnier, M. and Bové, J. M. 1976. Transmission of Indian citrus greening by *Trioza erytreae* (Del Guercio), the vector of South African greening. In: "Proceedings of the 7th Conference of the International Organization of Citrus Virologists" (ed. Calavan, E.C.). IOCV, Riverside, pp.18-20.

Matsumoto, T., Su, H. J. and Lo, T. T. 1968. Likubin. In: "Indexing Procedures for 15 Virus Diseases of Citrus. USDA Agriculture Research Services Handbook" No. 333: 63-67.

McClean, A. P. D. and Oberholzer, P. C. J. 1965. Citrus psylla, a vector of greening disease of sweet orange. South African Journal of Agricultural Science, 8: 297-298.

McClean, A. P. D. and Schwarz, R. E. 1970. Greening or blotchy-mottle disease of citrus. Phytophylactica, 2: 177-194.

McClean, A. P. D., Schwarz. R. E. and Oberholzer, P. C. J. 1969. Greening disease of citrus in South Africa. Proceedings of the 1st International Citrus Symposium, pp 1421-1425.

Michaud, J. P., McCoy, C. W. and Futch, S. H. 2002. Ladybeetles as biological control agents in citrus. Citrus Industry, 83 (3): 24-27.

Moll, J. N. and Martin, M. M. 1973. Electron microscope evidence that citrus psylla (*Trioza erytreae*) is a vector of greening disease in South Africa. Phytophylactica, 5: 41-44.

Moran, V. C. 1978. Preliminary observations on the choice of hosts plants by adults of the citrus psylla, *Trioza erytreae* (Del Guerico) (Homoptera:Psyllidae). Journal of the Entomological Society of Southern Africa, 31: 45-54.

Moreno, P., da Graça, J. V. and R. K.Yokomi. 1996. Preface. In: "Proceedings of the 13th Conference of the International Organization of Citrus Virologists" (eds. da Graça, J. V., Moreno, P. and Yokomi, R. K.). IOCV, Riverside, pp. v-vi.

Oberholzer, P. C. J., Von Staden, D. F. A. and Basson, W. J. 1965. Greening disease of sweet orange in South Africa. In: "Proceedings of the 3rd Conference of the International Organization of Citrus Virologists" (ed. Price, W. C.). University of Florida Press, Gainesville, pp 213-219.

Ôtake, A. 1990. Bibliography of citrus greening disease and its vectors attached with indices, and a critical review on the ecology of the vectors and their control. Japanese International Cooperation Agency, 161 p.

Planet, P., Jagoueix, S., Bové, J. M. and Garnier, M. 1995. Detection and characterization of the African citrus greening Liberobacter by amplification, cloning and sequencing of the *rpl*KAJL-

*rpo*BC operon. Current Microbiology, 30: 137-141.

Quilici, S. 1988. Biological control of citrus psylla in Reunion Island. In: "Proceedings of the 2nd FAO-UNDP Greening Workshop, Lipa, Philippines" (eds. Aubert, B., Ke, C. and Gonzales, C.), pp 39-42.

Reinking, O. A. 1919. Diseases of economic plants in southern China. Philippines Agriculture, 8: 109-135.

Raychaudhuri, S. P., Nariani, T. K., Ghosh, S. K., Viswanath, S. M. and Kumar, D. 1974. Recent studies on citrus greening in India. In: "Proceedings of the 6th Conference of the International Organization of Citrus Virologists" (eds. Weathers, L. G. and Cohen, M.). University of Florida Press, Gainesville, pp 53-57.

Roistacher, C. N. 1996. The economics of living with citrus disease: Huanglongbing (greening) in Thailand. In: "Proceedings of the 13th Conference of the International Organization of Citrus Virologists" (eds. da Graça, J. V., Moreno, P. and Yokomi, R. K.). IOCV, Riverside, pp 279-285.

Salibe, A. A. and Cortez, R. E., 1966. Studies on the leaf mottling disease of citrus in the Philippines. FAO Plant Protection Bulletin, 14: 141-144.

Samways, M. J. 1990. Biogeography and monitoring outbreaks of the African citrus psylla, *Trioza erytreae* (Del Guercio). In: "Proceedings of the 4th Asia Pacific International Conference on Citriculture" (eds. Aubert, B., Tontyporn, S. and Buangsuwon, D). FAO-UNDP, Chiang-Mai, Thailand. pp 188-97.

Samways, M. J., Tate, B. A. and Murdoch, E. 1986. Monitoring the citrus thrips and psylla using fluorescent yellow sticky traps - a practical guide. Citrus and Subtropical Fruit Journal, 629: 9-15.

Schneider, H. 1968. Anatomy of greening diseased sweet orange trees. Phytopathology, 58: 1155-1160.

Schwarz, R. E. 1964. An insect-transmissible virus trapped on sweet orange seedlings in orchards where greening disease is common. South African Journal of Agricultural Science, 7: 885-889.

Schwarz, R. E. 1967. Results of a greening survey on sweet orange in the major citrus growing areas of the Republic of South Africa. South African Journal of Agricultural Science, 10: 471-476.

Schwarz, R. E. 1968a. Indexing of greening and exocortis through fluorescent marker substance. In: "Proceedings of the 4th Conference of the International Organization of Citrus Virologists" (ed. Price, W. C.). University of Florida Press, Gainesville, pp 118-124.

Schwarz, R. E. 1968b. The distribution of greening in citrus areas of South Africa. In: "Proceedings of the 4th Conference of the International Organization of Citrus Virologists" (ed. Price, W. C.). University of Florida Press, Gainesville, pp 124-127.

Schwarz, R. E. 1970. Comparative indexing of the annual and seasonal incidence of greening in sweet orange fruits by external symptoms and by the albedo fluorescence test. Phytophylactica, 2:1-16.

Schwarz, R. E. and van Vuuren, S. P. 1970. Decreases in fruit greening of sweet orange by trunk injections with tetracyclines. Plant Disease Reporter, 55: 747-750.

Su, H. J., Hung, T. H. and Tsai, M. C. 1992. Detection of the fastidious bacteria causing the Asian citrus greening by DNA probes. In: "Proceedings of the 12th Conference of the International Organization for Citrus Virologists" (eds. Moreno, P., da Graça, J. V. and Timmer, L. W.). IOCV, Riverside, p 466.

Su, H. J., Hung, T. H. and Lim, W. H. 1995. Infection and spreading of citrus greening. In: "Abstracts of the International Symposium on Integrated Management of Insect-Borne Virus Diseases of Tropical Fruits", FFTC/ASPAC, Taipei, p 29.

Tirtawidjaja, S. 1980. Citrus virus research in Indonesia. In: "Proceedings of the 8th Conference

of the International Organization of Citrus Virologists" (eds. Calavan, E. C., Garnsey, S. M. and Timmer, L. W.). IOCV, Riverside, pp 129-132.

Tirtawidjaja, S., Hadiwidjaja, T., and Lasheen, A. M. 1965. Citrus vein phloem degeneration virus, a possible cause of citrus chlorosis in Java. Proceedings of the American Society for Horticultural Science, 86: 235-243.

Van den Berg, M. A. and Deacon, V. E. 1987. Predators of the citrus psylla, *Trioza erytreae* (Hemiptera:Triozidae), in the lowveld and Rustenburg areas of Transvaal. Phytophylactica, 19: 285-289.

Van der Merwe, A. J. and Andersen, F. G. 1937. Chromium and manganese toxicity. Is it important in Transvaal citrus greening? Farming in South Africa, 12: 439-440.

Van Vuuren, S. P. 1977. The determination of optimal concentration and pH of tetracycline hydrochloride for trunk injection of greening-infected citrus trees. Phytophylactica, 9: 77-81.

Van Vuuren, S. P. and da Graça, J. V. 1977. Comparison of the thin layer chromatographic methods for indexing citrus greening disease. Phytophylactica, 9: 91-96.

Van Vuuren, S. P., Moll, J. N. and da Graça, J. V. 1977. Preliminary report on extended treatment of citrus greening with tetracycline hydrochloride by trunk injection. Plant Disease Reporter, 61: 358-359.

Van Vuuren, S. P., van der Vyver, J. B., Luttig, M. and da Graça, J. V. 2000. Low incidence of huanglongbing fruit symptoms in Valencia sweet orange in the presence of a population of citrus tristeza virus. In: "Proceedings of the 14th Conference of the International Organization of Citrus Virologists" (eds. da Graça, J. V., Lee, R. F. and Yokomi, R. K.). IOCV, Riverside, pp 373-377.

Villechanoux, S., Garnier, M., Renaudin, J. and Bové, J. M. 1992. Detection of several strains of the bacterium-like organism of citrus greening disease by DNA probes. Current Microbiology, 24: 89-95.

Villechanoux, S., Garnier, M., Laigret, F., Renaudin, J. and Bové, J. M. 1993. The genome of the non-cultured, bacterial-like organism associated with citrus greening disease contains the *nus*G-*rpl*KAJL-*rpo*BC gene cluster and the gene for a bacteriophage type DNA polymerase. Current Microbiology, 26: 161-166.

Xu, C.F., Wang, D. C. and Ke, C. 1991. A report of implementation of integrated control programme of citrus huanglongbing aiming at renovating old infected orchard in epidemic zone and protecting new non-infected zone in non-epidemic zone. In: "Proceedings of the 6th International Asia Pacific Workshop on Integrated Citrus Health Management" (eds. Ke, C. and Shamsudin Osman). FAO-UNDP-MARDI, Kuala Lumpur, Malaysia, pp 55-61.

Yang, Z. N., Herron, C. M., Molina, J. J., da Graça, J. V. and Mirkov, T. E. 2001. Efficient genetic transformation of citrus for potential resistance to plant pathogenic viruses, bacteria and fungi. (Abstract). Phytopathology, 91: S97.

Zhao, X. Y. 1981. Citrus yellow shoot disease (Huanglongbing) - a review. Proceedings of the International Society of Citriculture, 1: 466-469.

Diagnosis and Management of Certain Important Fungal Diseases of Citrus

S.A.M.H.Naqvi

National Research Centre for Citrus, Indian Council of Agricultural Research,
PO Box 464, Amravati Road,
NAGPUR 440 010 (Maharashtra), India

Abstract : Citrus is susceptible to a number of fungal pathogens causing incalculable losses to the crop. Occurrence of a particular pathogen, its ability to cause disease, survival and subsequent spread to cross threshold level in order to damage the crop are governed by agro-climatic conditions, varietal susceptibility, soil type etc. Among fungal diseases, the soil borne diseases of Citrus are widespread whereas other foliar diseases are climate dependent. Once the soil-borne pathogen enters in a given locality, in presence of susceptible hosts and favourable environmental conditions for its growth and multiplication, it becomes the endemic problem of the area. Once the soil-borne pathogen is established, it is difficult to eradicate. The chemical treatments to control the pathogen become a recurring costly affair and environment concern. Regular use of chemical in control of soil-borne pathogens may also lead in development of resistance. Among the soil borne pathogens, the diseases caused by *Phytophthora* species are wide spread in all the citrus growing belts of the world and cause incalculable losses. Dry root rot caused by *Fusarium* species is also a serious problem in certain countries. To manage the soil borne pathogens, it is essential to raise or select disease free plant material. Wherever possible quarantine and sanitary measures should be followed to avoid introduction of disease. Good drainage and aeration of soil should be maintained for healthy and adequate growth of root system. Resistant and compatible rootstock should always be used considering the diseases prevalent in the locality. Correct diagnosis and regular monitoring of the diseases are required for their quick and economically feasible management. The use of fungicides in control of Citrus diseases is justified only if significant losses are anticipated and no other cultural options left to manage the disease. For foliar diseases, weather monitoring and its record in relation to disease eruption in earlier seasons will be helpful in developing strategies in control of the diseases. Predisposing factors for disease development which can be averted with cultural operations should be minimized or eliminated to control the diseases.

1. Introduction

Citrus is considered to be native of Himalayan foot hills of North-Eastern India, North Central China and its adjoining area from where a number of Citrus species/ varieties have their origin and later taken to different parts of the world. Presently Citrus has become an important fruit crop in world trade for fresh fruits and its processed products / by products with about 102.64 million tons total world production of 2001. More than 135 countries are growing Citrus commercially in different agro-climatic conditions for its diversified use and increasing demand world over. The countries having major

contribution to world Citrus production are Brazil (17.91 %), USA (14.32 %), China (11.70 %), Mexico (6.15 %), Spain (5.39 %) and India (4.74%). Thus, these top Citrus producers contribute more than 60 % to the world Citrus production. Citrus occupies an important place in the horticultural wealth and economy of India as the third largest fruit industry after banana and mango producing approximately 4.87 million tons per annum (FAOSTAT, 2002, Singh and Naqvi, 2001).

Identification of the diseases and their proper management is an important aspect for successful cultivation of any crop. Disease management in perennial crops differs from the annual ones in many respects where besides the prophylactic measures, the cure of individual plant is very important. Each and every plant requires due attention for its protection or cure from the diseases. Disease problems in Citrus, if not attended properly, may become a limiting factor for its successful cultivation. A number of fungal, viral and few bacterial pathogens right from nursery level to bearing stage attack citrus plants. *Citrus* spp. are prone to the attack of more than 100 diseases and disorders. Abiotic disorders are caused by adverse nutritional status, environmental conditions, inappropriate cultural practices etc. Fortunately, due to the geographical isolation, different climatic and edaphic factors, cultivation of wide range of Citrus cultivars, only a few diseases in any Citrus growing area cause significant damage and require due attention for their effective management. In this chapter, management of certain important fungal diseases of Citrus is discussed those are not covered in other chapters in this book and may become a limiting factor to sustain optimum production of Citrus in a given area.

2. *Phytophthora* diseases of Citrus

Phytophthora is known to cause destructive plant diseases much before its discovery in real scientific terms. Association of *Phytophthora* in destruction of Citrus plants was recorded in 1836 when highly flourishing Citrus plants of 200 to 300 years age started disappearing in Azore island, much before the famous potato famine of Ireland in 1845 and after 31 years of famine, in 1876, Anton de Bary described the fungus as *Phytophthora* means " Plant destroyer". He described potato late blight fungus, *Phytophthora infestans* (Montag) de Bary as the type species for the new genus. *Phytophthora* species are classified in the Kingdom Stramenopila and the phylum Oomycota and are actually more closely related to the golden brown algae than the true fungi (Gunderson *et al.*1987, Chesnick *et al.*, 1996). Species of *Phytophthora* have a number of unusual features that distinguish them from many other phytopathogenic fungi (Zentmyer,1983).

Bonavia (1888) probably reported the first *Phytophthora* epidemic of citrus in Azore island during 1832- 1836. Later *Phytophthora* epidemics of Citrus were reported in 1841 from France, 1845 in Portugal, 1855-1889 killed all lemon trees in Italy, 1869-1880 killed all lemon and citron trees in Greece, 1860 – 1879 in Australia, 1871 in Spain, 1875 in California, 1876 in Florida, 1906 in Cuba, 1911 in Paraguay, 1917 in Brazil and 1920 in Mexico and in 1935 in Trinidad (Fawcett, 1936). *Phytophthora* diseases were the most serious of all citrus diseases and decimated citrus worldwide, especially the seedling trees of sweet orange, grapefruit and lemon which were highly susceptible to the fun-

gus. Fawcett first reported that the fungus caused the Citrus gummosis (Fawcett, 1913,1936). An extensive damage was done by the fungus to the citrus plantation in Florida on rough lemon during 1920 – 1940 and in Australia where citrus plantation was replaced with *Phytophthora* resistant rootstocks like trifoliate orange and Benton citrange (Fraser, 1942, Broadbent, 1977).

Citrus group of fruits has been the matter of attraction to mankind for their various uses before the recorded history. Ancient Chinese writings reveal that the history of Chinese citriculture is over 4000 year old. During that period, Citrus plants mostly grew in their wild forms in their natural habitat in and around the land of their origin. Latest evidences and occurrence of certain *Citrus* species in wild forms suggest the origin of citrus in Asia which extends from the Himalayan foothills of North-eastern India to North Central China, Myanmar, Thailand, Indonesia in South-east. Later discoverers, conquerors and some of great explorations helped in dissemination of Citrus from its natural home to other parts of the world (Awtar Singh *et al.*2002). Before the invention of the terrarium in 1827 by Dr Nathanial Ward, probably the citrus is disseminated from its natural home to other parts of the world through seeds. Thus the seedling trees were virtually free from viruses and *Phytophthora,* having highly productive life up to 200 to 300 years or more. The invention and introduction of the Wardian case or terrarium markedly enhanced the worldwide distribution of *Phytophthora*. For the first time plants alongwith the soil could be transported long distances anywhere in the world. Thus the shipment of plants with soil also transported and disseminated dangerous *Phytophthora* species worldwide. It is probable that the great potato blight famine caused by *Phytophthora infestans* was caused by the introduction of this fungus in soil through Wardian case. Similarly, citrus *Phytophthora* was probably also introduced causing the worldwide epidemics and destruction of citrus seedling trees. The first appearance and notice of citrus gummosis in Azore islands was probably because Azores islands were the mid-ocean stop for providing vital supplies for ships returning from Africa, Asia, and the Americas (Naqvi, 1999a, Naqvi 1999c).

Phytophthora species are responsible for significant economic losses on many important food, fiber, and ornamental crops (Erwin and Ribiero, 1996.) and one of the major pathogens of many horticultural crops causing incalculable losses. Thousands of fruit trees succumb to *Phytophthora* diseases every year in India. Besides gummosis and brown rot, the root rot caused by *Phytophthora* alone reduces 46 % yield of Citrus plants in California and Citrus industry looses about $ 12.9 million annually due to this pathogen whereas to *P. citrophthora* $ 5 million annually (Anonymous, 1989, Menge, 1993). Recognizing the importance of this devastating pathogen, Indian Council of Agricultural Research has launched a National Network Project on *Phytophthora* diseases of Horticultural Crops (PHYTONET) in 1997 to combat the problem. *Phytophthora* is the major problems of Citrus industry world over. *Phytophthora* species cause most serious diseases of citrus and infect almost every part of citrus plants right from damping off of seedlings in nursery beds, decay of fibrous roots, crown rot, foot rot, gummosis, and brown rot of fruits in groves and as post harvest decay during storage and transport. Recently, *Phytophthora* diseases of citrus and management strategies have been reviewed (Naqvi, 2003).

2.1 Disease symptoms

2.1.1 Nursery diseases

Damping off of seedlings in nursery bed is wide spread problem of Citrus industry and more frequently occurs in field nurseries where maintenance of sanitary measures is difficult. More than 20 % seedling mortality has been observed in Central India due to the disease in nursery beds (Naqvi 1996; Naqvi, 2000a).

  Phytophthora nicotianae, *Phytophthora citrophthora* and *Phytophthora palmivora* cause damping off of Citrus seedlings in nursery beds / trays. Necrosis of tissue and typical damping off of seedling occur due to fungal infection just above the soil line. The pre-emergence rot of seeds and post emergence damping off of seedlings

Figure1: Damping off of seedlings showing mortality in patches in nursery bed

occurs in patches (Fig.1). The seedling mortality increases where excessive soil moisture accompanies the favourable temperature for the causal fungi (Klotz *et al.,* 1966). Pathogens survive in soil through chlamydospores/ oospores. Flood irrigation in flat bed system spreads the pathogen from one bed to the other. The infected / infested nursery stock causes further losses to seedlings in secondary nursery beds. The budded plants show stunted, chlorotic growth with development of poor feeder roots. The infected / infested seedlings in primary nursery beds become the major source of spread of *Phytophthora* diseases to orchards in virgin areas.

2.1.2 Orchard diseases

Phytophthora causes foot rot, root rot, crown rot, gummosis, leaf fall, brown rot and canopy blight diseases in Citrus. Foot rot lesions develop as high as 60 cm from the ground level on the scion portion and may extend up to bud union on resistant root-stock. In case of susceptible rootstocks, the infection may further extend to the soil line as collar rot or below on crown roots as crown rot. On scraping the dead bark of the

Figure 2: Gummosis caused by *Phytophthora nicotianae,* arrow indicates gum oozing and craking of bark

lesion, a brown, discolored, slippery area can be seen. Such active lesions start oozing gum known as gummosis (Fig. 2). In dry season, the dead-bark becomes firm, breaks away from healthy bark, curls and splits. In severe cases, when foot and significant portion of root system is damaged, the large branches of the same side of the affected plant are killed due to the rot of conducting tissues near the bark. Usually the disease is confined to feeder roots. In field, the decay of feeder roots due to *Phytophthora* may be

ascertain by carefully uprooting the affected feeder roots and pulling the affected part of feeder roots by lightly pressing between index finger and thumb. The affected feeder root sloughs the cortex leaving only stele (Fig.3). The feeder root rot due to *Phytophthora* usually remains unnoticed by the growers until the advanced symptoms of gummosis or chlorosis appear of canopy appear. Dull chlorotic foliage is the first symptom of such affected plants where mid rib, main lateral veins and bands of leaf tissue bordering them become yellow leaving rest of the leaf normal in colour. Such vein chlorosis is often confused with nitrogen deficiency. The diseased plants thus have comparatively fewer fibrous roots than healthy plants. In severe cases, where regeneration of feeder root does not cope with the rate of destruction, the affected plant will show starvation, less canopy volume with naked branches, die back, slow decline symptoms and result either in erratic bearing and yield loss or such plants bear heavily
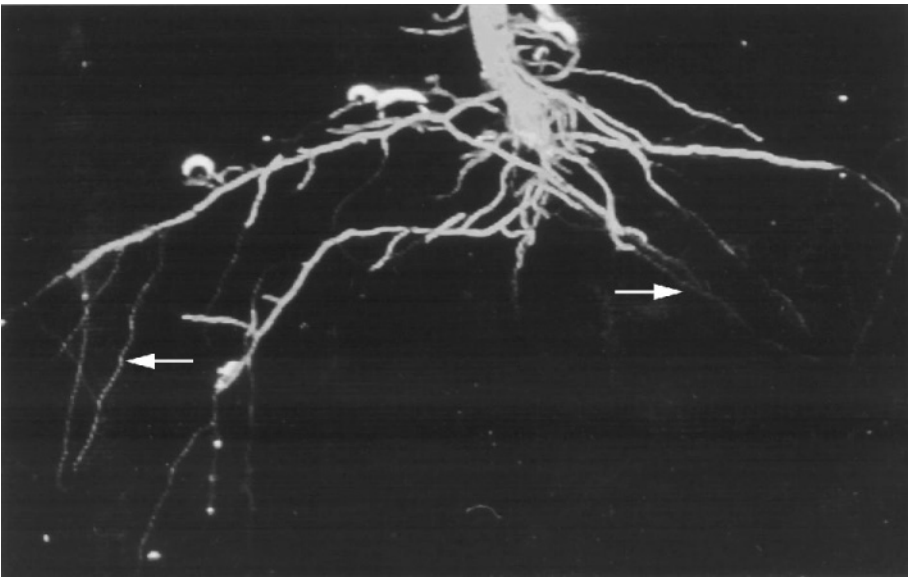
Figure 3: Cortex decay of roots by *Phytophthora* infection leaving the white fibres of stele

and collapse (Fig. 4), when fruits are still on the plant. (Naqvi, 2000a). In continuously wet weather conditions for about 24 hrs or more, *Phytophthora* splashes along with raindrops to low hanging fruits and causes a typical brown rot of fruits and leaf fall. If favourable conditions prolong, the secondary inoculum produced on above ground part of the plant may spread and the whole canopy may be blighted. Such spread of canopy blight is more severe where *P. palmivora* or *P. citrophthora* are prevalent. At some locations the complex of root weevil (*Diaprepes abbreviatus*) damage and *Phytophthora* infection complex makes the disease situation and tree decline faster and more grave. The larvae of weevil feed on the bark of structural roots and develop

injuries as root etching and *Phytophthora* spp. infect the margins of these etchings and destroy the bark and cambium of large roots (Brlansky *et al.,* 1998). *P. palmivora* has been found often associated with destruction of larger roots of citrus in Central India and Andhra Pradesh (Naqvi,2002a, 2002b).

2.2 *Phytophthora* spp. causing Citrus diseases

Figure 4 : Decline of Nagpur mandarin due to *Phytophthora* root and collar rot

Phytophthora spp. viz. . *P. boehmeriae* (Sawada),. *P. cactorum* (Leb. And Cohn) Schroeter, *P. cinnamomi* (Rands), *P. citricola* (Sawada), *P. citrophthora* (R.E.Smith and E.H. Smith) Leonian, *P. dreschleri* (Tucker), *P. hibernalis* (Carne), *P. megasperma* (Drechsler), *P. nicotianae*, Breda de Haan, *(= parasitica* Dastur*), P. palmivora* (Bulter) Butler, and *P. syringae* (Klebahn) Klebahn, have been reported pathogenic on Citrus from different Citrus growing areas of the world (Boccas and Leville, 1978, Erwin and Rebiero, 1996; Naqvi, 2002b). *P. citrophthora* and *P. nicotianae* are wide spread caus-

ing foot rot, root rot, gummosis and brown rot of citrus are common in tropical and sub
tropical areas of the world. *P. hibernalis* and *P. syringae* infect the citrus fruits occa-
sionally in cool and moist winter areas while *P. palmivora* and *P. citricola* are reported
to attack citrus in some tropical areas (Broadbent, 1997; Graham and Timmer, 1992). *P.*
citrophthora P. nicotianae and *P. palmivora* have been reported to cause Citrus dis-
eases in India (Kamat, 1927; Kumbhare and Moghe, 1976; Lele and Kapoor, 1982; Naqvi,
1988; Naqvi,2000b; Uppal and Kamat, 1936), and Florida (Graham and Menge, 2000;
Zitko and Timmer, 1994; Widmer *et al.*, 1998).

Recently, *P. capsici* was found causing severe root rot and brown rot of Khasi
mandarin in Tripura State of India (Naqvi, 2002b). *P. citricola* has been reported to
attack citrus fruits in Mediterranean and subtropical areas. *P. hibernalis* causes severe
gummosis and *P. hibernalis* and *P. syringae* cause brown rot to a limited extent in area
with cool and wet winters, and produce non papillate, deciduous sporangia and their
optimum growth temperature is less than 20°C. In addition to the above species, *P.*
colocasiae (Raciborski) was reported a severe outbreak of Citrus leaf fall of Coorg
mandarin in Kodagu region of Karnataka. *P. colocasiae* was consistently isolated
causing root rot of Coorg mandarin seedlings. Both A_1 and A_2 mating types were
present in this area. Presence of citrus isolate of *P. colocasiae* may be the result of
interspecific natural hybridisation, since the orchards from where the above species
was isolated were having Citrus, Colocasia, Cardamom, Black pepper and Arecanut
plantation. *P. arecae* (Coleman) Pethybridge, was reported causing brown rot of fruits
of *C. medica* and *C. limon* in Karnataka, India (Narasimhan, 1931) and found highly
pathogenic on roots and stems of citrus seedlings and fruit in Florida (Timmer *et al.,*
1990), further confirmation of these species is required using the molecular probes.
Kamat (1927) reported that the gummosis of mosambi was due to *P. palmivora* Bulter in
south India. Later Uppal and Kamat (1936) found *P. palmivora* in Maharashtra causing
citrus decline.

During the survey of Central and peninsular India the presence of *P. palmivora*
causing foot rot and gummosis Maharashtra and Coorg in Karnataka was further con-
firmed (Lele and Kapoor, 1982). Leaf fall of Nagpur mandarin was also reported due to *P.*
nicotianae in Vidarbha (Kumhare and Moghe, 1976). Recent survey of Central India for
Phytophthora diseases of Nagpur mandarin has revealed that besides *P. nicotianae*, *P.*
citrophthora was widely prevalent in the area alongwith *P.palmivora* causing root rot
and crown rot of *Citrus jambhiri* Lush (rough lemon)- a major root-stock of the area and
foot rot and leaf fall of Nagpur mandarin (Naqvi, 1988, Naqvi, 2000b, Naqvi, 2002a,
2002b). *P. citrophthora* has been a serious problem in Australia, USA and other parts of
the world and its presence was suspected in India by Fraser during her survey of Citrus
die back problem in India (Fraser, 1966; Fraser and Singh, 1966). *P. citrophthora* has
also been isolated causing severe diseases of Coorg mandarin. Both the mating type
A1 and A2 of *P. nicotianae* have been recorded from the orchards of Nagpur mandarin
in Nagpur district (Naqvi, 2000b; Naqvi 2002b). *P. parasitica* Dastur (= *nicotianae)* has
been a common species associated with Citrus diseases in Assam (Chowdhury, 1951)
and in North-west India (Bajwa, 1941 and Paracer and Chahal, 1962). Kapur and Bakshi
(1967) reported that 14-18 % decline in sweet orange was due to foot rot in Abohar
(Punjab).

2.3 Distribution of *Phytophthora* spp. in India

Phytophthora species are widely distributed in almost all the citrus growing belts of India and are the major cause of citrus decline (Naqvi, 2002b; Naqvi and Singh, 1999, Naqvi and Singh, 2002). *Phytophthora nicotianae, P. palmivora* and *P. citrophthora* are the main species of common occurrence in Citrus orchards and nurseries in India.

An extensive survey of citrus orchards and nurseries in India has been conducted under National Network Project on *Phytophthora* diseases of Horticulture Crops (Citrus) funded by Indian Council of Agricultural Research during 1997 – 2002. Citrus cultivation belts of Vidarbha and Marathwada region of Maharashtra Punjab, Madhya Pradesh, Andhra Pradesh and North Eastern States of India were surveyed to assess the impact of *Phytophthora* diseases, species involved and propagules density / cc soil (Naqvi,2000b; 2001a, 2002b).

Phytophthora nicotianae A_1 and A_2, *P. citrophthora*, and *P. palmivora* were found frequently associated with Nagpur mandarin, acid lime and Mosambi orchards in Central India with population ranging from 5 to >200-propagules/ cc soil. In Nagpur district, 61.2 % isolates of *P. nicotianae* were A2 type whereas 38.8 % of A1 type (Naqvi, 2000b).

In Madhya Pradesh adjoining to Vidarbha region of Maharashtra, India, 20 - 50 % Nagpur mandarin plants were found affected and causing severe decline due to *P. nicotianae, P. palmivora* and *P. citrophthora* (Naqvi, 2000b). In Andhra Pradesh, 20 – 100 % acid lime plantation was severely affected with *Phytophthora nicotianae* both A1 and A2 mating types were widely distributed in the area along with *P. citrophthora* and *P. palmivora* A2. Kinnow growing areas of Punjab State, 10 – 80 % plants of Jaffa (*C. sinensis* Obsbeck) and 10 – 100 % plants of Kinnow mandarin ranging from 12 – 25 year old, were showing collar rot/ root rot symptoms caused by *Phytophthora nicotianae* and *P. citrophthora* due to excessive flood irrigation (Naqvi, 2002a, 2002b).

Citrus cultivation in northeastern States of India is mostly on seedling trees of Khasi mandarin and the area is supposed to be the natural of home of citrus and place of origin of some *Citrus* species. In Tripura State, the citrus cultivation is mostly confined to Jampui hills and Sankan area about 140 km from Agartala. Almost all the citrus orchards in Jampui hills were infested with *P. nicotianae*, *P. citrophthora, and P. palmivora* causing citrus root rot and leaf fall. In Mezoram State, citrus orchards were found infested/ infected with *P. nicotianae, P. citrophthora* and *P. palmivora* causing gummosis and root rot (Naqvi, 2000b, 2002b). Wide spread of *Phytophthora* spp. in citrus groove caused severe damage to citrus plants at various stages of plant growth in form of root rot, collar rot, crown rot, gummosis and brown rot and appeared to be the major cause of Citrus decline in the surveyed areas. Presence of both the mating types of *P. nicotianae* in Vidarbha region of Maharashtra further poses the threat of natural hybridisation and development of new races in the area. Infested nurseries were the source of disease spread to new virgin areas (Naqvi, 2000b; Naqvi and Singh, 2002).

Phytophthora nicotianae (Breda de Haan) = *P. parasitica* (Dastur) have many morphological similarities and there is dispute in using these both the name for the same species. *P. nicotianae* is preferred in place of *P. parasitica* however, both the name are being used in many publications. *P. nicotianae* produces pear shaped to

spherical, non-deciduous sporangia. Cultures of opposite mating types produce spherical oospores and amphigynous antheridium. In water culture, the shape of sporangium sometimes is variable with hyphal swellings radiating mycelial growth. Isolates of *P. palmivora* from citrus show elliptical to ovoid, prominently papillate sporangia on sympodial sporangiophores. Sporangia are deciduous with short pedicel. *P. palmivora* forms oogonia and oospores when A1 and A2 mating types are paired. It forms spherical oogonia and amphigynous antheridia. *P. citrophthora* produces sporangia of variable shape ranging from spherical to ovoid, obpyriform and ellipsoidal, persistent usually single on irregularly branched sporangiophores. There is often a swelling at the point of sporangiophore branching. Occasionally more than one papillae are observed in sporangia. Chlamydospores and oogonia are not formed in Citrus isolates. However, some isolate may be crossed with other mating types to produce oospores. *P. capsici* produces characteristically ellipsoid, pyriform sporangia with predominantly tapering at the base. These sporangia are formed in sympodial sporangiophores and highly deciduous with long pedicel (much longer than *P. palmivora*). *P. capsici* is predominantly heterothallic and produces oospores with opposite mating types (Erwin and Ribeiro,1996).

All the four species of *Phytophthora* described above causing Citrus diseases in India can easily be distinguished by their morphological characteristics. *P. nicotianae* produces more rounded pear shaped sporangia that are noncaducous; *P. citrophthora* produces ellipsoidal elongated sporangia on irregularly branched sporangiophores with occasional more than one papillae. *P. palmivora* produces ovoid sporangia that are caducous but with short pedicel whereas *P. capsici* is distinguished from other species by production of ellipsoidal to elongated sporangia tapering at base, caducous in nature with long pedicel as compared to *P. palmivora*.

2.4 Isolation and detection of *Phytophthora* spp.

Phytophthora is a slow growing fungus on artificial medium and on routine isolating media used for fungus isolation, other fast growing fungi from the soil or infected plant part usually cover its growth. Hence one can face little difficulty in isolating *Phytophthora* spp. from old, badly decayed and dried tissues at advanced stage of disease or from naturally infested soil and thereby incorrect diagnosis of the causal agent. Leaf or fruit baiting methods are generally used for qualitative detection of the pathogen (Grimm and Alexender, 1973; Klotz *et al.*, 1958b) and may also be used for most-probable-number technique (Tsao, 1960) where required facilities are not available for selective isolation or other advanced techniques. With the development of a number of media for selective isolation of this pathogen, it has become easy to work with *Phytophthora* species. Presently more than 35 selective media have been developed and tried by various workers with the basic concept to suppress fast growing fungi, bacterial contamination including actinomycetes with selective antimicrobial agents, which are non toxic to *Phytophthora*. The topic has been reviewed in detail (Tsao, 1970, 1983).

Mere detection or isolation of *Phytophthora* spp. can be achieved with any selective medium devised for a particular species of *Phytophthora*. But to quantify the propagule density in a particular type of soil or area, it becomes mandatory to select the

best selective medium with required amendments to detect the target species. In our studies to detect and quantify *P. nicotianae, P. palmivora* and *P. citrophthora* in orange groves of Central India in black cotton swell and shrink soil over basaltic alluvium and other parts of India, out of six selective media viz. Modified Kerr's medium (Hendrix and Kuhlman, 1965), McCain's medium (McCain *et al.*, 1967), Masago medium (Masago *et al.*, 1977), PVPH medium (Tsao and Guy, 1977), PARPH medium (Mitchell *et al.,* 1986) and BHMPVR medium (Bist and Nene, 1988), PARPH was the best in detecting maximum number of active propagules /cc soil of *Phytophthora* species infecting citrus with a little modification in concentration of ampicillin and hymexazol. However, hymexazol may be excluded from the medium if it is prepared for the isolation of *P. palmivora,* which is highly sensitive to this chemical like *P. cactorum* and *P. infestans* (Naqvi, 1988; 2000a, 2000b, 2002b).

Specific monoclonal and polyclonal antibodies are also developed to detect *Phytophthora* species in infected plant parts and a small amount of inoculum in soil with ELISA test. ELISA method is very sensitive and quick in detecting the presence of *Phytophthora* species at lower densities (Miller and Martin, 1988, Miller *et al.,* 1990; Skaria and Miller, 1989). However the selective medium is also equally efficient to detect the small amount of inoculum present in the soil (Timmer *et al.,* 1993). Recently, for quick and reliable identification of *Phytophthora* species, internal transcribed spacer (ITS) based identification has been developed to identify known species of *Phytophthora* and unknown isolates quickly (Cooke *et al.,* 2000, Cooke *et al.,* 1996, Crawford *et al.,* 1996, Cooke and Duncan 1997, Lee et al 1993, Levesque *et al.,* 1998, Ristaino *et al.*1994, Trout *et al.*1997). An identification web site has also been developed to facilitate *Phytophthora* workers where user generated ITS digest profiles of any unidentified taxa may be entered into the search page for comparison with the database. The web site (http://www.Phytid.org) includes a database of ITS digest profile of 46 *Phytophthora* species. However, some species remain as polyphylectic assemblages awaiting more rigorous analysis (Brasier and Hansen, 1992). There is a need to optimize PCR assays for detection of different propagules types in soil, if these assays are to be used for precision agriculture application (Ristaino and Gumpertz, 2000). Molecular detection methods of *Phytophthora* and their impacts on plant disease management have been reviewed (Martin *et al.,* 2000).

2.5 *Phytophthora* population in citrus nurseries

Amravati district of Maharashtra, India constitutes the major propagating area of Nagpur mandarin in Central India where 150-250 private and Govt. citrus nurseries supply 5-8 million grafted Nagpur mandarin plants each year to different parts of India. Survey of these nurseries has revealed that almost all the nurseries were infested with *P. nicotianae, P. palmivora* and *P. citrophthora* and about 10 to 15% plants were killed at secondary nursery level due to *Phytophthora* diseases. Predisposing factors for this high population were: i).Use of rough lemon- a susceptible root-stock, ii).Flood irrigation and flat bed system iii). Retention of water for longer period in beds iv). Low budding v).Uniform planting of budded plants at 9" distance without leaving space between the rows vi). Repeated use of same land for nursery raising or location of nurseries near old orchards

vii). Regular contamination through soil and irrigation water and viii). No prophylactic measures (Naqvi,1988, 90, 97, 99b, 99d, 2000a).

2.6 *Phytophthora* population in orchards

It would be difficult to locate any orchard in Central India and other citrus cultivation belts of India, free from *Phytophthora* diseases. *Phytophthora* propagules have been recorded up to 250-350 /cc soil in highly infested orchards. There has been frequent death of plants in non-bearing orchards but toll increases as the plants start bearing. Every year 5- 10 % plants die due to severe root rot in bearing orchards (Naqvi, 1988; 99b, 2000a). Faulty cultural practices such as water stress for 30-45 days during December–January and during May-June followed by flood irrigation to induce flowering disturbs the balance of water uptake of plant with decayed feeder roots and water demand for excessive bearing which results in sudden decline of such plants (Naqvi, 2000b, 2002b). Population may vary from one time of the year to another and the significance of this fluctuating population in relation to root rot and yield loss remains to be determined (Timmer *et al.,* 1989b, Matheron *et al.,* 1997). In most of the citrus orchards of California and Florida, the population ranged from 1 to 20 propagules/ cc soil, but occasionally it may be 100 to 200 propagules/ cc soil. (Menge, 1986; Timmer, *et al.,* 1988, 1989b, Zitko *et al.,* 1987). It is difficult to decide the thresholds level but normally the population > 10 propagules /cc significantly affect the fibrous root density yield when treated with fungicides.(Sandler *et al.,* 1989; Naqvi, 1994 Matheron *et al.,* 1997).

2.7 Epidemiology of *Phytophthora* diseases

Phytophthora inhabits in soil and is a water loving fungus. It survives in soil through small thick walled spores (chlamydospores) and oospores, which can tolerate dry summer conditions. The chlamydospores are developed in cool and dry conditions with limited soil moisture and in paucity of actively growing roots since *Phytophthora* species are primarily parasites but are poor saprophytes in soil. Chlamydospore development also occurs in poorly aerated soil and in high CO_2 concentration in the soil. Resistant spores can survive for several months in unfavourable conditions (Tsao, 1969). Oospore development is not frequent and only occurs when both the mating types are present in the soil. These spores are thick walled and resistant to extreme dry and cool conditions. Besides survival in unfavourable conditions, oospores also become source of variation and development of new races. With onset of monsoon and in optimum temperature (25 - 32°C), flooding or excessive irrigation and soil aeration, these spores germinate indirectly to produce sporangium and zoospores or directly to produce mycelium. Chlamydospores also require nutrients in form of root exudates for germination. These zoospores swim in water for short distance by flagellar movement or may be carried away by rain or irrigation water (Duniway, 1983) and are attracted to root tips (Khew and Zentmeyer, 1973, 1974) and wounds to cause infection. A new generation of sporangia is formed within 24 hrs of their entrance in tissue and again liberates the zoospores to initiate new infection.

        This cycle repeats so far soil remains saturated and other favourable conditions

persist. In this way the reservoir of inoculum accumulates. Thus zoospores are the main propagules responsible for infection and spread of *Phytophthora* between roots or trees (Duniway, 1983). Recently, the mechanisms of dispersal of *Phytophthora* species have been reviewed (Ristaino and Gumpertz, 2000) where five types of mechanisms of dispersal are described for *Phytophthora* species viz. i. Dispersal from root to root in soil involves either root growth to inoculum, inoculum movement to roots or root-to-root contact. ii. Inoculum dispersal in surface water, iii. Splash dispersal from soil to aerial parts of plant, iv. Aerial dispersal from sporulating lesions on leaves, stems or fruit to other aerial parts of plant and v. dispersal by human or invertebrate activity including movement of soil, plants or propagules. All these modes of dispersal occur in *Phytophthora* diseases of citrus. The infested nurseries and infected / infested nursery stocks are the primary source of spread of *Phytophthora* diseases of citrus to virgin areas (Graham and Timmer, 1992; Naqvi,1999a; 1999e, 2000a, 2000c).

Environmental factors play an important role in disease development, severity, dispersal, and survival of *Phytophthora* in causing epidemics (Govindarao, 1954, Cheema *et al.,* 1954). *P. palmivora* thrives best at 25°C –28°C in Maharashtra (Uppal and Kamat 1936). Distribution and activity of *P. palmivora* was related with cooler and humid region of Maharashtra and Coorg where temperature remained below 28°C and rainfall was above 1500 mm, while *P. nicotianae* was prevalent in warmer and drier climate of Vidarbha, Ahmednagar and Kodur at 30° – 32°C temperature and rainfall below 1150-1300 mm (Lele and Kapoor 1982). In Citrus nurseries of Vidarbha region, *P. citrophthora* was active during winter season at 20-24°C temperature and ceased its activity with the increase of temperature while population of *P. nicotianae* and *P. palmivora* increases. The soil temperature in Central India remains between 20 – 29°C through out the year. Thus *Phytophthora* remained active throughout the year in nurseries and in irrigated orchards (Naqvi, 1990, Naqvi,2002b). Soil temperature plays important role in rate of sporangia formation, zoospore production, the duration of zoospore motility and ultimate root colonization by *Phytophthora* species. The critical threshold temperature for *P.citrophthora* was 27°C or above and for *P. nicotiane* 33°C or above in Arizona. A fivefold increase in duration of zoospore motility was observed for *P.citrophthora* at 24°C than at 30°C, temperatures that respectively favor and prevent rootlet colonization while an 11-fold increase was detected for zoospores of *P.nicotianae* at favorable compared to inhibitory soil temperatures of 30 and 36°C, respectively (Matheron and Porchas, 1996).

In Mediterranean climate, cool temperature around 15°C induce dormancy of chlamydospsores of *P. nicotianae* (Lutz and Menge, 1986a,b). In Florida, the soil temperature rarely falls below 15°C and hence population of *P. nicotianae* remains significantly active (Graham and Timmer, 1992). Besides other favourable edaphic factors, in Central and northwest India, *Phytophthora* population also increases with the increase in root mass in three season viz. January-February, May-June and September-October followed by shoot flush. In California, the increase in *P.nicotianae* populations also coincides with a root flush in May, which occurs after the spring shoot flush in March or April (Lutz and Menge, 1986b). Similarly, a spring flush of roots may occur in Florida when soil temperatures rise above 20 – 23°C followed by an increase in *P. nicotianae* populations (Graham and Timmer, 1992). Heavy soil with poor drainage, excessive irri-

gation and prolonged contact of water with tree trunk exacerbate *Phytophthora* diseases in nurseries and orchards and cause foot rot, collar rot and gummosis. Injuries to the trunk and prolong wetness makes it more susceptible to infection (Whiteside,1972). Soil moisture, aeration and temperature significantly influence the fibrous root infection, multiplication of the pathogen and its further spread. Stressed conditions due to dry or saturated soil, root damage due to water logging and depletion of oxygen predispose the roots for infection and increase their susceptibility (Duniway, 1983; Feld *et al.,* 1990). Damaged roots or under stress conditions increase the root exudates and restrict the root regeneration. Root exudates released in such condition also attract zoospores (Stolzy *et al.,* 1965). A general practice adopted by citrus growers in Central India to keep mandarin plants (budded on rough lemon) under water stress condition from 30 - 45 days followed by breaking the stress by flood irrigation or summer rains to induce flowering further aggravate the root susceptibility and increase the rate of fibrous root infection (Naqvi, 2002a).

2.8 Disease management

The management of *Phytophthora* diseases of Citrus is an integrated approach that includes (i). Production and use of *Phytophthora* free nursery stock, (ii). Use of resistant/ tolerant rootstocks, (iii). Appropriate cultural practices to mitigate disease development, (iv). Use of chemicals as prophylactic and/or cure of the diseases and (v). Use of biological antagonists.

2.8.1 Management of diseases at nursery level

Management of the disease at nursery level starts from selection of nursery site and seed extraction. Sanitary measures to exclude the pathogen from seeds and soil is utmost important to avoid disease establishment. The seeds should be extracted from healthy fruits picked up from the resistant variety of recommended rootstock tree from > 4 feet height to avoid *Phytophthora* infection/ infestation. Strict sanitary measures should be followed while extracting and cleaning the seeds (Fisher, 1993a). Seeds may be immersed in agitating hot water (51.7°C) for 10 min. to disinfect *Phytophthora* (Klotz *et al.,* 1960). Though the hot water treatment takes complete care of *Phytophthora* infection but development of this treatment plant may be cumbersome and just after treatment, the proper and timely cooling of seeds governs the viability of the seeds during storage and percent germination when sown immediately. It is has been experienced that if strict sanitary measures are adopted while picking and extracting the seeds, the hot water treatment may be avoided.

For storage seed should be treated with 8 hydroxyquinoline for 3 min. and may be stored in aerated mesh bags. Seed storage in plastic bags often encourages fungal development and spoilage of seeds. Raised seedbeds should be either solarized and/or fumigated in the areas where sufficient solar heat is available in summer. Nursery should be away from citrus plantation and operational equipments should be separate to avoid introduction of pathogens. The risk of introduction of pathogen through wind, soil and workers always remains in field nurseries. To avoid this risk, seedlings should be raised

in trays in green house. Disease severity often increases in fumigated soil, if re-infested with the pathogens than the native soil due to elimination of natural soil microbial competitors and antagonists. It is advisable to use metalaxyl spray just after seed sowing to ovoid the risk of *Phytophthora* infection, if any present in the trays or nursery beds (Singh *et al.,* 2001, Naqvi, 2002a).

2.8.2 Production of *Phytophthora*-free nursery stock

In citrus nurseries, *Phytophthora* caused diseases are the menace and may appear any time of plant growth in nurseries through contaminated water, soil and even through nursery workers and implements. In India, the management of *Phytophthora* diseases in Citrus nurseries is often overlooked and thus the plants raised in *Phytophthora* infested nurseries become the primary source of *Phytophthora* spread to other virgin areas and become the cause of general replant problem. Vidarbha region of Maharashtra is the largest Citrus propagating area in India. More than 8 million Citrus plants are propagated mainly on rough lemon every year. Similarly in north-western zone, Kinnow and other sweet orange cultivars are being raised.

In North-eastern states and southern India, Citrus is propagated by seedlings in field nurseries. Almost all the nurseries were badly infested with *Phytophthora* spp. *P. nicotianae, P. citrophthora* and *P.palmivora* could be isolated from these nurseries either alone or in combination of one or two species from a single nursery. The *Phytophthora* infected nursery stock when planted in orchard grows poorly, takes longer period to establish and in most of the cases die / decline at bearing stage (Naqvi, 1988; 1994; 1999a,b).

Production of *Phytophthora* free Citrus nursery stock is the most important aspect in Citriculture and is the basis for a sound and productive citrus industry. The strategies adopted for producing *Phytophthora*-free Citrus plants is an integrated approach includes mainly the exclusion of pathogen through strict sanitary measures at all the steps of nursery raising, minimizing the predisposing factors for growth and multiplication of *Phytophthora.* There is an urgent need to include the protocol in citrus certification programme in India in order to minimize the spread of this pathogen to virgin areas (Naqvi, 2003). *Phytophthora*-free plants can be raised in field nurseries and in containerized green house. In field nurseries, the risk of introduction of pathogen is higher as compared to well-protected green house nursery.

The site selection is an important criterion while opting for field nurseries. Besides the basic requirements like availability of clean water and transportation, the location of field nursery must be far away and isolated from the commercial plantation to avoid any contamination. In vicinity of commercial plantation; it is practically impossible to raise citrus plants free from disease and insect-pests. The soil of the site should be well drained. Poorly drained and heavy soils retard root development and favour infection by root rot fungi in the field nurseries. It is difficult to contain diseases in field nurseries because there are ample chances of introduction of pathogens through air, soil, water and the worker. Once introduced becomes very difficult to eradicate the soil borne pathogens (Klotz *et al.,* 1959). Soil solarization and fumigation has been used successfully in restricting *Phytophthora* from the nursery.

2.8.3 Soil solarization

The soil solarization is an important non-chemical, natural hydrothermal technique to disinfest the soil from a number of plant pathogens and insect-pests. The process is accomplished through passive solar heating integrating physical, chemical and biological mechanisms. Recently, the search for such non-chemical methods in controlling soil borne pathogens has been intensified either alone or as components in integrated disease management programme considering the phasing out of methyl bromide in 2005 (Katan, 1999; 2000; Stapleton, 2000). In tropical parts of the world, the intense solar radiation can be utilized in the process of soil disinfestation through this ecofriendly process. Besides disinfestation from soil borne pathogens, the solarized soil also undergoes various physical and chemical changes. The increase in the concentration of soluble mineral nutrients has been observed in solarized soil. The concentration of NH_4-N and NO_3-N has been recorded to increase from 26 – 177 Kg/ ha in the top 15 cm soil depth in soil types ranging from loamy sand to silty clay (Katan, 1987; Stapleton and DeVay, 1995). The biological environment of the solarized soil also changes with creation of biological vacuum after inactivating pathogenic micro-organisms and consequently, the soil micro-organisms survived the solarization grow faster and occupy the soil environment. The increased availability of nutrients and minimum competition by inactivating the other microflora enhances the activity of other soil micro-organism, which include mostly antagonists.

Soil solarization in Vidarbha region of Maharashtra was successful in reducing the soil borne pathogens. During April- May, the atmospheric day temperature rises up to $45 – 46^0C$ (Nagpur condition) which allowed the inside temperature to rise upto 54ºC. The solarization for 4 – 6 week time eliminates most of fungal, bacterial and nematodes pathogens from the soil (Singh *et al.,* 2001, Naqvi 2002a, and b).

2.8.4 Soil fumigation

The monitoring of solarised soil decides whether the soil is to be fumigated or not. The soil fumigation of the solarized soil can be done effectively with Dazomet granules, a soil fumigant, which releases methyl isothiocynate gas on hydrolysis and thereby, completely eliminates *Phytophthora* spp., *Pythium* spp*., Rhizoctonia* spp. and *Fusarium* spp. from the soil. The soil having 60-70 propagules of *Phytophthora* /cc when fumigated with Dazomet granule @ 50 g/M^3 of soil for a week could completely eliminate *Phytophthora* propagules from the soil (Naqvi, 1999d, 2002a,). Pre-plant application of methyl bromide at 500 to 600 kg/ha or metam-sodium at 425 litres/ ha reduces the pathogen population significantly or eliminate these fungi completely (Klotz and Clalavan, 1969). However, fumigation also eliminates or destroys the beneficial mycorrhizal fungi and may result in stunting of seedlings due to inadequate availability of mainly phosphorus, zinc and copper. The fumigated soil will therefore, require the recommended doses of dolomite and superphosphate in absence of mycorrhiza for normal seedling growth (Timmer and leyden,1978). Trifoliate and its hybrids are normally not affected with fumigation-induced stunting as compared to other rootstocks (Graham, 1986). Solarized and fumigated potting mixture / primary nursery beds in field

nursery should be used for seed sowing or filling the bags in the secondary nursery to transplant the rootstock seedlings.

In case of containerized nursery, surface sterilized plastic trays (60x40x12 cm) should be used, filled with solarized and /or fumigated pot mix to sow the seeds. The trays should be kept at least 1.5 – 2 feet above the ground on platform / benches to safeguard from splashing of soil from the ground and also to provide air circulation under the trays. In any case, the trays or its soil mix or the fertilizer which is to applied should not be allowed to come in contact with ground and should always be kept on clean concrete dry platform. The floor of the nursery should be covered with stone dust and boulders (2 – 4 inch thick) to avoid any splash of soil borne pathogens and should be regularly sprayed / dusted with copper + lime mixture. Irrigation water from pond or canal should be avoided as it may carry the pathogen (Klotz *et al.,* 1959). The well water is safe for irrigation provided it should not have any runoff contamination from the citrus groves. The irrigation hose should be kept off the ground to avoid contamination (Singh *et al.*2001, Naqvi, 2001a)..

Steam sterilization of potting mix can be done with the help of large sized steam sterilizing unit especially designed for this purpose. A jeep trolley usually converted in a sterilizing chamber by providing steam-releasing pipes at the base of the trolley. The temperature of the soil mix should rise up to 100ºC for about 40 min. (Olsen and Backer, 1968; Laing and Beattie, 1997, Naqvi, 2002b). The sterilized soil/ pot mix should be filled directly from the trolley to trays or bags.

2.8.5 Monitoring for *Phytophthora* diseases

In citrus nurseries, *Phytophthora* caused diseases are the menace and may appear any time of plant growth in nurseries through contaminated water, soil and even through nursery workers and implements. A regular and strict monitoring should be done for *Phytophthora* infection by collecting soil samples at least 20 samples from a bed to monitor the population of *Phytophthora* and its subsequent control. In case of infection, the infected/contaminated plants should immediately be removed along with the bag from the containerized system and destroyed to keep the nursery totally free from *Phytophthora* and other diseases. In field nursery, even the surrounding plants of the infected plants should be discarded. Prophylactic sprays of metalaxyl or fosetyl.al at monthly interval to control the introduction of *Phytophthora* is recommended. Nursery implements should be disinfected regularly with sodium hypochloride or bleach solution and at the entry of nursery, the arrangement must be made to disinfect the shoes of workers and visitors with copper sulphate and lime dust (Naqvi, 1994; 1999a,b; 2001a,b).

Application of metalaxyl and aluminum fosetyl effectively control the *Phytophthora* diseases, but these fungicides can not eradicate the problem or substituted for sanitation and excessive use of metalaxyl develops resistance in pathogen. Strict sanitary measures to exclude the pathogen, judicious use of irrigation water, avoiding excessive runoff, keeping nursery as much as possible dry, avoiding excessive wetting of plants is the key for success in producing *Phytophthora* –free Citrus nursery stock. Use of fungicides in management of *Phytophthora* diseases in nurseries

is not desirable (Timmer *et al.,* 1998; Graham and Menge, 2000; Naqvi, 2001a).

2.8.6 Use of resistant rootstocks

The primary reason for shifting citriculture from seedling to budded plants was the appearance of *Phytophthora* foot rot in the Azores islands. As the disease was recognized, the interest in rootstocks greatly increased because of the heavy losses experienced among the susceptible seedlings. The search for resistant rootstocks started and seedlings were gradually replaced so that today virtually all Citrus trees are propagated by budding on to location specific *Phytophthora* tolerant rootstock seedlings. Initially Sour orange and rough lemon dominated as rootstocks in citriculture but later due to their susceptibility to viruses and other diseases, the screening and development of new rootstocks became a vital aspect in citriculture (Graham, 1995a; 1995b; Hough, 1992; Robert and Edgar, 1993). *Phytophthora* remains a threat and a persistent problem wherever citrus is grown that can result in substantial tree loss particularly trees on susceptible rootstock (Whiteside, 1973).

Various methods have been adopted to screen the rootstocks against *Phytophthora* species with the aim to introduce the pathogen to the host in a conducive environment for disease development simulating field conditions. These methods include infestation of steam-pasteurized soil with cornmeal-sand inoculum of pathogenic isolates (Broadbent, 1977), tank-test where a tank filled with water or nutrient solution into which zoospores are released from sporangial culture and seedlings are dipped in this solution (Grimm and Hutchison 1973; Klotz *et al.,*, 1958a), inoculation of wounded stem (Whiteside, 1974) and nonwounded seedlings with zoospores (Cameron *et al.,* 1972), in pasteurized soil amended with chlamydospores produced in culture (Graham, 1990, Graham and Castle, 1994), infesting soil with mycelia followed by periodic water logging of pots (Grimm and Hutchison, 1973), planting in infested field (Klotz *et al.,* 1968) and inoculation of wounded stems with mycelial discs (Klotz *et al.,* 1958b).

In general none of the *Citrus* spp. and their hybrids with trifoliate orange is absolutely resistant to *Phytophthora* infections but show great variation in their susceptibility. Further, there is variation in tolerance to foot rot and to root rot (Carpenter and Furr, 1962; Grimm and Hutchison, 1977; Graham, 1990). For example, Carrizo citrange and sour orange exhibited tolerance to foot rot but were found susceptible to root rot. This indicates that the correlation with foot rot is not good to root rot. However, both Swingle citrumelo and trifoliate orange are tolerant to root rot and resistant to foot rot/ stem infection. Thus root rot tolerance may be an acceptable indicator of bark resistant. The significance to root rot screening for evaluation of rootstocks becomes more important as the foot rot screening takes more time, space and some time inconsistent development of the symptoms as compared to root rot evaluation.

Recently, to make the rootstock screening more easy and reliable, rooted cuttings of rootstocks have been tried and given reproducible results as compared to seedlings (Graham and Timmer, 1992; Graham, 1995a; Naqvi, 2000a, 2002b). Rooted cuttings of Citrus can be used in screening programme of citrus germplasm against *Phytophthora* species effectively as against seedlings. This technique reduces screening time, space and reproducible results can be obtained by avoiding barrier of nucellar

and zygotic seedlings. Earlier, Carrizo citrange was used in evaluation trial as standard for tolerance but now Swingle citrumelo is a preferred standard for comparison in identification of *Phytophthora*-resistant germplasm. For root rot screening, the term 'tolerance' is suggested rather than using the 'resistance' because roots of virtually all commercial rootstocks become infected under artificial inoculations and in groves (Graham, 1995a). A tolerant rootstock should possess comparatively high degree of tolerance to root rot, capacity to regenerate roots faster at regular intervals to cope with the *Phytophthora* root rot. The rootstock should not support the multiplication of the pathogen in the rhizosphere (Graham, 1995b).

The use of tolerant rootstock with desirable horticultural characteristics is the best management strategy of *Phytophthora* diseases in order to reduce the costly applications of fungicides. Currently, tolerant rootstocks have undesirable traits and rootstocks with desirable horticultural characteristics are susceptible. The efforts are being made worldwide to combine the desirable traits of rootstocks through breeding and other biotechnological means (Grosser and Gmitter, 1990, Grosser *et al.,* 1994, Grosser *et al.,* 1997, Roose, 1997, Broadbent, 1997). Thorough screening of rootstocks mostly against *P.nicotianae* and *P.citrophthora* among the mandarins and hybrids, sweet oranges, citranges, citrumelos, sour orange types and lemon types has given some clear indications for their susceptibility to *Phytophthora* root rot. Among the mandarins and hybrids, Cleopatra, Sun Chu Sha, Calamandarin and Changsha are least tolerant to root rot except 639 (Cleopatra x trifoliate). Among trifoliate and its hybrids, Swingle citrumelo, C-32, C-35, Benton and Yuma citrange and African Shaddock x Rubidoux have shown resistance to bark infection and tolerance to root rot in order to root regeneration with minimum population support of pathogen in rhizosphere. In general, sour orange group has tolerance to foot rot but susceptible to root rot in spite of greater ability to regenerate roots but also greater population support. Other sour orange type, Gou Tou and Smooth Flat Seville also behave similarly except *C. obovoidea. C. volkamariana* and *C. macrophylla* show intermediate reactions to foot rot and root rot (Graham, 1995a, Graham and Menge, 1999, 2000, Hough, 1992, Matheron *et al.,* 1998).

There is a need to screen the tolerant rootstocks against all the three species viz. *P. nicotianae, P. citrophthora* and *P. palmivora* separately as well as in combination. It is observed in many citrus orchards and nurseries in India that all the three species are present in the groves and even in rhizosphere of single plant. Among the three species, *P. palmivora* is more aggressive and voracious in colonizing the roots and causes damage to even larger roots and severe canopy blight as compared to others. The spread of *P. palmivora* is also expected to be faster than *P.nicotianae* and *P.citrophthora* due to deciduous sporangial production (Graham *et al.,* 1998, Zitko and Timmer, 1994, Widmer *et al.,* 1998, Naqvi, 2002b). It is also prudent to study the effect of scion varieties on susceptibility of rootstock and population of pathogen (Ippolito *et al.,* 1997a).

In India, almost all the citrus plants are being raised on rough lemon and very few on other rootstocks like Rangpur lime etc. A large number of Citrus cultivars have been screened in search of resistance against *Phytophthora* diseases in India, mostly based on field trial in naturally infested soils. Uppal and Kamat, (1936) found lime and rough lemon (*C. jambhiri*) resistant to gummosis while mosambi, pummelo and manda-

rin were susceptible. Similarly pummelo, sweet orange, adajamir and acid lime were highly susceptible in Assam whereas rough lemon was moderately susceptible and mandarin and sour orange were found resistant to *Phytophthora* (Chowdhury, 1951). In peninsular India, lemon, citranges, sathgudi, jambhiri, grapefruit, mandarin and acid lime were susceptible except sour orange which was found resistant (Ramakrishnan, 1954). Vadlapudi, and Kichili were more susceptible to collar rot disease (Govindarao 1954). Sweet lime, Karnakhatta and Italian 76 root-stocks were resistant to collar rot for the Hill variety of mandarin at Saharanpur whereas jambhiri and Florida rough were moderately susceptible (Singh 1962) .

In Gujarat, Kagzi lime was found susceptible to *P. nicotianae* (Somani and Patel, 1969, 1972). From Srirampur Citrus germplasm collection, out of 34 citrus cvs from 12 citrus spp., only *C. reshini* cv Cleopatra mandarin Morocoo and *Poncirus trifoliata* were resistant to *P. palmivora,* rest others were susceptible, mildly tolerant or tolerant except *P. limonia* cv L-19 Rangpur lime, *C. reshini* cv Cleopatra mandarin Australia and Jamberi local were highly tolerant (Kumbhare and Choudhari, 1978). Hybrids of Cleopatra and trifoliate were most tolerant to *Phytophthora* and vigourous than hybrid of Rangpur lime x trifoliate where as hybrid of rough lemon x trifoliate was highly susceptible   In similar studies with the strains of *Poncirus trifoliata* and its hybrids, a high degree of resistance to *P. parasitica* was observed in *P. trifoliata* strain rubidaux and Srirampur whereas trifoliate hybrids were generally less resistant (Prasad and Rao 1983). Ten strains of rough lemon were evaluated against *P. parasitica* and were ranked Jatti Khatti, Jambhiri Pune, Kansu orange, Jambhiri Kodur, RLL-II, RL-I, South African RL and K 17 in their decreasing resistance order (Sawant *el al.* 1993).

The root-stock trials at different locations in the country under AICRP on tropical fruits have shown variability among the strains of Rangpur lime and rough lemon for the resistance to *P. parasitica.* Linocriyo Brazil, Rangpur lime Knorr and Souranthan strains of Rangpur lime and Jattikhatti strain of rough lemon were having better ranking for resistance (Anonymous, 1991). There is urgent need in order to save the declining citrus industry of India, to replace the susceptible rootstocks like rough lemon and Rangpur lime with well known tolerant rootstocks like Swingle citrumelo, citranges, trifoliate hybrids like x 639 (Cleopatra x trifoliate), *C. volkamariana* and *C. macrophylla* examining the soil suitability of the area.

2.8.7 Cultural practices

Being soil borne nature, once *Phytophthora* enters in a nursery or orchard, it becomes an endemic problem and difficult to eradicate. 'Prevention is better than cure' should be followed strictly. Manipulations in certain cultural practice help upto a great extent in limiting the problems. Plant should be selected from *Phytophthora* free certified nurseries and with high budding, above 22- 50 cm height or more (Whiteside, 1972). While planting care should be taken to keep bud union as high as possible from the soil line so that irrigation water should not touch the scion. In areas of high rainfall, trees are often budded 50 cm or more above the soil line. Soil should be kept well drained. Double ring method should be adopted for irrigation and fertilization to keep the trunk of the tree and the area around the trunk as far as possible dry. Flood irrigation and stagnation of

water for longer period in the basin should be avoided. Use of herbicides in citrus orchards also help in reducing humid conditions under the tree and eliminating the chances of injuries to trunk and root system by farm operation. (Naqvi, 2001a,b).

Phytophthora is a water loving fungus and requires abundant water supply for the growth, multiplication, dispersal and infection. Hence irrigation methods and ultimately water management strategies play the key role in management of *Phytophthora* diseases. In saturated soil with water, zoospore is able to move only less than a centimeter and in flood irrigation they can move with water wherever it goes. Thus water run off is the problem. Only adequate irrigation is to be given for optimum growth and production of citrus plant and management of *Phytophthora*. Irrigation water should be applied to wet the top 60-90 cm of soil, and the top 30 cm should be allowed to dry to –600 to –700 hpa before re-irrigation. Intermittent wetting and drying of soil, variable soil temperature and host tolerance helps in zoospore encystment, inactivation and ultimate escape from infection. Thus water management strategies can help immensely in management of *Phytophthora* diseases of Citrus (Lutz and Menge, 1989, Naqvi, 2002b).

In orchards, if old budlines or elite mother plants got infection and a considerable portion of root or collar has been destroyed by *Phytophthora*, in such cases inarching with resistant rootstock can effectively rescue the affected plants. Such infected plants in the orchard can be rejuvenated for further long productive life (Fig.5).

2.8.8 Chemical control

Use of fungicides can mitigate the problem up to a great extent but can not eradicate it. Copper fungicides are used as foliar spray and as trunk paste to control *Phytophthora* diseases. Copper fungicides are effective in controlling foot rot and gummosis of Citrus if used at correct time. Bordeaux paste should always be applied before onset of monsoon and after the monsoon on tree trunk as prophylactic measure. Foot rot or gummosis affected portions should be scraped out with sharp knife taking care not to damage the wood before application. However, this procedure is laborious and time consuming and not always successful in controlling the foot rot (Timmer,1977; Naqvi, 1994).

Availability of systemic fungicides like metalaxyl (Ridomil MZ 72) and fosetyl-al (Aliette) has given the Citrus growers additional and more effective options for the control of *Phytophthora* diseases. Both the fungicides are registered in India and available in market. Due to systemic in nature and long term persistence of these fungicides in plant system, the foliar spray and drench treatment or pasting the affected trunk can save labour and time consuming operations like opening of root system and surgery of the affected parts for the control of diseases using copper fungicides (Timmer, 1977; Naqvi, 1994). These fungicides can also be applied through drip and micorsprinkler irrigation systems.

Before application of these fungicides, it is pertinent to know that which *Phytophthora* species in involved in disease development and what is its quantity in the soil. Because different species of the pathogen act at different time of the year and thus to understand the optimum timing for the application of fungicide. Knowledge of the quantity of the fungus will help in determining action for control measure. Whether

the fungicidal treatment is warranted or not. A threshold of 15 – 20 propagules/ cc soil indicates the need for a fungicide application. Identification of the fungus is also required because these fungicides are specific in their action only against *Phytophthora* and related fungi and also higher in cost. Ridomil MZ 72 and Aliette both are effective in controlling the *Phytophthora* diseases of Citrus. However it would be prudent to alternate the use of each fungicide in order to inhibit potential development of resistance to either fungicides by *Phytophthora* (Fisher, 1993b; Timmer *et al.,* 1998).

Figure 5: Inarching with resistant rootstock seedlings to save collar affected citrus plant

Foliar sprays + drench of Metalaxyl or foliar spray of fosetyl.al were effective in controlling gummosis, foot and root rot diseases and in increasing the feeder root density by more than 50 % or even 100 % of the treated plants (Davis, 1982, Naqvi, 1994, Sandler *et al.,* 1989, Timmer, 1977). Since both the fungicides have different mode of action against the pathogen, Ridomil may be used effectively as spray; drench and trunk paste while Aliette should be used as foliar spray and trunk paste only. In three years field trials, Ridomil drench was significantly effective in bringing down the popu-

lation of *Phytophthora* in soil while in case of Aliette drench though the root growth was at par with Ridomil treatment but population of *Phytophthora* also increased with out any check (Naqvi, 1994). Regular foliar applications of fosetyl.al and soil applications of metalaxyl increased yield and fruit size in some orchards (Timmer *et al.,* 1989a, Sandler *et al.,* 1989, Menge, 1986; Matheron *et al.,* 1997; Ippolito *et al.,* 1997b).

The best period to apply fungicides to have maximum control of root rot would be when major root activity takes place during the year and at these periods of year roots need to be protected. *Phytophthora* grows actively at temperatures between 10ºC and 35ºC (optimum 26ºC). Citrus root growth ceases below soil temperature of 13ºC or above 36ºC. The optimum temperature for root growth is similar to that of *Phytophthora*. Citrus roots have distinct growth and dormant periods, which alternate with the periods of foliage growth (Lutz and Menge,1986b). Citrus trees in summer rainfall areas have three distinct periods of alternate growth of shoots and roots (Schutte, 1994). The three shoot flushes (February-March, June –July and October-November) precede the root flushes (March-April, July- August and December-January). The timings for these flushes may vary at different places. The application of systemic fungicides would be best utilized if applied at the peak of each vegetative flush, just as the expanded new leaves start to harden off. The downward translocation of carbohydrates to the roots at the commencement of root flush improves the mobility of fungicide (Aliette) to the roots or spray + drench of Ridomil.

2.8.9 Biocontrol

In order to develop eco-friendly management of *Phytophthora* diseases, screening of bio-control agents against Citrus *Phytophthora* has become a vital research aspect and is being worked out all over the world. Certain strains of Psuedomonas putida are found to reduce *Phytophthora* population up to 96 % in green house. This bioagent when tried in field with seedlings reduced 41 - 66 % population of *Phytophthora citrophthora* and controlled 22 % root infection for 3 months (Menge, 1993). *Pythium nunn* and *Penicillium funiculosum* suppressed the root rot and gummosis in pot experiments (Tsao *et al.*, 1997). *Trichoderma harzianum* has been advocated to have a potent antagonistic action against *Phytophthora* root rot of Coorg mandarin when applied along with Coffee waste, poultry manure and FYM in pot culture experiments (Sawant and Sawant,1989). *Myrothecium roridum* also inhibited *Phytophthora* infection in Citrus (Tuset *et al.,* 1990). These bioagents have not been commercialized so far but in future the integrated use of biocontrol agents either alone or in combination with low doses of fungicides, host resistance and with improved culture practices will be the long term solution of *Phytophthora* diseases of Citrus.

John Menge, Professor at University of California worked for several years to find out biocontrol of Citrus *Phytophthora* diseases did not find *Trichoderma* as promising biocontrol agent against Citrus *Phytophthora* diseases (Personal communication). In my experiments, some strains of *T.harzianum* do cause lysis of *Phytophthora* mycelium *in vitro* but *in vivo* they grow in harmony and there is no significant control of root rot or suppression of *Phytophthora* propagules in rhizosphere of Citrus. *T. viride, T. harzianum* and *T.hamatum* also cause fruit rot of Nagpur mandarin. Certain

Trichoderma spp. viz. *T. viride, T. hamatum, T. koningi* and *T. piluliferum* induced sex organs in A^2 mating type of *P. nicotianae*. Certain strains of *T. harzianum, T. hamatum* and *T. rescei* act as root growth promoter of Citrus but not as antagonists against Citrus *Phytophthora* or root rot suppressor.

3. *Fusarium* root rot and dry rot

The destruction of root system of Citrus by *Fusarium solani* (Mart.) Appel.& Wr. emend. snyd. & Hans. is underestimated when compared with the diseases caused by *Phytophthora* spp. of Citrus (Labuschagne,1994). Fusarium root rot and dry rot are destructive diseases of Citrus plantation. The diseases have been reported to cause serious damage to Citrus in North Arcot district of Tamil Nadu, Chittor and surrounding

Figure 6: Dry root rot affected larger root of citrus plant

areas of Andhra Pradesh (Ramakrishnan, 1954). The disease causes sporadic losses in Central India, North-Eastern region and in Nepal (Ghosh and Singh, 1993). Dry root rot can be distinguished from *Phytophthora* foot and root rot as it affects larger roots and trunk below the bud union without oozing any gum. It is characterized by moist dark decay of bark in early stages and dry shredded cankers at later stage with hard, dried dark brown to grey colour of wood (Fig. 6). The disease may progress in the wood for many years with the only symptom being a slight wilting under dry conditions (Bender *et al.,* 1982). Chronic rotting of feeder roots and scaffold roots by *F. solani* is often associated with gradual decline of the canopy. The affected feeder roots are characterized by cortical sloughing exposing the light coloured stele in the centre of the root.

Canopy symptoms comprise wilting (leaf curl), defoliation, die back of twigs. The appearance of the remaining leaves is dull green in contrast to the lush green appearance of leaves on healthy trees (Labuschagne, 1994).

Fusarium solani is one of the components contributing to Citrus decline. The fungus often acts in association with other pathogens like Citrus nematodes and *Phytophthora* resulting in increased disease severity compared to the individual pathogens acting alone (Dandurand and Menge, 1992; Labuschagne *et al.,* 1989). Usually the disease develops gradually showing yellowing and wilting of affected plants in dry condition but if plant is under stress due to poor drainage, excessive fertilizer application of ammonium nitrate and urea, waterlogging, poor soil aeration, rootstock and scion incompatibility, attack of insect pests, viral diseases, nematodes, *Phytophthora* and depletion of starch reserves in the roots, the disease aggravates and plant die suddenly while chlorotic leaves remain attach to dead plant.

3.1 Management of the disease

The most effective control measure against the disease comprises elimination of conditions that cause stress in the trees. Maintaining soil conditions favourable for root development is of the utmost importance. Faulty cultural practices like damaging root and trunk during farm operations, water logging in orchard favour the disease development and should be avoided. Regular disease monitoring of the orchard to identify the initial stages of the disease is necessary to cure the affected plants. At early stages of the disease, removal of one or two infected roots when tap root and crown are still in good condition can control further disease development. Correction of stress factors predisposing the disease by providing good drainage, soil aeration and treatment with benzimidazole fungicides provide effective control. An integrated control strategy is most effective in the long run. Since *F. solani* interacts with other root pathogens such as Citrus nematodes and *Phytophthora* spp., controlling the latter two pathogens will also automatically reduce the damage (Dandurand and Menge, 1992). Biocontrol with *Trichoderma* spp. and avirulent strains of *F. oxysporum*, soil solarization, and selection of resistant rootstock would be long term solution for the disease.

4. Mushroom root rot

Mushroom rot or *Armillaria* rot occurs in localized areas in Citrus plantation. During the survey in India, certain pockets of Amravati and Nagpur district of Maharashtra were found affected with this disease. It is also frequent in Northeastern and southern states causing sudden wilt and collapse of sweet orange and mandarin plants on rough lemon rootstock or seedling trees. The disease is caused by *Armillaria mellea* (Vahl:Fr.) P.Kumm. and also by another species *A. tabescens*. Sporophores of *A. mellea* have an annulus on the stipe that is not present in *A. tabescens*. Generally the causal organism grows on forest trees in temperate and tropical regions of the world and it may transmit to Citrus plants from the area which is cleaned from the forest trees for Citrus plantation. The pathogen grows beneath the bark and destroys the structural root system of plant. Rotting of structural roots induces slow declining in the affected tree. Chlorosis and

abscission of leaves are the common symptoms when the sufficient amount of large roots is destroyed by the pathogen. Sometimes the symptoms are confusing with foot rot caused by *Phytophthora* spp. however mushroom rot does not extend much on above ground portion of the trunk. Growth of mushroom sporophores at the base of affect tree, mycelial growth beneath the bark and on the wood and destruction of structural root system are some positive identification symptoms of the disease (Fig 7). The declining symptoms appear on whole canopy and affected plants collapse sud-

Figure 7: Mushroom growth near trunk causing root rot of mandarin

denly. Weakening of plants due to drought or nutritional stress is the predisposing factor for the disease (Broadbent, 1981).

The disease spreads to other trees through the contact of infected roots to the healthy roots of the plants growing in proximity. Dispersal of basidiospores from the basidiocarps is not the potent threat for spread of the disease. However, the lefts over root system of other native trees in the soil while cleaning the land become active source of inoculum and disease occurrence. The pathogen grows actively in roots and stumps of cut forest trees and Citrus tree gets infection from this reservoir when the roots of growing citrus plants reaches to this inoculum.

4.1 Management of Mushroom rot

Adopting sanitary measures in excluding the inoculum from the affected site is the best practice to contain the further spread of the disease. The rhizosphere soil and affected root system should be removed carefully by deep excavation. Removal of affected plants and as much as possible the deep root system helps in controlling the further spread of the disease. The pits should be left fallow and open for more than a year before replanting. These pits may also be fumigated to kill the fungus.

5. Powdery Mildew

The disease is prevalent in sub-mountain tracts of Coorg, Nilgiris, Wynad and Shevoy hills in Southern India causing severe losses to mandarin and sweet orange plantation. It has also been reported in North-Eastern states, Darjeeling, Sikkim, Bhutan, Dhankuta and Jankpur area of Nepal, Srilanka, Java and Philippines on almost all the Citrus cultivars (Roy and Das, 1995; Roy and Ghosh, 1991; Ghosh and Singh, 1993; Mukherjee, 1949). The disease is the major cause declining and death of mandarin plantation in Jampui hills of Tripura and Mezoram States (Fig 8). Repeated attack of the pathogen on new flush has virtually vanished the khasi mandarin plantation from a number of locations in Jampui hills (Naqvi, 2002b).

Powdery mildew is caused by *Acrosporium tingitaninum* (Carter) Subram. (Syn. *Oidium tingitaninum* (C.N.Carter). The pathogen attacks all aerial parts of the Citrus plants in nurseries and orchards. White powdery patches of mildew appear on upper part of young leaves and on twigs (Fig.9). The leaf tissue at infection court in initial stages of infection turns darker watery green than normal and later becomes yellow. In severe conditions, infected leaves and premature fruits drop off and twigs show die back symptoms (Ramakrishnan, 1954). High humidity and cloudy whether favour the disease development and spread. The fungus overwinters as dormant mycelium in the buds and with the onset of favourable conditions it multiplies and causes the disease. The conidia are formed in chains, colourless and vary in size depending on host and climate and disseminate by wind. Development of perithecia is not reported. In Southern India, the disease is severe from October to March. The attack of powdery mildew drastically reduces the vigour and yield of the plants.

5.1 Management of the disease

Since the pathogen attacks all the Citrus cultivars and no Citrus cultivar is thought to be immune to the disease (Reddy *et al.,* 1984), regular monitoring of nurseries and orchards is required after summer rains during predisposing climate to note first appearance of the disease. Water shoots should be pruned regularly which are supposed to catch early infection. The disease can effectively be controlled by spray applications of tridemorph, triadimefon, dinocap and benzimidazole fungicides at first sign of attack and should be repeated at 10-day interval (Narasimhan *et al.*1984). Dusting of sulphur at 8-day interval is also recommended to control the disease effectively (Mukherjee, 1949).

6. Twig blight

Twig blight is a common problem of mandarin plantation in India. The survey of manda-
rin orchards in Central India has revealed that twig blight is a problem of negligence and
mismanagement of the orchard. Nagpur mandarin plants show drying of twigs after one
or two bearings. A number of stress factors like nutritional deficiencies, drought, attack
of insect pests, virus and virus like diseases and root rot infection by *Phytophthora*

Figure 8: Completely defoliated orchard of Khasi mandarin due to severe powdery mildew
          attack in Jampui hills of Tripura State, India

spp. together contribute to the problem. The plants affected with one or more of the
above factors show drying of twigs starting from the tip and die back (Fig 10). In wet
weather, during and after rains, *Colletotrichum gloeosporioides*(Penz.) Sacc.,
Botryodiplodia theobromae Pat. and in some cases *Fusarium* spp. multiply on the
dead tissue of twigs. Under stressed condition of plant, *Colletotrichum gloeosporioides*
and *Botryodiplodia theobromae* Pat. together increase the intensity of mandarin twig

blight gradually from season to season and after 2 - 3 years, the twig blight becomes prominent. Reduction of canopy volume due to this malady reduces the yield considerably. Blighted trees on bearing suffer heavy fruit drop. The fruit harvested from such trees develop high percentage of stem end rot during storage.

6.1 Management of the disease

The best management strategy of this problem is to remove the predisposing factors responsible for weakening the plant vigour (Fawcett, 1936). Regular pruning of dead twigs 1 - 2 cm below the dead portion after harvest and spray of benzimidazole fungi-

Figure 9: Powdery mildew growth on young Darjeeling mandarin leaves

cides twice at monthly interval after pruning keep the problem under control.

7. Felt disease

The disease develops on Citrus in humid climate and has been reported from many countries. In India, the disease is reported from Southern and North-Eastern zone on many Citrus cultivars. The disease normally does not cause severe damage to the plants and seems to be harmless since there is no fungal penetration of the bark (Reddy and Murti, 1985; Whiteside *et al.,* 1988; Timmer *et al.,* 2000).

Septobasidium pseudopedicellatum Burt and some other species of *Septobasidium* cause the disease. The affected twigs and branches get encircled by soft felt-like leathery fungal growth light brown to grey in colour which may extend to the petiole, leaf bases and fruit stalks (Fig. 11). The fungus grows on the colonies of scale insects. The disease is generally considered as harmless since the fungus does not penetrate the host tissue but in neglected conditions if proper control measures are to implemented, the disease may cause drying and death of twigs and even large branches. The disease was observed very severe in acid lime plantation of Tenali area of Guntur district of Andhra Pradesh. Heavy infestation of the pathogen induced die back symptoms in affected plants. The disease is also observed severe on Khasi mandarin.

7.1 Management for the disease

Figure 10 : Twig blight in Nagpur mandarin

The disease does not cause damage to the plants and economic losses if occur in sporadically. However, in severe infestation, it may lead to die back and death of twigs. Pruning of affected branches and spray of copper fungicides provides satisfactory control of the disease.

8. Pink disease

The disease is very destructive in tropical high rainfall areas. This disease attacks all Citrus cultivars. Infected trunk, limbs and twigs turn pink in colour due to pink colour of

mycelial covering, hence the disease is named Pink disease (Fig. 12). In India, it causes severe damages to Citrus plantation in Southern zone and North-Eastern (Reddy and Murti,1985; Whiteside *et al.,* 1988). The disease is caused by *Pellicularia salmonicolor* (Berk. & Br) Syn *Corticium salmonicolor* (Berk.& Br.). The pathogen also attacks surrounding plantation of rubber, cacao, coffee, mango, Jackfruit etc. (Fawcett, 1936; Ramakrishnan,1954).

The disease appears during or just after rains. The bark of trunk and limbs of mature trees is attacked and in humid conditions, the infected portion rapidly covered with white silvery mycelium, which later turns pink. The infected portion of bark is killed and fungus invades into the wood to impede translocation in vascular tissue. The infected plants die when complete girdling of trunk and limbs takes place. The fungus perpetuates in the callus formed around the decayed bark during dry season and in wet

Figure 11: Soft leathery felt-like fungal growth on branch of acid lime

season all the stages (mycelium, pustules, stromatic tissue, spores) are capable to spread the disease. The basidiospores formation has been reported on other collateral host but not on Citrus.

8.1 Management of the disease

Infected and damaged plants should be identified before monsoon and the affected portions of such plants should be pruned and burnt. This operation should also be done on other collateral hosts near the orchard. Cut ends and scrapped portion of plants should be covered with Bordeaux paste (Reddy and Murti, 1985).

Benzimidazole fungicides require their testing in field condition for the control of the disease.

9. Citrus decline in India

Citrus decline is the major limiting factor for the sustainable citrus cultivation in India. It has been recognized as a national problem of Citriculture. The Citrus decline is used as an umbrella term which includes all the symptoms and disorders induced by a number of biotic factors like diseases caused by viruses, virus like pathogens, fungi, bacteria and nematodes or initial sickness of nursery stocks due to these disease, besides the attack of insect pests and some of the undesirable abiotic stresses. This is not a single disease or disorder. Thus the syndrome develops due to various undesirable factors

Figure 12: Pink disease on Khasi mandarin in Mezoram State of India.

due to mere ignorance of scientific Citrus cultivation and / or incompetence in diagnosing the causal factor, has been regarded as Citrus decline or Citrus die-back in India. The Citrus decline (CD) may result by one factor or more than one factors in a given locality. In general, the citrus plants after few years of good growth often start declining with gradual decrease in vigour, productivity and the orchards exhibit a sick look. The magnitude of decline usually increases with the age of the affected plant if proper diagnosis and thereafter recommended remedial measures are not taken in time to correct them and thus after 10 -15 years or even early, such affected plants become uneconomical to maintain and often die.

9.1 Symptoms

The symptoms of CD are nonspecific and therefore resemble to the symptoms induced by various citrus diseases caused by viruses and virus like pathogens, fungi, bacteria, nematodes, attack of insect pests, deficiencies of macro and micro- elements and stress due to inadequate irrigation. Thus the symptoms described by various workers vary with the causal factor, however in general, the affected trees were described to show gradual loss in vigour with chlorotic and weathered leaves, naked branches, water stress like reaction, drying of twigs and branches, deterioration in quality of produce and yield.

No specific diagnostic tests for decline are prescribed and all the symptoms are based on visual above ground abnormal and sick condition of the plant affected in comparison with the apparently healthy ones. According to some scientists CD signifies a continuous dying of twigs. The leaves may be small with light green inter-veinal areas, with the midrib and lateral veins remaining dark green. The new flush of leaves remains smaller in size on affected twigs. In some leaves, only basal portion of the midrib is green while in others, splotches of yellow or pale green are present between the lateral veins. The shoots have tendency to die from the growing tip downwards. The cropping and fruit quality of such trees also deteriorate seriously with conspicuous reduction in fruit size. In severe cases, affected trees appear abandoned (Randhawa *et al.,* 1966) while in others view, die back is a syndrome which refers to a particular condition of the plant. It involves the characteristic mottling of the leaves, the defoliation of the young branches and dying back of twigs from tip downwards resulting in loss of vigour, general health, production, and ultimate death of the tree. Primary symptoms of the disease are the yellowing of the midrib and lateral veins of the old mature leaves, invariably the interveinal areas alongwith the veins also diffuse yellowing and ultimately the leaf may sometime turn yellow. Most of the affected leaves fall with onset of summer or autumn and the die-back of the defoliated twigs commences. This is followed by secondary growth from the auxiliary buds which consists of short, upright, small weak shoots which may be bushy. The defoliation and die-back of the weak shoots continues and such shoots may occasionally show excessive and premature flowering (Nariani and Raychaudhuri, 1986).

The decline or die-back is also described as deterioration of health conditions of the tree and there is considerable reduction in production. Usually trees suffering from this disorder make excellent growth for 5 - 6 years, bear commercial crops for first few years and then decline gradually. Trees show sparse yellow and variously symptomatic foliage, stunted growth, sickly appearance with dried up top growth. Young twigs at the top usually begin to dry fast and gradually dead shoots become very prominent. Such declining trees may be noticed in isolated patches or in the entire orchard. Decline is faster with advancing age of the tree (Ghosh and Singh, 1993). Variations in symptoms described by various workers indicate that the decline syndrome varies with the pathogen(s) causing the disease(s) either alone or in combination with more than one pathogen and its expression depending upon the citrus species/ cultivars and local agro-climatic conditions. The survey conducted by a committee of specialists from Punjab Agricultural University indicated that much of the decline was due to neglect,

mismanagement or absence of plant protection measures (Chadha *et al.,* 1965). I have not seen any citrus orchard declining in India after giving all the recommended package of practices and keeping them free from diseases and insect pests. Neglect, mismanagement and ignorance in plant protection measures are the cause of citrus decline in India (Fig 13). Hence such declining orchards rejuvenate when given proper treatments. It is not like the 'declino' or 'citrus decline' in western countries where following all the recommended package of practices, citrus plant decline and the etiology of the disease now being understood with latest investigations.

9.2 Distribution and losses

The area and production of citrus over last 30 years has increased at the rate of 11 %

Figure 13: Decline of Jaffa orange due to severe *Phytophthora* root rot and collar rot in Punjab

and 9 % respectively, however, the production per ha has been very low due to wide spread problem of CD. The decline of citrus has been noticed as early as 1888 from Assam and in 1912 from Bombay (Aiyappa *et al.,* 1971). Decline has been the persistent threat to all the citrus growing areas of India viz. North-Eastern region, North-Western region, Central India and peninsular southern India. Millions of Citrus plants succumb to the problem every year ranging from young non-bearing trees to bearing trees of various age group and thus for a citrus grower, the expected average productive age of citrus plant is not more than 15 years.

It is difficult to determine accurately the losses caused by CD and no such

authentic data of losses is available. It would be difficult to locate any orchard free from the problem and declining tree can be seen either in patches or randomly distributed throughout the orchard.

9.3 Potential causes of Citrus decline

The potential causes of decline vary from region to region depending on climatic and edaphic factors of different regions which are decisive for the predisposition of different diseases, attack of insect pests and for nutritional deficiencies. Relative susceptibility of the *Citrus* species cultivated in the area, selection of mother plant and rootstock for propagation and cultural practices also contribute enormously to the declining factors in the region. High rainfall areas have witnessed altogether different diseases than medium or arid irrigated region (Dass, 1978). Similarly manifestation of different diseases in sweet orange growing belt differs from mandarin and acid lime growing areas.

Besides the location specific diseases, there are some diseases of international importance like *Phytophthora* diseases of citrus, tristeza and greening which have been reported from almost all the citrus growing States and play the major role in citrus decline. Several plant pathogens and some non-parasitic disorders have been claimed to be the causes of CD.

Among virus and virus like diseases have been reported from different parts of the country (Ahlawat, 1997; Kapur, 1992). Many of the above reported diseases were not studied in detail to investigate their epidemiology, synergistic effect, strainal variation, biotypes, viruliferous nature of the vectors and their impact on citrus production and thus, remained as preliminary reports. Most of the reports about the occurrence of virus diseases in a given area are based on visual symptoms and /or biological indexing on a small scale. Hardly one or two research centres have facilities to use the advanced technologies of diagnosis of virus diseases in India. Complete mapping for the distribution and magnitude of damage due to these diseases in citrus industry is still lacking.

Among fungal diseases, besides some location specific diseases like powdery mildew and scab, *Phytophthora* diseases were recorded widely distributed in all the citrus growing areas and identified as the major cause of decline (Chowdhury, 1951, Kapoor, and Bakshi, 1967, Naqvi and Singh, 2002).

In Tripura and Mezoram, powdery mildew is the major cause of citrus decline and death. Seasonal infections of powdery mildew (*Acrosporium tingitaninum* (Carter Subram (syn. *Odium tingitaninum* (Carter), scab (*Elsinoe fawcettii* Bitancourt & Jenkins), anthracnose (*Botryodiplodia theobromae* Pat.,*Colletotrichum gloeosporioides* Penz.),and Felt (*Septobasidium pseudo-pedicellatum* Burt.) in high rainfall areas like North-Eastern region and southern parts of India exacerbate citrus decline along with other major diseases.

Sooty mould (*Capnodium citri*) due to infestation of insect pests in all the citrus growing areas also intensifies the decline process. Citrus canker (*Xanthomonas axonopodis* pv *citri*) has been a highly destructive disease of acid lime in all parts of India. Canker also attack Kinnow mandarin heavily.

9.4 Integrated Management

The review of available literature on CDI revealed that citrus orchards suffer adversely with the attack of a number of diseases causing CDI in different parts of the country. Among these diseases, virus diseases and diseases caused by *Phytophthora* spp. are prevalent in all the citrus growing areas and have been assessed as major causes of CDI where as powdery mildew and scab are confined to high rainfall areas only and rest other diseases reported to be associated with CDI are sporadic in their distribution.

Recognizing CDI as a problem of national importance, comprehensive research efforts were made to identify and manage the causes of CDI. Voluminous information has been generated during last five decades on diagnosis and management of citrus diseases associated with CDI. CDI has been reviewed by several workers (Aiyappa and Srivastava,1967; Chadha, 1970a, 1970b; Nariani and Raychaudhuri, 1986; Randhawa *et al.,* 1966; Raychaudhuri *et al.,* 1967, 1969) and it has been categorically emphasized to produce and supply disease free planting material to citrus growers. Unfortunately in last 50 years, such programme of supplying disease free nursery stocks of citrus could not be implemented successfully probably due to huge demand of nursery plants every year. Efforts have been made to supply disease free citrus planting material on commercial scale at NRC for Citrus for last few years to conquer the Citrus decline at faster pace. Chadha (1970a) suggested that 'much useful information (regarding CDI) is at hand. A good deal of this can immediately be put to use in the solution of decline problem in many orchards. What remains to be done is to develop proficiency in the diagnosis of the various troubles having known etiologies. Once such competency is gained, the proper corrective can be applied and the problem solved. The various corrective measures - improved irrigation and drainage, use of virus free budwood and approved rootstocks, control of insect pests (diseases), nematodes, proper application of nutrients are already known. What remains to be done is to understand when each is called for to rejuvenate old orchards and to insure the prosperity of new planting'. It is envisaged that best management of CDI can only be achieved by implementing IPM paradigm of disease management which includes i. Production of disease free elite planting material, ii. use of resistant rootstocks iii. Integrated management of diseases, insect pests and vectors through chemical and biological means.

10. References

Anonymous, 1989. $ 5 million lost to *Phytophthora citrophthora*. Calif. Citrog. 75(2): 43.

Anonymous 1991. Research report of AICRP on tropical fruits. Tech.Doc. No.41 IIHR, Bangalore.

Ahlawat, Y.S. 1997. Status of virus and virus like pathogens infecting citrus in India and future strategies for citrus improvement. Indian Phytopathology. 50(2): 192-193.

Aiyappa, K.M. and Srivastava, K.C. 1967. Citrus dieback in India. ICAR Tech. Bull. Agric. 14 pp. 77.

Aiyappa, K.M., Bakshi, J.C., Srivastava, K.C., Capoor, S.P., Nagpal,R.L., Rao, D.G. and Nanaya, K.A. 1971. A report of the study team on citrus, Nat. Commission on agric. Doc.4 pp 51.

Awtar Singh, Naqvi, S.A.M.H. and Shyam Singh, 2002. Citrus germplasm – Cultivars and Rootstocks. Kalyani Publishers, Ludhiana.166 p.

Bajwa, B.S. 1941. Gummosis in fruit trees. Punjab Fruit J. 889-891.

Bender, G.S., Menge, J.A., Ohr, H.D. and Burns, R.M. 1982. Dry root rot of Citrus. Its Meaning for the grower. Citrograph, 67(11): 249-254.

Bist, V.S. and Nene, Y.L. 1988. A selective medium for *Phytophthora* Causing pigeon pea blight. Pigeon Pea Newsletter, 8:12-13.

Boccas, B. and Leville, E. 1978. Phytophthora diseases of Citrus (Les maladies a Phytophthora des agrumes. Institute de recherches sure les Fruits et Agrumes) 162 p.

Bonavia, E. 1888. Cultivated oranges and lemons, etc. of India and ceylon with researches into their origin and the derivation of their names, and other useful information. London Atlas, (reprinted by M/s Bishen Singh Mahendra Pal Singh and M/s Periodical Experts, New Delhi Appendix 32, pp. 276-77, 384 .

Brasier, C.M. and Hansen, E.M. 1992. Evoluationary biology of *Phytophthora*. Annu. Rev. Phytopathol. 30:173-200.

Brlansky, R., Derrick, K., Graham, J., Lee, R. and Timmer, P. 1998. Important diseases of Florida citrus. Citrus Ind. 79(6): 20-24.

Broadbent, P. 1977. *Phytophthora* diseases of Citrus: A review. Proc. Int. Soc. Citric. 3: 986-992

Broadbent, P. 1981. Armillaria root rot of citrus in New South Wales, Australia. Proc. Int. Soc. Citric. 1: 351-353.

Broadbent, P. 1997. Rootstock tolerance to biotic stress. Proc. Int. Soc. Citriculture pp.1255-1257.

Cameron, J.W., Klotz, L.J., DeWolfe, T.A. and Soost, R.K. 1972. Estimates of the resistance of *Citrus* x *Ponscirus* hybrids to feeder root infection by *Phytophthora* spp. by a green house seedling test. Plant Dis. Rep. 56: 927-931.

Carpenter, J.B. and Furr, J.R. 1962. Evaluation of tolerance to root rot caused by *Phytophthora parasitica* in seedlings of citrus and related genera. Phytopathology 52: 1277-1285.

Chadha, K.L. 1970a. Introduction. In : "Citrus decline in India, Causes and control." (eds. Chadha, K.L., Randhawa, N.S., Bindra, O.S., Chohan, J.S. and Knorr, L.C.), a joint publication of Punjab Agric. Univ. Ohio State Univ.USAID, PAU, Ludhiana, pp. 1-4.

Chadha, K.L. 1970b. Rootstocks. In: "Citrus decline in India, Causes and control." (eds. Chadha, K.L., Randhawa, N.S., Bindra, O.S., Chohan, J.S. and Knorr, L.C.), a joint publication of Punjab Agric. Univ. Ohio State Univ. USAID, PAU, Ludhiana, pp.9-25.

Chadha, K.L., Randhawa, N.S., Bindra, O.S. and Chohan, J.S. 1965. Report of the survey under taken to investigate causes of citrus decline in Punjab. Punjab Agri. Univ. Dec. 1965,12 p.

Cheema, G.S., Bhat, S.S. and Naik, K.C. 1954. Commercial fruits of India. Macmillan & Co. Bombay.

Chesnick, J.M., Tuxbury, K., Coleman, A., Burger, G. and Lang, F. 1996. Utility of the mito-chondrial nad4L gene for algal and protistan phylogenetic analysis. J. Phycol. 32: 452-456.

Childs, J.F.L. 1947. Foot rot in Florida: Its habits and suggestions for its control. Citrus Ind. 28(9),: 5-9.

Chowdhury, S.1951. Gummosis of Citrus in Assam. Indian J.Agric. Sci.16: 570-571.

Cooke, D.E.L. and Duncan, J.M. 1997. Phylogenetic analysis of *Phytophthora* species based on the ITS1 and ITS2 sequences of ribosomal DNA. Mycol. Res. 101: 667-77.

Cooke, D.E.L., Drenth, A., Duncan, J.M., Wagels, G. and Brasier, C.M.. 2000. A molecular phylogeny of *Phytophthora* and related Oomycetes. Fungal Genetics and Biology 30: 17-32.

Cooke, D.E.L., Kennedy, D.M., Guy. D.C., Russel, J., Unkles, S.E. and Duncan, J.M. 1996. Relatedness of Group I species of *Phytophthora* as assessed by RAPDs and sequences of ribosomal DNA. Mycol. Res. 100: 297-303.

Crawford, A.R., Bassam, B.J., Drenth, A., Maclean, D.J. and Irwin, J.A.G. 1996. Evolutionary relationships among *Phytophthora* species deduced from rDNA sequence analysis. Mycol. Res. 100: 437-43.

Dandurand, L.M. and Menge, J.A. 1992. Influence of *Fusarium solani* on citrus root rot caused by *Phytophthora parasitica* and *Phytophthora citrophthora* . Plant and Soil 144: 13 -21.

Davis,R.M. 1982. Control of *Phytophthora* root and foot rot of citrus with systemic fungicides metalaxyl and phosetyl aluminium. Plant Dis. 66:218-220.

Duniway, J.M. 1983. Role of physical factors in the development of *Phytophthora* diseases. In: "Phytophthora: Its Biology, Taxomony, Ecology and Pathology". (eds. Erwin,D.C, Bartnicki-Garcia, S. and Tsao, P.H.) American Phytopathological Soc., St. Paul, MN. pp. 175-187.

Erwin, D.C. and Ribiero, O.K. 1996. Phytophthora Diseases Worldwide. St. Paul, MN: Am. Phytopathol. Soc. 562 p.

FAOSTAT 2002. Database results. Food and Agricultural Organisation of United Nations, http://fao.org. 8p.

Fawcett, H.S. 1913. Two fungi as causal agents in gummosis of lemon trees in California. *Phytopathology*.3: 194.

Fawcett, H.S. 1936. Citrus diseases and their control. McGraw-Hill Book Co. New York and London, 656 p.

Feld, S.J., Menge, J.A. and Stolzy, L.H. 1990. Influence of drip and furrow irrigation on Phytophthora root rot of citrus under field and greenhouse conditions. Plant Dis. 74: 21-27.

Fisher, J. 1993a. Guideline proposed for producing *Phytophthora* free trees. Citrus Ind. 74(6):22-25.

Fisher, J. 1993b. Ciba Geigy addresses *Phytophthora* resistance to Ridomil. Citrus Ind. 74(6): 28-29.

Fraser,L.R. 1942. *Phytophthora* root rot of citrus. J. Austral. Inst. Agric. Sci. 8:101-105.

Fraser, L.R.1966. Citrus die back in India. Report to the dept. of External Affair, Canberra, Australia. Pp.95

Fraser, L.R. and Singh, D. 1966. Root rot of Citrus in India. Indian Hort.11: 15-16 & 26.

Ghosh, S.P. and Singh, R.B. 1993. Citrus in South Asia. FAO Regional Office for Asia and the Pacific, Bangkok 1993/24, 70 p.

Govindarao, P. 1954. Citrus diseases and their control in Andhra Pradesh. Andhra agric. J.187-192.

Graham, J.H. 1986. Citrus mycorrhizae: Potential benefits and interactions with pathogens. Hort-Science 21: 1302-1306.

Graham, J.H. 1990. Evaluation of tolerance of citrus rootstocks to *Phytophthora* root rot in Chlamydospore-infested soil. Plant Dis. 74:743-746.

Graham, J.H. 1995a. Screening for rootstock tolerance to *Phytophthora*: progress and prospects. Citrus Ind. 75 (5): 18-21.

Graham, J.H. 1995b. Root regeneration and tolerance of citrus rootstocks to root rot caused by *Phytophthora nicotianae*. Phytopathology 85:111-117.

Graham, J.H. and Castle, W.S. 1994. Screening citrus genotypes for tolerance to *Phytophthora* root rot in chlamydospore-infested soil. Proc. IV Congress Intern. Soc. Citrus Nurserymen, Johannesburg, South Africa.pp. 307-315.

Graham, J.H. and Menge, J.A. 1999. Root diseases. In: "Citrus health management". (eds. Timmer, L.W. and Duncan,L.W.) American Phytopathological Society, St. Paul, MN. pp. 126-135.

Graham, J.H. and Menge, JA. 2000. *Phytophthora*-induced diseases In: "Compendium of Citrus diseases", (eds. Timmer,L.W., Garnsey, S.M. and Graham, J.H.) American Phytopathlogical Society, St. Paul, MN., pp. 12-15.

Graham, J.H. and Timmer, L.W. 1992. *Phytophthora* diseases of Citrus. In: "Plant diseases of international importance", (eds. Kumar, J., Chaube, H.S., Singh,U.S. and Mukhopadhyay, A.N.) New Jersey, Prentice-Hall Inc., pp. 250-269.

Graham, J.H., Timmer, L.W., Drouillard, D.L. and Peever, T.L. 1998. Characterization of

Phytophthora spp. causing outbreaks of citrus brown rot in Florida. Phytopathology 88: 724-729.

Grimm, G.R. and Alexander, A.F. 1973. Citrus leaf pieces as traps for *Phytophthora parasitica* from soil slurries. Phytopathology 63: 540-541.

Grimm, G.R. and Hutchison, D.J. 1973. A procedure for evaluating resistance of citrus seedlings to *Phytophthora parasitica*. Plant Dis. Rep. 57: 669-672.

Grimm, G.R. and Hutchison, D.J. 1977. Evaluation of Citrus spp. relatives and hybrids for resistance to *Phytophthora parasitica* Dastur. Proc.Int. Soc.Citri. 3: 863-865.

Gunderson, J.H., Elwod, H., Ingold, H., Kindle, A. and Sogin, M.L. 1987. Phylogenetic relationship between chlorophytes, chrysophytes and oomycetes. Proc. Natl. Acad. Sci. USA 84: 5823-5827.

Grosser, J.W. and Gmitter, Jr F.G. 1990. Protoplast fusion and citrus cultivar improvement. Plant Breeding Rev. 8: 339-374.

Grosser, J.W., Louzada, E.S., Gmitter, Jr F.G. and Chandler, J.L. 1994. Somatic hybridization of complementary citrus rootstocks: Five new hybrids. Hort. Sci. 29: 812-813.

Grosser, J.W., Gmitter, Jr F.G., Castle, W.S. and Chandler, J.L. 1997. Production and evaluation of citrus somatic hybrid rootstocks: Progress report. Proc. Int. Soc. Citriculture 3: 1246-1250.

Hendrix, F.F. Jr. and Kuhlman, E.G. 1965. Factors affecting direct recovery of *Phytophthora cinnamomi* from soil. Phytopathology 55: 1183-1187.

Hough, A. 1992. Citrus rootstock reaction to *Phytophthora* root rot. Citrus Journal 2(5):40-43.

Ippolito, A., Nigro, F. and Lima, G. 1997a. Influence of the scion on the response of sour orange rootstock to experimentally induced Phytophthora gummosis and root rot. Proc. Int. Soc. Citriculture 385-388.

Ippolito, A., Nigro, F. and Lima, G. 1997b. Effectiveness of fosetyl.al and metalaxyl against Phytophthora root rot in sour orange grafted with clementine. Proc. Int. Soc. Citriculture 389-393.

Kamat, M.N. 1927. The control of mosambi gummosis. Agric. J.India. 22: 176-79.

Kapur, S.P. and Bakshi, J.C. 1967. Foot rot or gummosis a serious disease in Citrus orchards. Punjab Horticulture 7:85-89.

Kar, P.C. and Saha, J.C. 1943. Controlling of fruit scab of pummelo. *Sci. Cult*. 8(10) 422-423.

Katan, J. 1987. Soil solarization. In: "Innovative approaches to plant disease control". (ed. Chet.I.) Wiley, New York, pp. 77-221.

Katan, J. 1999. The methyl bromide issue: problems and potential solutions. J. Plant. Pathol. 81: 153-159.

Katan, J. 2000. Physical and cultural methods for the management of soil-borne pathogens. Plant Protection, 19: 725-731.

Khew, K.L. and Zentmyer, G.A. 1973. Chemotactic response of zoospores of five species of *Phytophthora*. Phytopathology 63: 1511-1117.

Khew, K.L. and Zentmyer, G.A. 1974. Electostatic response of zoospores of seven species of *Phytophthora*. Phytopathology 64: 500-507.

Klotz, L.J., DeWolfe, T.A, and Wong, P.P. 1958a. Decay of fibrous roots of citrus. Phytopathology 48: 616-22.

Klotz, L.J., DeWolfe, T.A. and Wong, P.P. 1958b. Influence of two varieties of Citrus scions on the pathogenicity of three isolates of *Phytophthora parasitica* to sweet orange rootstocks. Phytopathology 48:520-521.

Klotz, L.J., Wong, P.P. and DeWolfe, T.A. 1959. Survey of irrigation water for the presence of *Phytophthora* spp. pathogenic to citrus. Plant Dis. Rep. 43:830-832.

Klotz, L.J., DeWolfe, T.A., Roistacher, C.N., Nauer, E.M. and Carpenter, J.B. 1960. Heat treatments to destroy fungi in infected citrus seeds. Calif. Citrog. 46: 63-64.

Klotz, L.J., De Wolfe, D.A., Newcomb and Platt, R.C. 1966. Control of damping off of citrus seedlings. Calif. Citrog. 51: 314, 322, 324.

Klotz, L.J. and Calavan, E.C. 1969. Gum diseases of citrus in California. Calif. Agric. Exp. Stn. Ext. Serv. Circ. 396. 26pp.

Klotz, L.J., Bitters, W.P., DeWolfe, T.A. and Garber, M.J. 1968. Some factors in resistance of citrus to *Phytophthora* spp. Plant Dis. Rep. 52: 952-955.

Kumbhare, G.B. and Moghe, P.G.1976. Leaf fall disease of Nagpur orange caused by *Phytophthora nicotianae* var *parasitica* Waterhouse. Curr.Sci. 45: 561-562.

Kumbhare, G.B. and Choudhari, K.G. 1978. Reaction of few citrus Cultivars to root rot caused by *Phytophthora palmivora*. Indian J. Mycol. Pl.path. 8: 211-212.

Labuschagne, N. 1994. *Fusarium solani* as a root pathogen of citrus - an overview. Citrus Journal 4(5): 22-24.

Labuschagne, N. Van der Vegte, F.A. and Kotze, J.M. 1989.Interaction between *Fusarium solani* and *Tylenchulus semipenetrans* on citrus roots. Phytophylactica 21, 29-33.

Laing, M.D. and Beattie, H.V. 1997. Control of soilborne pathogens of citrus seedlings in composted pine bark medium. Proc. Int. Soc. Citriculture 397-401.

Lee, S.B., White, T.J. and Taylor JW. 1993.Detection of *Phytophthora* species by oligonucleotide hybridization to amplified ribosomal DNA spacer. Phytopathology 83: 117-181.

Lele, V.C. and Kapoor, J.N. 1982. *Phytophthora* on Citrus in central and peninsular India. Indian Phytopathology. 35: 407-410.

Levesque, C.A., Harlton, C.E. and DeCock, W.A.M. 1998. Identification of some oomycetes by reverse dot blot hybridization. Phytopathology 88: 213-222.

Lutz, A. and Menge, J.A. 1986a. Seasonal growth of citrus feeder roots and shoots and rhizo-sphere population fluctuations of *Phytophthora* parasitica. Phytopathology 76: 1093-1094.

Lutz, A. and Menge, J.A. 1986b. Citrus root health II. Phytophthora root rot. Citrograph 72(2):33-36.

Masago, H., Yoshikawa, M., Fukada, M. and Nakanishi, N. 1977. Selective inhibition of *Pythium* spp. on a medium for direct isolation of *Phytophthora* spp. on a medium for plants. Phytopathology 67:425-428.

Matheron, M.E. and Porchas, M. 1996. Colonization of citrus roots by *Phytophthora citrophthora* and *P. parasitica* in daily soil temperature fluctuations between favorable and inhibitory levels. Plant Dis. 80: 1135-1140.

Matheron, M.E., Porchas, M. and Matejka, J.C. 1997. Distribution and seasonal population dynamics of *Phytophthora citrophthora* and *P.parasitica* in Arizona citrus orchards and effect of fungicides on tree health. Plant Dis. 81: 1384-1390.

Matheron, M.E., Wright, G.C. and Porchas, M. 1998. Resistance to *Phytophthora citrophthora* and *P.parasitica* and nursery characteristics of several citrus rootstocks. Plant Dis. 82: 1217-1225.

McCain, A.H., Holtsmann, O.V. and Trujillo, E.E. 1967. Concentration of *Phytophthora cinnamomi* chlamydospores by soil sieving. PhytoPathology 57: 1134-1135.

Martin, R,R., James, D. and Levsque, C.A. 2000. Impacts of molecular diagnostic technologies on plant disease management. Annu. Rev. Phytopathol.38: 207-59.

Menge, J.A. 1986. Use of Systemic fungicides on citrus. Citrograph 71: 245-252.

Menge, J.A. 1993. Improved root rot control with mulches. Calif.Citrog. 79(1): 16-17.

Miller, S.A. and Martin, R.R. 1988.Molecular diagnosis of plant diseases. Annu. Rev. Phytopathol. 26: 409-432.

Miller, S.A., Rittenburg, J.H., Petersen, F.P. and Grothaus, G.D.1990. Development of modern diagnostic techniques and benefits to the farmer. In: "Monoclonal antibodies in agriculture" (eds Schots, A.), Pudoc, Wageningen pp15-24.

Mitchell, D.J., Kannwischer-Mitchell, M.J. and Zentmeyer, G.A. 1986. Isolation, identification

and producing inoculum of *Phytophthora* spp. In: "Methods for evaluating pesticides for control of Plant Pathogens." (ed. Dickey, K.D.), APS Press, St.paul, MN pp. 63-66.

Mukherjee, J.N. 1949. Trace elements deficiencies in citrus. Sci.Cult. 15(6): 235-237.

Naqvi, S.A.M.H. 1988. Prevalence of *Phytophthora* spp. pathogenic to citrus in orange groves of Vidarbha, Maharastra. Indian J. Mycol. & Pl.path. 18: 274-276.

Naqvi, S.A.M.H. 1990. Survey of Nagpur mandarin nurseries for *Phytophthora* spp. Annual Report 1989-90, NRC for Citrus Nagpur.pp 84.

Naqvi, S.A.M.H. 1994. Efficacy of some fungicides in control of *Phytophthora* diseases of Nagpur mandarin in Central India. Indian Phytopathology. 47(4): 430 -434.

Naqvi, S.A.M.H. 1996. Management of *Phytophthora* diseases of Citrus in India. In: "Perspectives in Biological Sciences" (eds. Rai, V., Naik, M.L. and Mohanacharry, C.) School of lifesciences, Ravishankar University, Raipur,India. pp 271-281.

Naqvi, S.A.M.H. 1997. Phytophthora diseases of Citrus and their management. Ext. Bulletin No. 10. NRC for Citrus, Nagpur, pp.7.

Naqvi, S.A.M.H. 1999a. Integrated management of fungal diseases of Citrus. In: "IPM system in Agriculture- Cash Crops" vol.6. (eds. Upadhyay, Rajeev K., Mukerji, K.G. and Dubey, O.P.) Aditya Books Pvt. Ltd. New Delhi, India. pp. 489-503.

Naqvi, S.A.M.H. 1999b. *Phytophthora* - a serious threat to Citrus industry. Intensive Agriculture, 36 (11 & 12): 23-27.

Naqvi, S.A.M.H. 1999c. Major pathogens of Indian Fruits and Vegetables. In: "IPM System in Agriculture -Cash Crops" vol.6. (eds. Rajiv Upadhyay, Mukerji, K.G. and Dubey, O.P.) Adiya Publishers, New Delhi, pp. 505-524.

Naqvi, S.A.M.H. 1999d. Production of Disease free planting material – control of soil borne diseases. In: "Citriculture" (ed. Shyam Singh), NRC for Citrus, Nagpur. pp.86-90.

Naqvi, S.A.M.H. 1999e. Major diseases of Citrus and their management. In: "Citriculture", (ed. Shyam Singh), NRC for Citrus, Nagpur. Pp.273-284.

Naqvi, S.A.M.H. 2000a. Managing *Phytophthora* diseases of Citrus. Indian Horticulture, 44(4): 5-9.

Naqvi, S.A.M.H. 2000b. Distribution of *Phytophthora* spp. and mating types pathogenic to citrus in Vidarbha and Marathwada region of Maharashtra and Northeastern States of India. In: "Hi-Tech Citrus management- Proc. Int. Symp. Citriculture, Nagpur India". (eds. Shyam Singh and Ghosh, S.P.) pp. 1073-1080.

Naqvi, S.A.M.H. 2000c. Recent trends in disease management of citrus. In: "Hi-Tech Citrus management- Proc. Int. Symp. Citriculture, Nagpur India." (eds. Shyam Singh and Ghosh, S.P.) pp. 785-799.

Naqvi, S.A.M.H. 2001a. Diagnosis and management of fungal diseases of Citrus. In: Citrus (eds. Singh, Shyam and Naqvi, S.A.M.H.) International Book Distributing Co. Lucknow, India, pp. 375-391.

Naqvi, S.A.M.H. 2001b. Citrus diseases and management in NEH region. In: Citrus decline and management in NEH region. (ed. Shyam Singh) NRC for Citrus, Nagpur, India, pp. 90-105.

Naqvi, S.A.M.H. 2002a. *Phytophthora* diseases of Citrus in India and their integrated management. National Symp. on Perspectives in integrated plant disease management organised by Indian Soc. of Plant Pathologists, Ludhiana, NRC for Citrus, Nagpur and CICR, Nagpur at NRC for Citrus, Nagpur. 13-14th Feb. 2002 (Abstract). pp. 21-22.

Naqvi, S.A.M.H. 2002b. Fungal Diseases of Citrus – Diagnosis and Management. Technical Bulletin No. 5. NRC for Citrus, Nagpur. 61p.

Naqvi, S.A.M.H. 2003. Production of *Phytophthora*-free nursery-stock – mandatory to avert decline and death of citrus plants in India. In: " Souvenir and Abstracts of National Symposium on Plant pathogens diversity in relation to plant health, January, 16-18th, 2003, Hyderabad, India"p. 139.

Naqvi, S.A.M.H. 2003. *Phytophthora* diseases of citrus and management strategies. Annual Review of Plant Pathology, vol. 2, (in press)

Naqvi, S.A.M.H. and Shyam Singh 1999. Phytophthora diseases - the major cause of citrus decline in Central India. Proc. of Nat. Symp. on Citriculture, Nov. 1997, Nagpur, pp 430-435.

Naqvi, S.A.M.H. and Shyam Singh, 2002.Citrus Decline -Present Status and Management Strategies. In: "IPM System in Agriculture, Key Pathogens and Diseases", vol. 8 (eds. Rajiv Upadhyay, Arora, D.K. and Dubey, O.P.) Adiya Publishers, New Delhi pp. 209-236.

Narasimhan, M.J. 1931. Sexuality of the koleroga fungus *Phytophthora arecae* (Cole.) Pethy. J. Mysore Agric. Exp. Union. 12:4-7.

Narasimhan, V., Subramanian, K.S., Shanmugam, N. and Jeyarajan, H.1984. Efficacy of certain fungicides in the control of powdery mildew of mandarin. Pesticides 18(1): 61-64.

Nariani,T.K. and Raychaudhuri, S.P. 1986. Citrus die-back problem in the tropics. Review of tropical Plant Pathology 2: 29 - 53.

Olsen, C.M.and Baker, K.F. 1968. Selective heat treatment of soil, and its effect on the inhibition of *Rhizoctonia solani* by *Bacillus subtilis*. Phytopathology 58: 79-87.

Paracer, C.S.and Chahal, D.S. 1962. Diseases of root, crown and trunk of Citrus. Punjab Hort. J. 2: 92-95.

Prasad, M.N.V.and Rao, N.N.R. 1983. Reaction of some Citrus root Stocks hybrids for tolerance to *Phytophthora* root rot. Indian Phytopathology. 36: 726-728.

Ramakrishnan, T.S. 1954. Common diseases of Citrus in madras state. Govt. of Madras. Madras.

Randhawa, N.S., Bhumbla, D.R. and Dhingra, D.R. 1966. Citrus decline in the Punjab -A review. Punjab Hort. J. 6: 35-44.

Raychaudhuri, S.P., Nariani, T.K. and Lele, V.C. 1967. Citrus dieback complex -a serious threat to citrus industry in India. In: "Proceedings Int. Symposium on Subtropical and tropical Horticulture, New Delhi", pp. 714-722.

Raychaudhuri, S.P., Nariani, T.K. and Lele, V.C. 1969. Citrus dieback problem in India. In: "Proceedings Ist int.citrus Symp. III" (ed. Chapman, H.D.), Riverside,CA. pp. 1432-1437.

Reddy, G.S.and Murti,V.D. 1985. Citrus diseases and their control. ICAR Publication, New Delhi,7 -8.

Reddy, M.R.S., Naidu, P.H. and Raju, D.J. 1984. Screening of citrus germplasm for resistance to powdery mildew. Curr. Sci. 53: 1163-1164.

Ristaino, J.B., Larkin, R.P. and Campbell, C.L. 1994. PCR amplification of ribosomal DNA for species identification in the pathogen genus *Phytophthora*. Appl. Environ. Microbiol. 68: 948-954.

Ristaino, J.B. and Gimpertz, M.L. 2000. New frontiers in the study of dispersal and spatial analysis of epidemics caused by species in the genus *Phytophthora*. Annu. Rev. Phytopathol. 38: 541-576.

Robert, E.R. and Edgar, D.H. 1993. Survey of rootstock susceptibility to foot rot in the immokalee foundation grove. Citrus Ind. 74(3): 44-45.

Roose, M.L. 1997. Rootstock breeding at the university of California, Riverside. Proc. Int. Soc. Citriculture 1254.

Roy, S.D. and Das, S. 1995. Infectivity potential of powdery mildew on mandarin orange (*Citrus reticulata* Blanco) in the hills of Darjeeling. Adv. Plant Sci. 8: 83-85.

Roy, S.D. and Ghosh, S.K. 1991. Global status of powdery mildew (*Oidium tingitaninum*) disease on Citrus spp. J. Mycopathol. Res. 2: 127-132.

Sandler, H.A., Timmer, L.W., Graham, J.H. and Zitko, S.E. 1989. Effect of fungicide applications on populations of *Phytophthora parasitica* and on feeder root densities and fruit yields of citrus trees. Plant Dis. 73:902-906

Sawant, Indu, S., Sawant, S.D. 1989. Coffee fruit skin and cherry husk as substrates for mass

multiplication of *Trichoderma harzianum* an antagonist of Citrus *Phytophthora*. Indian Phytopathology. 42: 336.

Sawant, S.D., Sulladhamath, V.V. and Ganapathi, M.M. 1993. A method to rank collection/ strains of same *Citrus* species on the basis of susceptibility to *Phytophthora*. Golden Jubilee Symposium of Horticultural Society of India.

Schutte, G.C. 1994. The timings of fosetyl-al (Aliette) treatments for *Phytophthora* root rot control in the summer rainfall regions. Cirus Journal 4(2): 20-21.

Skaria, M. and Miller, S.A. 1989. A rapid test for detecting *Phytophthora* spp. in citrus. J. RioGrande Valley Hortic. Soc. 42:63-67.

Somani, R.B. and Patel, A.J. 1969. Note on gummosis of lime (*C. aurantifolia* (Christian) Swing) and its control. Indian J. Agric. Sci 40: 533-534.

Somani, R.B. and Patel, A.J. 1972. Susceptibility of Kagzi lime to *Phytophthora nicotianae* Breda de Haan in Gujrat. Indian J.MicroBiol. 12: 205-206.

Singh, L.B. 1962. Studies on the root-stock for mandarin in the wet Subtropics (variety Hill). Indian J. Hort. 19: 1-9.

Singh, Shyam and Naqvi, S.A.M.H.(eds.) 2001. Citrus. International Book Distributing Co. Lucknow, India, 588 p.

Singh Shyam, Shivankar,V.J., Naqvi, S.A.M.H., Singh, I.P., Ghosh, D.K. Das, A.K. 2001. Production of disease free planting material of Citrus. In: "Citrus" (eds. Shyam Singh and Naqvi, S.A.M.H.) International Book Distributing Co. Lucknow, India, pp. 153-163.

Stapleton, J.J. 2000. Soil solarization in various agricultural production systems. Plant Protection, 19: 837-841.

Stapleton, J.J. and DeVay, J.E. 1995. Soil solarization: A natural mechanism of integrated pest management. In: "Novel approaches to Integrated Pest management" (ed. Reuveni, R.) Lewis Publishers, Boca, Raton, pp. 309-322.

Stolzy, L.H., Letey, J., Klotz, L.J. and Labanauskas, C.K.1965. Water and aeration as factors in root deay of *Citrus sinensis*. Phytopathology 55:270-275.

Timmer, L.W. 1977. Preventive and curative trunk treatments for control of *Phytophthora* foot rot of citrus. Phytopathology 67:1149-1154.

Timmer, L.W., Graham, J.H., Sandler, H.A. and Zitko, S.E. 1988. Population of *Phytophthora parasitica* in citrus orchards and tree response to fungicide applications. Citrus Ind. 69: 40-4, 54.

Timmer, L.W., Sandler, H.A., Graham, J.H. and Zitko, S.E. 1989a. Phytophthora feeder root rot of bearing citrus: Fungicides effects on soil populations of *Phytophthora parasitica* and citrus tree productivity. Proc. Fla. State Hortic. Soc. 102: 5-9.

Timmer, L.W., Zitko, S.E., Sandler, H.A. and Graham, J.H. 1989b. Seasonal and spatial analysis of populations of *Phytophthora parasitica* in citrus orchards. Plant Dis.73: 810-813.

Timmer, L.W., Zitko, S.E. and Sandler, H.A. 1990. An isolate of *Phytophthora arecae* from Florida pathogenic to citrus (abstr.) Phytopathology . 80:1025.

Timmer, L.W., Menge, J.A., Zitko, S.E., Pond, E., Miller, S.A. and Johnson, E.L. 1993. Comparison of ELISA techniques and standard isolation methods for Phytophthora detection in citrus orchards in Florida and California. Plant Dis. 77:791-796.

Timmer, L.W., Graham, J.H. and Zitko, S.E. 1998. Metalaxyl resistant isolates of *Phytophthora* nicotianae: Occurrence, sensitivity, and competitive parasitic ability on citrus. Plant Disease 82: 254-261.

Timmer, L.W., Garnsey, S.M. and Graham, J.H. (eds.) 2000.Compendium of Citrus diseases. American Phytopathlogical Society Press Inc., St. Paul, MN.92p

Trout, C.L., Ristainom J.B., Madritch, M.and Wangsomboondee, T. 1997. Rapid detection of *Phytophthora infestans* in late blight infected tissue of potato and tomato using PCR. Plant Dis. 81:1042-1048.

Tsao, P.H. 1960. A serial dilution end-point method for estimaating disease potential of citrus phytophthoras in soil. Phytopathology 50: 717-724.

Tsao, P.H. 1969. Studies on the saprophytic behavior of *Phytophthora parasitica* in soil. Proc. Ist Int.Citrus Symp. (ed. Chapman, H.D.), pp.1221-1230.

Tsao, P.H. 1970. Selective media for isolation of pathogenic fungi. Annu. Rev. Phytopathol. 8: 157-186.

Tsao, P.H. 1983. Factors affecting isolation and quantitation of *Phytophthora* from soil. In: "Phytophthora, its biology, taxonomy, ecology and pathology" (eds. Erwin,D.C., Bartnicki-Garcia, S. and Tsao, P.H.) APS press, St.paul, MN, USA, pp. 219-236.

Tsao, P.H. and Guy, S.O. 1977. Inhibition of *Mortierella* and *Pythium* in a *Phytophthora* isolation medium containing hymexazol. Phytopathology 67: 796-801.

Tsao, P.H., Fang, J.G., Sztejnberg, A. and Daft, G.C. 1997. Biological control with fungi antagonistic against Phytophthora root rot of citrus. Proc. Int. Soc. Citriculture 394-396.

Tuset, J.J., Hinarejos, C. and Garcia, J. 1990. *Phytophthora* foot rot control in citrus with *Myrothecium roridum.* Bulletin OEPP 20:169-76.

Uppal, B.N. and Kamat, M.N. 1936. Gummosis of Citrus in Bombay. Indian J. Agric. Sci. 6: 803-820.

Whiteside, J.O. 1972. Foot rot of citrus trees – the importance of high budding as a preventive measure. Citrus Ind. 53(4): 14-17.

Whiteside, J.O. 1973. *Phytophthora* studies on Citrus root-stocks. In: "Proc. First int. Citrus short course". (eds. Jackson, L.K., Krezdron, A.H. and Soule, J.) Gainesville, FL pp. 15-21.

Whiteside, J.O. 1974. Zoospore inoculation techniques for determining the relative susceptibility of citrus rootstocks to foot rot. Plant Dis. Rep. 58:713-17.

Whiteside, J.O.1975. Biological characteristics of *Elsinoe fawcettii* pertaining to the epidemiology of sour orange scab. Phytopathology 65:1170-1175.

Whiteside, J.O.1981. Evolution of current methods for citrus scab control. Proc.Fla.State Hortic.Soc. 94: 5-8.

Whiteside,J.O., Garnsey,S.M. and Timmer,L.W.(eds.) 1988. Compendium of citrus diseases. APS Press, St.Paul. MN,77 p.

Widmer, T.L., Graham, J.H. and Mitchell, D.J. 1998. Histological comparison of fibrous root infection of disease-tolerant and susceptible citrus hosts by *Phytophthora nicotianae* and *P. palmivora*. Phytopathology 88: 389-395.

Zentmyer, G.A. 1983. The world of *Phytophthora*. In: "Phytophthora- its biology, taxonomy, ecology and pathology" (eds. Erwin, D.C., Bartinicki-Garcia, S. and Tsao, P.H). APS Press, pp. 1-7.

Zitko, S.E., Timmer, L.W. and Castle, W.S. 1987. Survey of Florida Citrus nurseries for *Phytophthora* spp. Proc. Fla. State Hort. Soc.100: 82-85.

Zitko, S.E., and Timmer, L.W. 1994. Competitive parasitic abilities of *Phytophthora parasitica* and *P.palmivora* on fibrous roots of citrus. Phytopathology 84: 1000-1004.

Certification Programs for Citrus

Richard F. Lee

University of Florida, Citrus Research and Education Center
Lake Alfred, FL 33850, U.S.A.

Abstract: Certification programs are important for the long term sustainability of citrus and other crops which are vegetatively propagated. The certification program provides a basic platform for all integrated pest management practices, it is important for the management of insect and fungal pests, and the program ensures that the grower is planting healthy germplasm of the highest horticultural quality. Graft transmissible pathogens without insect vectors are effectively controlled, and graft transmissible pathogens having insect vectors or other means of natural spread can be controlled and their damage minimized. To be effective, a certification program must be mandatory. The components of a certification program include a quarantine program, a clean stock program, and a certification program to provide for distribution of the high quality, virus testes propagating material into the citrus industry for the benefit of the citrus industry as a whole. Sustainability of a certification program and other considerations are summarized. The cost of planting trees healthy trees of the highest genetic potential which originate from a certification program is very small, considering that a healthy citrus planting may outlive the person who plants it.

1. Introduction

There are many diseases which can limit the productivity of a citrus planting. Most diseases caused by fungi and bacteria can be controlled by chemical applications and cultural practices; they are not passed from generation to generation of trees by the multiplication of budwood. However graft transmissible pathogens remain in all offspring if they are present in the propagating materials during propagation. These graft transmissible pathogens include viruses, viroids, systemic prokaryotes such as *Xylella fastidiosa* and the systemic bacterium causing Huanglongbin (citrus greening), phytoplasmas, and spiroplasma. Often these pathogens occur as latent infections due to trees being propagated on tolerant rootstocks or residing in tolerant scions, but suddenly become destructive if propagated on a susceptible rootstock. For example, sour orange has been the most popular rootstock in the Caribbean Basin area, accounting for nearly 90 per cent of the total tree population in the region (Lee *et al.,* 1994). Following the introduction of the exotic pest *Toxoptera citricida,* the most efficient vector or citrus tristeza virus (CTV), in the late 1970 into Venezuela (Ochoa Corona *et al.,* 1994), this exotic pest continued its geographic spread throughout the Caribbean Basin area (Rocha-Pena *et al.,* 1995). Following the establishment of this pest, commonly called the brown citrus aphid (BrCA), in new areas, new severe strains of CTV occurred usually in 2-5 years. The first sign of the presence of new severe strains of CTV is

usually an outbreak of decline on sour orange rootstock which becomes widespread in the growing area (Rocha-Pena *et al.,* 1998). Because the decline on sour orange root-stock aspect of CTV can be controlled by growing trees on a CTV-tolerant rootstock, farmers in the region begin to propagate citrus on CTV-tolerant rootstocks. In each country, it is when the newly propagated trees on CTV-tolerant rootstocks do not grow well that the growers realized that sour orange was a rootstock of preference because of it's tolerance to many graft transmissible pathogens which are commonly present in the budwood. When the budwood harboring these pathogens is propagated on a suscep-tible rootstock, the symptoms are then expressed with devastating results. Mandarin (*Citrus reticulata* Blanco) rootstocks are very susceptible to cachexia, lemon (*C. limon* (L.) Burm. f.) type rootstocks are susceptible to woody gall and to citrus blight, *Poncirus trifoliata* (L.) Raf. and hybrid rootstocks of citrange [*C. sinensis* (L.) Osbeck X *P. trifoliata* (L.) Raf.] and/or citrumelo [*P.* trifoliata (L.) Raf. X *C. paradisi* Mac.] where one of the parents is *P. trifoliate* are susceptible to citrus tatterleaf virus, exocortis and other citrus viroids, and citrus blight (Rocha-Pena *et al.,* 1998). Thus, the need for pathogen-free propagating materials has become readily apparent in the Caribbean Basin.

While certification programs have in common a general scheme, they can be flexible so that special needs of a country or growing area can be meet. With planning, they can provide control of other pests which are not graft-transmissible pathogens but which can be spread through a region by movement of nursery materials. For example, the Florida nursery certification program regulates nematodes which affect citrus, all nurseries, not just citrus nurseries, are regulated as well as monitoring of fill dirt and borrow pits to keep nematodes from becoming widely distributed in the state. More recently citrus root weevils, *Diaprepes abbreviatus* (L.), have been included because this exotic pest, present in isolated locations since the 1960s, began increasing its geographic area in Florida by the movement of infested container nursery material (http://doacs.state.fl.us/~pi/5b-2.htm). In other countries certification programs moni-tor nurseries to keep *Phytophthora* propogules low, especially in areas where the irriga-tion water is of reservoir origin (Lee *et al.,* 1999). Horticultural standards may be imposed on the nursery propagations, such as establishment of a minimum height for budding and a minimum size for plants before they can be planted.

2. Interrelatedness of quarantine, clean stock and certification programs

The term "certification program" is often used in a wrong sense as it commonly is applied to refer to a clean stock program which if used as a propagating source, would enable propagation of clean nursery plants. A true certification program is a marriage of three separate, but well integrated, programs which ensures the production of healthy and high quality nursery trees, and provides protection against unknowing importation of additional exotic pests which may threaten continued production of citrus in the future. The three programs which comprise a certification program are:
(i). quarantine program for the safe introduction of select horticultural germplasm,
(ii). clean stock program for the testing and therapy treatments to identify domestic sources and to produce pathogen free material for commercial use, and (iii). a certifica-

tion program to provide a method of maintaining the pathogen free materials and to making them available to nurseries and growers to benefit the industry. The healthy plants from the quarantine program and the clean stock program provide the necessary material for the certification program.

2.1 Quarantine programs

Citrus growers worldwide are always looking for new citrus germplasm which may appeal to consumers or offer a competitive advantage. For example, an earlier or later maturing variety which may extend an already recognized market, such as navel oranges for the fresh fruit market, or better fruit pigmentation, unique fruit shape, seedlessness, or a newly released cultivar from another part of the world. These are common reasons growers are interested in importing citrus germplasm. Plant breeders want additional cultivars and varieties for use in their breeding program for the development of new rootstocks and scions. In each country a constant demand exists for exotic germplasm for use in the citrus industry. Uncontrolled importation of such exotic germplasm can result in the importation of new pests and pathogens which may cause important economic damage. These risks may be minimized by carefully controlled introduction through quarantine stations which allow for safe importation of foreign germplasm without the introduction of new pests and diseases.

Quarantine programs operate under the jurisdiction of the ministry of agriculture of a country or commissioner of agriculture in a state or province. Usually the quarantine programs are operated by the plant protection services of the government.

Two different approaches to quarantine have been developed with citrus. The traditional approach is to locate quarantine greenhouses in an isolated area away from the commercial citrus (Lee *et al.,* 1999). The imported budwood is tested for presence of pathogens, and either thermotherapy and/or shoot tip grafting methods are applied to free the introduced germplasm from the pathogens present in the original germplasm. It is important to remember that the germplasm is only free of the pathogens for which it has been tested for. Other graft transmissible pathogens may be present which have not been tested for. These other pathogens may cause problems which have not yet been recognized. For example, the decline of Roble orange on Swingle citrange rootstock in Florida appears to be due to the presence of citrus leaf blotch virus, a pathogen not recognized until recently (Galipienso *et al.,* 2001; Vives *et al,* 2003). This traditional method requires expensive facilities and it is difficult to justify the expense in countries where citrus is a minor crop.

The second approach to quarantine is the *in vitro* method where the imported budwood is kept under quarantine in glass test tubes (Navarro *et al.,* 1984). This procedure was developed in Spain for the safe introduction of citrus varieties and has proven to be effective. Budsticks, upon receipt from the exotic source, are thoroughly cleaned and surface sterilized, then placed immediately into test tubes in culture media and placed in a growth chamber. When shoots begin to develop, shoot tip grafting, taking only a very thin section (about 0.2 mm thick) of the meristematic bud are grafted to a healthy seedling plant, using the receptor seedling plant as a rootstock for the shoot tip bud (Roistacher, 1991). This procedure minimizes the risk of introduction of pathogens,

requires less space than the traditional quarantine greenhouse, and enables rapid processing of new entries.

The *in vitro* method of quarantine has several advantages. Pests and diseases are eliminated early, thus shortening the time in quarantine. The test tubes serve as a substitute for the traditional quarantine greenhouse. Shoot tip grafting is commonly used to therapy local germplasm of graft transmissible pathogens, so the expertise is usually already available. Spain has introduced well over 100 cultivars using the *in vitro* method of quarantine, and the method is used in California also (Navarro, 1993; Lee *et al.,* 1999).

Importation of citrus germplasm from areas where high risk diseases are present should be avoided, if possible. Examples of high risk diseases are huanglongbin, or citrus greening, common in many Oriental and Asian countries (Bove and Garnier, 1984; Bove 1995) and citrus variegated chlorosis (CVC), caused by a strain of *Xylella fastidiosa*, present in several countries in Brazil and neighboring countries in South America (Beretta *et al.,* 1993, Lee 1998; Lee *et al.,* 1991). CVC has been reported in Costa Rica, Central America, recently (Moreira *et al.,* 2002). Other diseases which should be avoided include witches' broom disease of lime (Garnier *et al.,* 1991) having a leaf hopper vector, and citrus chlorotic dwarf (Korkmaz *et al.,* 1995) having a whitefly vector. Care must be taken not to import these diseases into new areas. If citrus germplasm is imported from high risk areas, extra caution must be taken to ensure the introductions are pathogen free.

While citrus seed is commonly imported without concern of its carrying graft transmissible pathogens, this is not a good practice. There are now reports of CVC and witches' broom of lime being seed transmitted (El-Kharbotly *et al.,* 2003) as well as psorosis and psorosis-like pathogens in *P. trifoliata* and in hybrids having *P. trifoliata* as one of the parents (Roistacher, 1991). Seed should only be imported from countries which have recognized, certified seed source trees. Citrus certification programs allow for propagation and certification of true-to-type rootstock seed source trees which have been tested for freedom from known graft transmissible pathogens.

2.2 Clean stock program

It is desirable to recover healthy plants from the locally grown varieties and cultivars in addition to importing desirable exotic germplasm. The clean stock program provides for the testing and therapy to identify and produce sources of pathogen-free propagating stock from domestic sources. Often local varieties are best adapted to the local climate, soil and markets. If these locally selected sources are cleaned of graft-transmissible pathogens, they can be productive and useful.

Several steps are involved in the establishment of a clean stock program. (i). selection of mother trees from the local cultivars, (ii). indexing of the selected mother trees, (iii). recovery of pathogen-free plants by *in vitro* shoot tip grafting and/or thermal therapy, (iv). indexing of the plants recovered, (v). horticultural evaluation of the healthy plants, and (vi). maintenance of healthy plants.

The selection of mother trees should be done based on documentable criteria, such as superior productivity, higher fruit color, early or late ripening. Growers and

industry personnel are often the ones most likely to identify desirable characteristics in the field. The absence or presence of graft transmissible pathogens should not be a factor, as these pathogens will be eliminated by therapy. The field selections should be propagated in the greenhouse on vigorous rootstocks for a source of material for virus indexing and therapy procedures.

Because most infections by a graft-transmissible pathogen are latent, indexing must be performed on the selected mother trees in order to know what pathogen(s) need to be eliminated. Most indexing is done using indicator plants under a controlled environment; see the summary of indicator plants and growing conditions in Roistacher (1991). This biological indexing should be supplemented by laboratory procedures using serology, polymerase chain reaction assays or microscopy methods (Roistacher 1991). Graft-transmissible pathogens found in the domestic trees should be retained as a virus collection, and they are useful as positive controls for future biological/laboratory indexing. Healthy plants are recovered from the selected mother trees. This most commonly is done by shoot tip grafting procedures, but thermotherapy and *in vivo* or *in vitro* nucellar embryony methods are used also. Nucellar embryony is effective for elimination of graft-transmissible pathogens because most pathogens are not seed transmissible. However, nucellar plants express juvenile characteristics such as thorniness, late bearing, up-right growth, and excessive vigor. Additionally, not all seedlings are true-to-type.

Thermotherapy produces true-to-type plants without juvenile characteristics. However, some pathogens thrive under warm growing conditions, such as exocortis, cachexia, and other viroids, and citrus stubborn. Pathogens which require warm growing conditions are difficult to eliminate by thermotherapy.

Shoot tip grafting is the most commonly method used to therapy plants. This method is effective at eliminating all graft-transmissible pathogens including ones difficult to eliminate by thermotherapy. Because shoot tip grafted plants do not undergo an embryonic stage, there are no juvenile characteristics displayed.

It is important to index the recovered plants to ensure they are freed of the graft-transmissible pathogens found in the mother tree sources from which they were derived. It is always a mistake to assume that a plant which has been shoot tip grafted is automatically freed of graft-transmissible pathogens.

Horticultural evaluation of the recovered plants is very important. There has never been a problem reported with shoot tip grafted plants which resulted in abnormal recovered plants, but there can always be a spontaneous bud sport or labels may be placed on the wrong plants. A program does not want to release an off-type clone. Additionally the horticultural evaluation provides useful production information over a period of time enabling growers to choose the most productive clonal selections. If the horticultural evaluation is performed a several different rootstocks, the same plants can also serve as long term indexes for troublesome diseases. For example using Orlando tangelo rootstocks provide a long term index for cachexia, citrange rootstocks provide a long term index for citrus tatterleaf virus, and rough lemon rootstocks provide a long term index for woody gall.

Maintenance of the healthy plants to avoid re-infection by graft-transmissible pathogens is important. In most areas there are some graft-transmissible pathogens

which have insect vectors. This requires that the healthy plants be maintained either in a screenhouse or a greenhouse where they are protected from infestation by viruliferous vectors. Because of the time and effort devoted to developing a clean stock program, there should be back up plants maintained under protected conditions at a separate location in case something happens which threatens the virus-free status of the source plants in the collection. The plants in the clean stock program need to be re-indexed on a regular, recurring basis for the graft-transmissible pathogens which are present in the country.

In countries where citrus is not a major crop, consideration should be given to organizing a regional clean stock program. It is expensive to build quarantine quality screenhouses and greenhouses in which to house the clean stock program, and trained personnel are hard to find. If these expenses could be shared by two or more countries, it becomes much more affordable. Arrangements would need to be made to transport the clean budwood material over international borders. Also in countries where citrus is not a major crop, the certification program should consider importing a limited number to buds from desirable varieties from internationally recognized certification programs. Even then, these importations should be tested at the minimum for freedom from CTV, psorosis and psorosis-like pathogens and citrus viroids and be evaluated for horticulture trueness-to-type before being released to the growers. If indicator plants and adequate greenhouses are available, the biological indexing for psorosis and psorosis-like pathogens and citrus viroids can be completed within a few months. The freedom from CTV may be verified by serological testing. Locally selected varieties and cultivars also can be therapied by internationally recognized clean stock programs for a fee. This fee is usually less that the cost of developing in-country capacity for therapy. The local selections are returned as virus-free budsticks. If the decision is made to import clean stock from internationally recognized programs, there is still a need to confirm the freedom from virus infection by local indexing and to verify trueness-to-type. The need for re-indexing for virus and virus-like pathogens as well as the need for horticultural evaluation cannot be overemphasized.

2.3 Certification Programs

The purpose of certification programs is to guarantee the sanitary status and trueness-to-type of the nursery material during the process of commercial propagation through the nurseries. The programs are usually operated by a state or provincial agency having the legal authority to impose restrictions and to inspect nurseries. The programs are governed by legal regulations which detail the different steps of nursery operations and call for periodic indexing and inspection of trees being used for nursery propagations. The quarantine program and clean stock program provide the source of clean material which is distributed by the certification program. While many areas have clean stock programs, there are fewer certification programs. The programs in California, Florida, Spain, South Africa, Taiwan, and Australia are the longest running programs. Recently certification programs have been started in Belize, Jamaica, Brazil (Sao Paulo State), Uruguay, Argentina, and Oman.

Certification programs can be structured to the specific needs of each citrus area

(Lee *et al.,* 1999). Organization of the citrus industry, the graft-transmissible pathogens present, especially the presence of insect vectored pathogens, and grower interest are important considerations. Certification programs usually call for recurring indexing to verify the freedom from the graft-transmissible pathogens present in the area, not for exotic pests.

Certification programs provide the platform for an integrated pest management approach for growing citrus (Lee and Rocha-Pena 1992). The beginning point of a successful citrus operation is to plant healthy trees. The certification program can serve as the focal point for the release of new varieties and cultivars, for gathering yield and horticulture information, and for the distribution of extension-type information. In areas where CTV is managed by use of mild strain cross protection, the certification program provides the mechanism by maintaining plants infected with the specific strains for cross protection in the foundation block for use for propagation. Record keeping is an important part of a certification program. This allows for tracing problems which might appear at a later time back to a budwood source tree and/or specific nursery.The propagation components of certification programs are similar regardless of the country. The certification scheme is structured so that the time consuming, expensive indexing, and horticultural evaluations are limited to the protected, primary foundation trees, yet all plants being propagated benefit from the high horticultural quality and freedom from graft-transmissible pathogens. There are usually four blocks of trees in a certification program: (i). protected primary foundation blocks, (ii). foundation blocks which may be located in the field and/or under protected conditions depending on the local situation in regard to vectored pathogens, (iii). budwood increase blocks, and (iv). blocks of certified nursery trees to be distributed and planted in the industry.

In figure 1, Cartoon showing a general scheme of a certification program. The Protected Primary Foundation Block receives virus-free plants from either the Clean Stock Program (cleaned domestic selections) or the Quarantine Program (cleaned exotic selections). From the Protected Primary Foundation Block, Seed Source Tree Blocks are established, also Varietal Blocks to verify the horticultural trueness-to-type. The plants in the Protected Primary Foundation Block are the primary source of all buds for the countries citrus industry, and the plants are indexed on a recurring basis and continuously inspected for freedom from abnormalities. Buds from the Protected Primary Foundation Block may be used to establish Foundation Blocks, Budwood Increase Blocks, and/or Certified Nursery Trees. The Foundation Blocks may be in the field in locations where no insect vectored diseases are present; usually they are protected under screen. In some countries, the Foundation Blocks are owned by private nurseries while in other countries, they belong to the agency running the certification program. Plants in the Foundation Blocks are indexed for freedom of viruses on a recurring basis, inspected regularly for freedom from abnormalities, and budwood is only cut after fruit production to verify horticultural trueness-of-type. Buds from the Foundation Blocks may be used to propagate Budwood Increase Block trees or Certified Nursery Trees. Budwood Increase Blocks provide for exponential increase of budwood to be used to propagate Certified Nursery Trees. Budwood Increase Blocks have a relative short time limit, usually up to three years, when buds can be cut because these trees are not allowed to fruit. Indexing is usually for vector transmitted pathogens

Figure 1

Protected Primary Foundation Block

Clean Stock

Quarantine

Seed Source Trees

Varietal Block

Foundation Block

Budwood Increase Block

Certified Nursery Trees
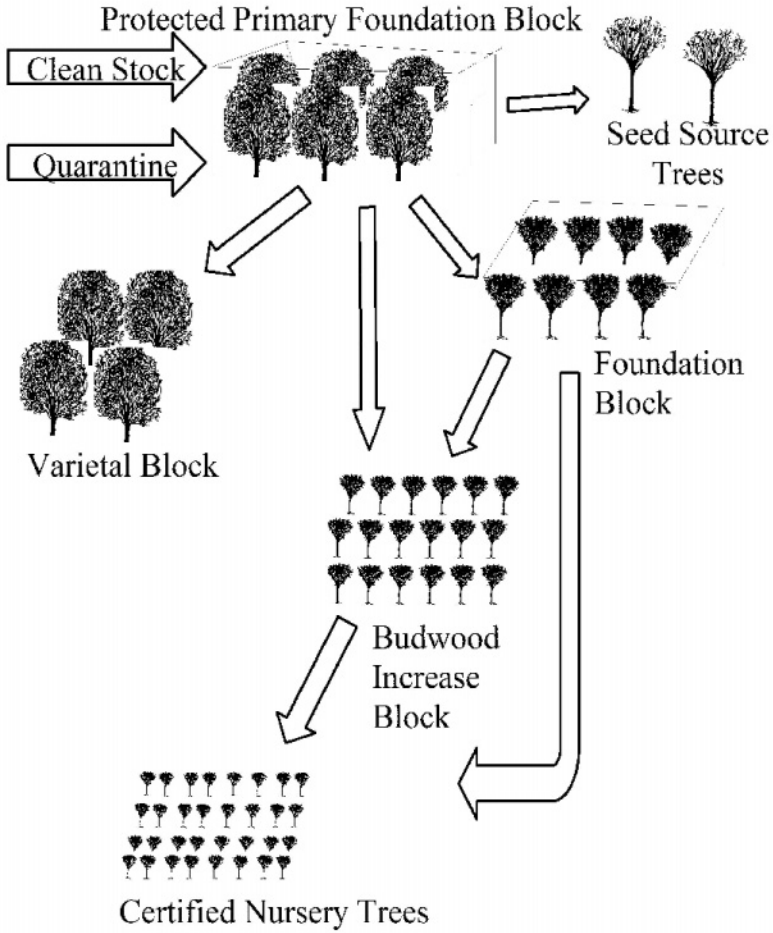
Figure 1: Cartoon showing a general scheme of a certification program.

which may be present in the area. Budwood Increase Blocks may be located in the field or under protected conditions, depending on the amount of challenge by vectors. Certified Nursery Trees will ultimately be planted through the citrus industry in the country. Records should be maintained to show they have been propagated according to the regulations and to allow tracing of the source material if an abnormality should occur.

2.3.1 Protected primary foundation blocks

Protected primary foundation blocks are composed of virus-free plants recovered through the clean stock and quarantine programs. They have been verified to be of the highest horticultural quality and are subjected to recurring indexing to verify their virus-free status over time. They are grown in protected conditions, usually in an insect proof screenhouse. Often they are grown in containers, sometimes they are planted in the ground if nematode or other soil pathogen problems are not present. The trees in the protected primary foundation block are the primary source of budwood for the establishment of foundation blocks. The trees should be allowed to fruit regularly so aberrant sprouts can be found it they should occur. These blocks are usually maintained by a public agency.

2.3.2 Foundation blocks

Foundation blocks usually belong to private owned nurseries, although they also may be maintained by public agencies. They may be field planted, but if insect vectored pathogens are present (Table 1), they must be maintained in screenhouses under protected conditions. These trees must be indexed on a regular recurring basis and allowed to fruit so horticulture trueness-to-type of the fruit can be monitored. These trees should be visually inspected at least once a year. The trees in this block may only be propagated using budwood which comes from the protected primary foundation block.

2.3.3 Budwood increase blocks

Budwood increase blocks are used to provide a catalytic increase of budwood from either the foundation block sources or from the protected primary foundation block sources. The budwood collected from these blocks are used to propagate the certified nursery trees which will be planted in the citrus growing area. There is a time limit for collection budwood from budwood increase block trees to avoid the possible propagation of undetected mutations, usually three years at the maximum. The trees are not allowed to bear fruit. The trees are usually indexing for the presence of insect vectored pathogens, such as CTV. Increase blocks also should be inspected to detect any possible growth abnormality.

2.3.4 Certified nursery trees

Certified nursery trees are usually propagated using budwood from the budwood increase blocks, but they may also be propagated using budwood from the foundation block trees. They are usually grown under regular field conditions or in different types of screenhouses/greenhouses according to the specific needs and technology available at each nursery. The trees undergo an inspection to guarantee that they meet the horticultural quality required in the certification regulations, and nurseries also must keep records to show they have complied with the regulations.

Table 1: Graft-transmissible pathogens of citrus having vector or other means of natural spread

Graft-transmissible Disease	Causal agent	Vector or means of spread
Citrus blight	unknown	unknown
Citrus chlorotic dwarf	Unknown virus-like	*Parabemisia myricae* Kuwana, agent whitefly
Citrus variegated chlorosis	Strain of *Xylella fastidiosa* Wells	Several species of sharpshooters (Hemiptera: Cicadellidae)
Huanglongbing (citrus greening)	*Candidates Liberobacter asiaticum & L. africanum* (Asian & African greening, Respectively)	*Diaphorina citri* Kuwayama, *Trioza erytreae* Del Guercio
Indian citrus mosaic	Indian citrus mosaic badnavirus	*Planococcus citri* Risso, the citrus mealy bug
Leprosis	Citrus leprosis rhabdovirus	*Brevipalpus spp.* mites (Acari : Tenuipalpidae)
Naturally spread psorosis	Citrus psorosis virus	Unknown
Satsuma dwarf	Satsuma dwarf nepovirus	Unidentified soilborne agent
Stubborn	*Spiroplasma citri* Saglio	Leafhoppers: *Scaphytopius nitrides* Baker, *Neoaliturus tenellus* Gillette and Baker, *N. haemoceps* Muls. & Reyl.
Tristeza	Citrus tristeza closterovirus	Several aphid species, *Toxoptera citricida* Kirkaldy, *Aphis gossypii* Glover, and *Aphis citricola* van der Goot are the most common
Witches' broom	*Candidates* Phytoplasma aurantifolia	*Hishimonus phycitis* Distant suspected, not confirmed
Woody gall (Luteovirus-like)	Citrus vein enation virus	Aphids, *T. citricida* and *A. gossypii*

2.3.5 Seed source trees

Seed source trees should be included in the certification program. Budwood is avail-

able from internationally recognized clean stock programs for most common rootstocks used with citrus. This material has been evaluated to ensure the source trees which provide the budwood are true-to-type, additionally the trees have been indexed for freedom from psorosis and psorosis-like pathogens as these pathogens can be seedborne in *P. trifiolata* and hybrids having *P. trifoliata* as one of the parents (Roistacher 1991). Caution needs to be used on importing seed from countries having citrus variegated chlorosis (CVC) caused by *Xylella fastidiosa* and Witches' broom disease of lime (WBDL) caused by a phytoplasma as there are unconfirmed reports of seed transmission of these pathogens (Pria *et al.,* 2003; El-Kharbotly *et al.,* 2003). Seed source trees should be inspected annually for freedom from abnormalities and diseases, as with other foundation trees.

3. Considerations regarding the establishment and operation of citrus certification programs

3.1 Root pathogens

Should root pathogens be included? Even though the root pathogens are large enough to be visualized, unlike the virus and virus-like pathogens which are graft-transmissible, the root pathogens are often forgotten about because they are hidden in the soil. Once root pathogens have been spread to a new location, they are expensive to control. The chemical treatments for control of nematodes and other root pathogens are rapidly diminishing due to environmental concerns. Additionally, the cost of treatment may be more than the losses sustained. Continued use of pesticides to control root pathogens can lead to the development of resistant strains of the pathogen, such as has occurred with the fungus *Phytophthora* which causes foot and root rot of citrus. Host resistance can be broken by the development of resistance breaking biotypes of the pathogen over a period of time and with continuous challenge. Resistance breaking biotypes have developed for economically important citrus nematodes. The presence of resistance breaking biotypes can have a great economic impact on perennial crops such as citrus. Certification programs should address the exclusion of these pathogens and pests to prevent spread to new areas in which citrus is being planted.

The sugarcane root weevil, *D. abbreviatus* was introduced into Florida in the early 1960's. In recent years this pest has spread rapidly throughout the state resulting in the infestation of about 160,000 acres in 20 counties including 30,000 acres of citrus (Lee 2000). The root weevil has a serious negative impact on citrus because the larvae feed on the tree roots. If the rootstock is Swingle citrumelo, this feeding by the root weevil larvae breaks the resistance of this rootstock to *P. palmivora* in areas of the state where this soil fungus is present. This usually results in decline and eventual death of the tree (Rogers *et al.,* 1996). The rapid spread of this pest in Florida was due to the movement of the larval stage by transport of containerized nursery material. By carefully monitoring of nurseries for root weevil infestations, this pest is no longer spreading rapidly.

In the past forty years, citrus production worldwide has increased about three

fold (FAOSTAT, FAO, Rome), most of the increase was due to increased area being used to cultivate citrus. In most countries, the citrus certification program does not consider root pathogens. As a result, citrus nematodes such as *Tylenchulus semipenetrans,* commonly called the citrus nematode, have been distributed into most of the newly planted citrus areas worldwide; the exception is Florida where nematode certification programs were implemented in the 1950's (Lee *et al.,* 1999). The Florida nematode certification programs began following the finding that spreading decline was caused by the nematode *Radopholus similis*, commonly called the burrowing nematode. The nematode programs included surveys to define infested areas, removal of infested trees, and the establishment of barriers. The barriers were initially chemical, but more recently mechanical root pruning is being used because of environmental concerns of the chemicals used. All nurseries, not just citrus, as well as soil pits, must be tested for freedom of citrus nematodes before the nursery site can be used or soil moved from the soil pit.

In 1954 when the nematode certification programs began there were about 5,400 acres infested with the burrowing nematode in Florida, now there is about 10,000 acres. There has not been a burrowing nematode found in a citrus nursery since 1970. The originally infested area has not greatly increased because of the program. Citrus nematodes were included in the program in the early 1960s when this nematode infested much of the land used for citrus at that time. Because of severe recurring freezes resulting in the southern expansion of the citrus growing area in Florida, now only about 30 percent of the total citrus area is infested, the new citrus growing areas are nematode-free. When *Pratylenchus coffeae* , commonly called the coffee lesion nematode, was found at an isolated location in the early 1980's, this nematode was included in the program and has not spread (Lee *et al.,* 1999). A cost-benefit analysis of the burrowing nematode regulatory program from 1960 through 1994 when chemical barriers could be used has been calculated. Considering the rate of spread if there had been no program, and the average yield loss per tree, over the 35 years of the program operation, for every $70,000 invested in the program there was a return of $1 million. For the year 1994-1995, the first year when chemical barriers could no longer be used, there was a return of $1 million for every $1403 invested in the program (Lee *et al.,* 1999). These calculations do no consider the benefits of reduced need for chemical control of nematodes which also reduces the possibility of pesticide resistant biotypes developing, reduce tree replacement costs, and increase production from non-nematode affected trees.

3.2 How will the certification program be sustained?

A well run certification program needs resources to sustain the program over a period of time. The methods of funding of certification programs are quite variable. When Florida had a voluntary certification program, most of the funding was supplied by the State through the Florida Department of Agriculture and Consumer Services. When the Florida program became mandatory in January 1997, the extra costs of operation were largely met by increasing fees for services.

Many of the countries who have implemented citrus certification programs have

received help for the establishment of the program from the Food and Agriculture Organization (FAO) of the United Nations through the Technical Cooperation Program (TCP). The TCP is designed to enable FAO to provide technical expertise to governments formally requesting help to deal with emergency situations relating to agricultural production. TCP projects have specific guidelines; in general they must help solve an urgent problem in agriculture, they are limited to 18 months in duration, and the total budget must be less than US$400,000. TCP projects have provided the start for certification programs in Uruguay, Argentina, Belize, Jamaica, Oman, and currently in Trinidad/Tobago.

The regional FAO officer is the best source of information on the TCP program. After certification programs have been established, they may be sustained by a tax on the citrus industry or by charging for services which are required by a mandatory program or with support from the private sector, or by a combination of these means of support. A certification program must be sustained or it does not contribute to the long term productivity of the local citrus industry.

3.3 Mandatory or voluntary program?

In areas where graft transmissible pathogens with insect vectors are present, the certification program must be mandatory to be effective. When vector transmitted diseases are present, one grower can do everything correctly, but his investment will be lost due to the actions of a neighboring grower who plants infected plants nearby. Even with chronic tree declines such a Huanglongbin (citrus greening) which is spread by psyllids, a return on the investment may be realized if the plants are propagated pathogen free (Roistacher, 1997). The Florida certification program was voluntary from 1952 through 1996, and then it was made a mandatory program (Rucks, 1994; Lee, 2000). The action to make the Florida program mandatory began with the Florida Nurserymen's Association and the Florida Production Managers' Association due to concern about the impending arrival of the BrCA in Florida.

There were also documented instances where "as good as certified" budwood containing viroids were propagated on susceptible rootstock, registered budwood sources were found to harbor psorosis-like viruses, and exotic pests, such as citrus canker [*Xanthomonas axonopodis* pv. *citri* (Hasse) Dye] were being found near international airports providing evidence that exotic pests were getting into the state (Schoulties *et al.,* 1987, Roistacher, 1993, Rucks, 1994, Powell, 1998). The same reasons are compelling reasons why all certification programs should be mandatory, not voluntary.

3.4 Nursery site selection

Planting locations should be approved by the agency in charge of the certification program. They should be in areas with the minimum risk of infection and suitable of growing citrus plants of good quality. Consideration should be given of the location and the proximity of citrus or crops known to be infested with nematode pests, proximity of public or private thoroughfares, slopes than influence drainage, and probability

of contamination from adjacent areas.

Foundation blocks, increase blocks, and certified nursery plants should be located away from established citrus groves where possible. To maintain sanitation in the nursery, it is recommended that all soil, growing media, mulch, manure, etc introduced should be free of known plant pests, such as nematodes and soil fungi. It is preferable that plants be grown on benches raised at least 18 inches above the ground, or if placed on the ground, be placed on a plastic mat or liner so that roots do not come into direct contact with the soil.

4. Summary

The biology, host range, and life cycles of the pathogens commonly present must be understood and considered. Graft transmissible pathogens not having insect vectors are easily controlled by a certification program. The pathogens having insect vectors present more of a challenge, but data indicate the best way to have a productive grove in the presence of severe disease spread by efficient vectors is to begin with healthy plants.

5. References

Beretta, M. J. G., Garcia, A. J., Lee, R. F., Derrick, K. S., Barthe, G. A. and Neto, J. T. 1993. Observations on citrus variegated chlorosis in Brazil, pg Proc. 12th Conf. Int. Org. Citrus Virol., New Delhi.

Bove, J. M. 1995. Virus and virus-like diseases of citrus in the Near East region. FAO, Rome. 517 p.

Bove, J. M. and Garnier, M. 1984. Citrus greening and psylla vectors of the disease in the Arabian peninsula, In: Proc. 9th Conf. IOCV. IOCV, Riverside. pp. 109-114.

El-Kharbotly, A., Al-Shanfari, A. and Al-Subhi, A. 2003. Molecular evidence of the presence of the Phytoplasma aurantifolia in lime seeds and possible transmission to the seedlings. 2000 Proc. International Soc. Citriculture. Orlando, FL. (In press).

Galipienso, L., Vives, M.C., Moreno, P., Milne, R.G., Navarro, L. and Guerri, J. 2001. Partial characterization of citrus leaf blotch virus, a new virus from Nagami kumquat. Arch. Virol. 146:357-368.

Garnier, M., Zreik, L. and Bove, J.M. 1991. Witches' broom, a lethal mycoplasma disease of lime trees in the Sultanate of Oman and the United Arab Emirates. Plant Disease 75:546-551.

Garnier Korkmaz, S., Cinar, A., Kersting, U. and Garnsey, S.M. 1995. Citrus chlorotic dwarf: a new whitefly-transmitted viruslike disease of citrus in Turkey. Plant Disease 79: 1074.

Lee, R.F. 1998. Citrus variegated chlorosis, EcoPort slide show number 9. http://www.ecoport.org

Lee, R. F. 2000. Why have mandatory citrus certification programs? In: Proc. 14th Conf. Intl. Org. Citrus Virol.. IOCV, Riverside, pp. 311-325.

Lee, R. F., Derrick, K.S., Beretta, M.J.G., Chagas, C.M. and Rosetti, V. 1991. Citrus variegated chlorosis: a new destructive disease of citrus in Brazil. Citrus Industry 72: 12-13, 15.

Lee, R.F. and Rocha-Pena, M.A. 1992. Citrus tristeza virus. In: Plant Diseases of International Importance. Vol. III. (eds. A.N. Mukhopadhyay, H.S. Chaube, J. Kumar and U.S. Singh) Prentice Hall, New Jersey, pp. 226-249.

Lee, R.F., Baker, P.S. and Rocha-Pena, M.A. 1994. Citrus tristeza virus (CTV): an introduction to current priorities, with special reference to the worsening situation in Central America and the Caribbean. International Institute of Biological Control, Centre for Agriculture and

BioSciences (CAB) International, and Food and Agriculture Organization (FAO), United Kingdom. 197 p.

Lee, R. F., Lehman, P.S. and Navarro, L. 1999. Nursery practices and certification programs for budwood and rootstocks. In: Citrus Health Management, APS. APS, St. Paul, MN., pp 35-46.

Moreira, L., Villalobos, W., Rodriguez, C.M. and Rivera, C. 2002. Presence of *Xylella fastidiosa* in several species in Costa Rica. In: Proc. 42nd Conf. Caribbean Division American Phytopathological Society, Guatemala, 17-19 June 2002, p. 75.

Navarro, L. 1993. Citrus sanitation, quarantine and certification programs, In: Proc. 12th Conf. IOCV. IOCV, Riverside, pp. 383-391..

Navarro, L., Juarez, J., Pina, J.A. and Ballester, J.F. 1984. The citrus quarantine station in Spain. In: Proc. 9th Conf. IOCV. IOCV, Riverside. Pp. 365-370.

Ochoa Corona, F.M., Rocha Pena, M.A. and Lee, R.F. 1994. Impact of citrus tristeza closterovirus on Venezuelan citriculture: chronology of events. Revista Mexicana de Fitopatologia 12: 97-105.

Powell, C. A., Pelosi, R.R., Sonoda, R.M. and Lee, R.F. 1998. A psorosis-like agent prevalent in Florida's grapefruit groves and budwood sources. Plant Disease 82:208-209.

Pria, W. D. Jr., Li, W.B., Teixeira, D.C., Miranda, V.S., Franco, C.F. and Palma, R.R. 2003. Bacterium Xylella fastidiosa in sweet orange [*C. sinensis* (L) Osb.] seeds and its translocation from seeds to seedlings. 2000 Proc. International Society of Citriculture. Orlando, FL. in press

Rocha-Pena, M.A., Lee, R.F., Lastra, R., Niblett, C.L., Ochoa-Corona, F.M., Garnsey, S.M. and Yokomi, R. K. 1995. Citrus tristeza virus and its aphid vector Toxoptera citricida: threats to citrus production in the Caribbean and Central and North America. Plant Disease 79:437-445.

Rocha-Pena, M.A., Ochoa-Corona, F.M., Martinez-Soriano, J.P., Roistacher, C.N. and Lee, R.F. 1998. Citrus tristeza virus: events that occur before, during and after the disease epidemics. Subtropical Plant Science 50:26-36.

Rogers, S., Graham, J.H. and McCoy, C.W. 1996. Insect-plant pathogen interactions: preliminary studies of Diaprepes root weevil injuries and Phytophthora infections. Proc. Fl. State Hort. Soc. 109:57-62.

Roistacher, C.N. 1991. Graft-transmissible Diseases of Citrus. Handbook for detection and diagnosis. IOCV, Riverside and FAO, Rome. 286 p.

Roistacher, C.N. 1993. Arguments for establishing a mandatory certification program for citrus. Citrus Industry 74 (10): p. 8.

Roistacher, C.N. 1997. The economics of living with citrus diseases: huanglongbing (greening) in Thailand,. In: Proc. 13th Conf. IOCV. IOCV, Riverside, pp. 279-285.

Rucks, P. 1994. Quality tree program for Florida citrus. Proc. Fl. State Hort. Soc. 107:4-8.

Schoulties, C. L., Civerolo, E.L., Miller, J.W., Stall, R.E., Krass, C.J., Poe, S.R. and DuCharme, E.P. 1987. Citrus canker in Florida. Plant Disease 71: 388.

Vives, M. C., Gallpienso, L., Navarro, L., Moreno, P. and Guerri, J. 2003. Citrus leaf blotch virus: a new citrus virus associated with bud union crease on trifoliate rootstocks. Proc. 15th. Conf. IOCV. IOCV, Riverside.

People, Arthropods, Weather and Citrus Diseases

Mani Skaria

*Texas A &M University-Kingsville Citrus Center, 312 North International Blvd.
Weslaco, TX 78596, USA*

Abstract: Citrus trees are grown all over the world. The important diseases of citrus are catego-
rized as soil-borne, foliar, postharvest, insect-borne, and graft-transmissible. Some diseases are
developed and/or intensified as a result of weather-related factors. Graft-transmissible diseases
are mainly distributed by humans when contaminated plant material is used for new tree propa-
gation in nurseries. Therefore, establishing a virus-free budwood program would reduce the
spread of all graft-transmissible pathogens. Government agencies with statutory authority to
take action to limit the spread of graft-transmissible viruses should use quarantines to regulate
the movement of vectors and infected plant material. An education program to inform the public
of the rules and regulations restricting importation of plant materials, and enlisting public help in
identifying new vectors and diseases would help prevent certain diseases. Similarly, soil-borne
problem such as citrus nematode is distributed in commercial orchards when trees from nematode
contaminated nurseries are used for planting. Some diseases are found only in certain geographic
locations, *e.g.* citrus greening is found mainly in Asia and Africa and the citrus variegated
chlorosis in South America. However, exotic diseases can get established when people import
plant materials illegally. Eradication is the most important strategy that needs to be adopted
should an exotic disease or vector ever be detected for the first time in a citrus growing area.
However, there are several obstacles such as cost, personnel, and time that play a major role in
the success of an eradication program. Root feeding insects such as the sugarcane root stalk borer
weevil (*Diaprepes abbreviatus)*, can compound the effect of tree decline as a result of *Phytophthora*
infection. Moreover, these insects may produce thousands of offspring. Cultural practices like
leaving old stumps of a dead tree in soil can introduce infection by fungus *Ganoderma* that can
kill small replants. Hurricanes can result in extensive diseases of citrus. Disease such as melanose
is commonly associated with the presence of dead twigs, especially on trees with mild freeze
damage. The role of humans is significant in spreading numerous graft-transmissible diseases of
citrus.

1. Introduction

The genus *Citrus*, which originated in Asia, is now grown all over the world (Burke
1967, Davis and Albrigo 1994). This genus includes trees such as: sweet orange, man-
darins, grapefruit, lemons, limes etc. Grapefruit, a hybrid between the pummelo and
sweet orange is an exception with its origin in the West Indies area. A beautiful citrus
tree, its fruit, flowers, and aroma have attracted people from very early times. It is one
of the most important food items in many countries. Apart from direct food value,
citrus peel oil is used in perfumes. Citrus fruit is also being studied for human health
benefits, especially for cancer prevention (Liu *et al.,* 2001, Tian *et al.,* 2001). The

following description outlines the different types of citrus fruit grown, as well as lists the diseases that affect them, and explain how people, weather, and arthropods influence some important citrus diseases.

Important diseases of citrus (Timmer *et al.,* 2000) can be categorized in the following groups. They are: a) soil-borne diseases and problems caused by: *Phytophthora* spp., *Fusarium* spp., citrus nematode (*Tylenchulus semipenetrans* Cobb.), burrowing nematode (*Radopholus citrophilus* Huettel, Dickson, & Kaplan]; b) foliar diseases caused by: *Mycosphaerella citri* Whiteside), *Deuterophoma tracheiphila* Petri, *Diaporthe citri* Wolf; c) postharvest diseases caused by *Phytophhtora* spp., *Diplodia* spp., *Penicillium* spp., *Geotrichum candidum* Link ex Pers.; d) insect-borne diseases such as tristeza spread by brown citrus aphid (*Toxoptera citricida*), and *Aphis gossypii*, greening disease spread by psyllids *Trioza erytreae*; citrus variegated chlorosis caused by *Xylella fastidiosa* and spread by sharpshooters such as: *Acrogonia gracilis, Oncometopia facialis, Dilobopterus costalimai, Plesiommata corniculata*, and *Bucephalogonia xanthopis*; and e) graft-transmissible diseases like *Citrus tristeza virus*, citrus tatter leaf virus, *citrus psorosis virus*, *citrus exocortis viroid*, etc.

People and arthropods play the key role in dissemination of economically important citrus diseases, not only from one plant to another or from one orchard to another but globally. Most of devastating citrus diseases like Citrus tristeza, diseases caused by *Phytophthora* spp., nematodes and bacterial canker were spread globally due to mere ignorance of mankind, from the place of origin of the disease to virgin areas of the world. Subsequently these diseases became the cause of epidemic supported by favorable weather conditions and spread due to arthropods in certain areas causing huge economic losses.

Citrus disease development is influenced by geographic area, presence of insect vectors, and the scion-rootstock combination. Some graft-transmissible diseases such as *Citrus exocortis viroid* and citrus tatter leaf virus may be present as symptomless carriers in certain scion-rootstock combinations. The following brief description would help to understand important citrus types and rootstocks.

2. Important citrus types (scions)

2.1 Sweet orange (*C. sinensis* {L.} Osb.)

Sweet orange is the most widely cultivated citrus species. The commercially important types are: the navel oranges, regular round oranges, and the blood oranges. Based on fruit maturity, sweet oranges can be grouped as early, mid-season, or late varieties. Some important cultivars in this group are: the 'Washington' navel, Hamlin, Valencia, Pineapple, Pera, Jaffa, etc. These are consumed fresh and/or juiced.

2.2 Mandarin and tangerine oranges (*C. reticulata* Blanco)

Mandarins and tangerines are soft-skinned and easy to peel varieties compared to other citrus types. In the U.S., the soft-skinned oranges are commonly known as tangerines. Some important cultivars in this group are: the Owari, Clementine, Dancy,

Willowleaf, Temple, Murcott, and the Nagpur Santra, etc.

2.3 Tangeloes (*C. reticulata* x *C. paradisi*)

Tangeloes are man-made interspecific hybrids between mandarin and grapefruit. These are soft-skinned and easy-to-peel compared to other citrus types. Examples of important tangeloes are the Orlando and Minneola.

2.4 Pummelo or shaddock (*C. grandis* {L.} Osb.)

This is more popular in southeast Asia; however, demand for pummelo in the U.S. is increasing among people of Asian origin. The fruit is bigger than grapefruit with a white or pink flesh.

2.5 Grapefruit (*C. paradisi* Macf.)

Grapefruit is a hybrid between a pummelo and sweet orange. The best grapefruits are grown in tropical and subtropical regions. Grapefruit is the largest citrus fruit grown commercially in many countries. Grapefruit can be seedy, white, pink, or red. The popular varieties are pink or red and seedless. Examples of important red varieties are the Rio Red, Star Ruby, and Flame. Most well-known grapefruits are developed in Florida or Texas. Two hybrids between the pummelo and grapefruit have been developed in California, the Melogold and the Oroblanco. These are grown currently in California and Israel.

2.6 Limes (*C.aurantifolia* L.)

Limes are the most cold-sensitive of all types of citrus, and can be with acid or acidless. Important cultivars are West Indian (=Mexican) lime, and Tahiti lime.

2.7 Lemons (*C. limon* Burmf.)

Lemons are mostly grown in semiarid or arid subtropical conditions. Lisbon and Eureka are well-known lemon cultivars.

2.8 Kumquat

Kumquat belongs to a different genus called *Fortunella* which is not grown commercially. It is freeze hardy and is grown for its small fruit eaten whole. Two important varieties are the Nagami and Meiwa.

3. Rootstocks

World wide, commercial citrus is based on scions budded onto adaptable rootstocks.

Citrus grown as seedlings maintain juvenile characteristics for many years, and some cultivars may have problem with true-to-type (if grown from seed). Moreover, the use of a rootstock prepares the budded tree to be more tolerant to soil-borne nematodes and the fungus *Phytophthora* (Tables 1 and 2).

4. Disease spread by people

The most significant citrus diseases that are spread by humans are the graft-transmissible diseases caused by viruses, viroids, bacteria, etc. Graft-transmissible pathogens

Table 1: Important disease attributes of some rootstock used commercially.

Rootstock	Tolerance to	Other attributes
Sour orange (*C. aurantium*)	*Phytophthora* fungus, exocortis and cachexia viroids	Adaptable in most soils
Rangpur lime (*C. limonia*)	tristeza virus	Drought resistant
Rough lemon (*C.jambhiri*)	Tristeza virus, exocortis and cachexia viroids	Scion trees grow vigorously
Volkamer lemon (*C. volkameriana*)	Tristeza virus, exocortis and cachexia viroids	Not widely used
Cleopatra mandarin *C. reticulata*)	*Phytophthora* fungus, exocortis and cachexia viroids	Produces large scion trees
Sweet orange (*C.sinensis*)	Tristeza virus, exocortis and cachexia viroids, and *Phytophthora* fungus	Ideal for areas with blight and tristeza virus problems
Trifoliate orange (*Poncirus trifoliata*)	Tristeza virus, exocortis and cachexia viroids, and *Phytophthora* fungus	Trees grown on this root stock can be dwarfed by citrus exocortis viroid
Citranges (hybrid between sweet orange and trifoliate orange)	Tristeza virus, exocortis and cachexia viroids, and *Phytophthora* fungus	produce an overgrowth at the bud union
Citrumelo (hybrid between grapefruit and trifoliate orange)	Tristeza virus, cachexia viroid, and *Phytophthora* fungus	Does not grow well in high pH, water-logged soil

are distributed well in the plant so that they are transmitted through buds taken for new tree propagation (Roistacher 1991). There are reasons for this type of phenomenon of disease spread through grafting. For example, some pathogens such as the exocortis

viroid can be non-symptomatic in a grapefruit tree grown on sour orange rootstock (Calavan *et al.,* 1964, Roistacher *et al.,* 1977). Sour orange rootstock is tolerant to exocortis, whereas, citrange rootstocks are not. Therefore, new trees propagated on citrange rootstock with buds taken from this grapefruit tree would show dramatic symptoms a few years after being planted in the field. This is common in several countries with the tristeza virus threat and with growers wanting to use rootstocks such as citranges that are tolerant to tristeza. In addition to graft-transmission, diseases such as exocortis and tatter leaf virus can be spread by tools used in pruning (Garnsey and

Table 2:   Reaction of sour orange and hybrids of trifoliate oranges to different citrus pathogens.

Rootstock	*Phytophthora*	Citrus nematode	CTV	CEV	CTLV
Carrizo citrange	Tolerant	Tolerant	Tolerant	Susceptible	Susceptible
Troyer citrange	Tolerant	Tolerant	Tolerant	Susceptible	Susceptible
Swingle citrumelo	Resistant	Resistant	Tolerant	Tolerant	Susceptible
Sour orange	Tolerant	Susceptible	Susceptible	Tolerant	Tolerant

Jones 1967, Garnsey 1974, Roistacher *et al.,* 1980). A major cause of graft-transmissible disease spread is the result of no programs for virus-free budwood certification in several citrus producing areas.

4.1 Citrus tristeza disease

Tisteza is a very destructive disease of citrus caused by *Ctrus tristeza virus* (CTV) (Bar-Joseph *et al.,* 1989, Rocha-Pena *et al.,* 1995, Costa and Grant, 1951). The word tristeza originates from Spain and means Asadness. It was appropriately used to name one of the most destructive diseases of citrus (Moreira 1942).

CTV originated in Asia and citrus trees contaminated with CTV were spread to countries such as South Africa (Webber 1925, McClean 1963, Bar-Joseph *et al.,* 1981). This was followed by the natural spread of CTV in the field by aphid vectors such as the brown citrus aphid *Toxoptera citricida* (Kirkaldy) in many countries (Mendt 1992, Meneghini 1946, Yokomi *et al.,* 1994, Aubert *et al.,* 1992, Garnesy *et al.,* 1996, Gottwald *et al.,* 1996, Knorr and DuCharme, 1951, Lastra *et al.,* 1992). In the 1930s, countries such as Argentina, Brazil, and later Venezuela experienced a catastrophic decline and death of citrus trees that were grown on sour orange rootstock (Bitancourt 1940, Moreira 1942). The symptoms and the effect of the CTV infection on different citrus grown on various rootstocks were different. Based on field symptoms and disease severity, the following two major types of symptoms of tristeza virus are recognized in commercial orchard.

4.1.1 Sour orange Decline

Infection with CTV causes severe decline and tree death of cultivars such as sweet oranges and grapefruit on sour orange rootstock (Swingle 1909, Halma *et al.,* 1944, Grant and Schneider, 1953). These isolates produce the most dramatic symptoms of CTV infection and a threat to citrus production (Rocha-Pena *et al.,* 1995). It was the sour orange decline that first caught the attention of citrus pathologists. Effects of these types of CTV isolates can be a slow decline of trees or rapid tree death.

4.1.2 Stem pitting

As the name indicates, cultivars such as grapefruit, sweet orange, and limes develop wood pitting that are readily visible when the bark is removed (Bar-Joseph *et al.,* 1979a, Garnsey *et al.,* 1991, Garnsey *et al.,* 1996). The fruit on infected trees can be reduced in size and quality and the trees often look debilitated (Fig.1). The pits can be small or large. There may or may not be a visible gum association. In some cases, the stem becomes brittle and breaks off easily. Tristeza is present in all citrus producing areas of the world. However, the severity of field symptoms varies with the CTV strains and the rootstocks. For example, in some places where the decline-inducing strains are present in orchards with sour orange rootstock, tree decline and even tree death is quite normal. In areas such as Texas (Solis-Gracia *et al.,* 2001), Mexico, and certain parts of the Mediterranean, field symptoms of quick decline are not present. On the other hand, severe symptoms of CTV are readily found in areas such as Florida, California, South Africa, Brazil, Spain, India, China and several other eastern countries (Bar-Joseph 1989).

4.1.3 Vectors

Several aphid species transmit CTV, which has proven destructive to citrus in Africa, China, India, South America, Central America, the United State of America, and the Caribbean Islands (Yokomi *et al.,* 1994). Though CTV is spread by several aphids, the most destructive one is the brown citrus aphid (BrCA) *Toxoptera citricida* (Lastra *et al.,* 1992). Reports and concerns from scientists have been numerous regarding the rapid movement of BrCA from South America through: Central America (Panama in 1989, Nicaragua in 1991, Belize in 1996), the Caribbean Islands (Trinidad in 1985, Puerto Rico in 1992, Cuba and Jamaica in 1993), and to the continental U.S (Rocha-Pena *et al.,* 1995, Borbon *et al.,* 1992, Gottwald *et al.,* 1994, Gottwald *et al.,* 1996). The BrCA was first detected in Florida dooryard citrus in the fall of 1995 (Hardy 1995). Large-scale infestations of BrCA were observed in Florida commercial citrus in 1997. Florida has citrus tree-killing tristeza strains in orchards, and tristeza disease symptoms are wide spread in commercial and dooryard trees. This means that severe strains are available for the aphid to spread as they feed on new plants.

The BrCA is a very efficient vector of CTV compared to indigenous citrus aphids including: the green citrus aphid, *Aphis spiraecola*, cotton or melon aphid, *A. gossypii*, and the black citrus aphid, *Toxoptera aurantii* (Rocha-Pena *et al.,* 1995). The BrCA not only does it feed on young, tender flushes causing leaf and twig stunting, epinasty, and

overall loss of tree vigor, but more importantly, BrCA is the most efficient vector of CTV. Epidemiological studies with BrCA as the primary vector, showed a 95% increase in CTV incidence took place in 2-4 years, whereas without BrCA it took 12-14 years (Yokomi *et al.,* 1994). Moreover, the BrCA can transmit 'severe' CTV strains more efficiently than the other aphid species. The reproductive potential for the BrCA is enormous. Colonies of the aphid are exclusively female and no males are needed for reproduction. A mature female can produce 7-8 offspring per day with nymphs maturing in 7 days at 25 °C, and up to be 30 generations can be produced per year. Generally, the reproductive rate for BrCA increases as temperature reach 25 °C. The BrCA also has been seen to displace the native citrus aphid populations when it invades a new geographic area. Winter survival of the BrCA is mainly on root sprouts, new resets, dooryard citrus, and abandoned groves in Florida. The summer survival of the BrCA in Florida is mainly on
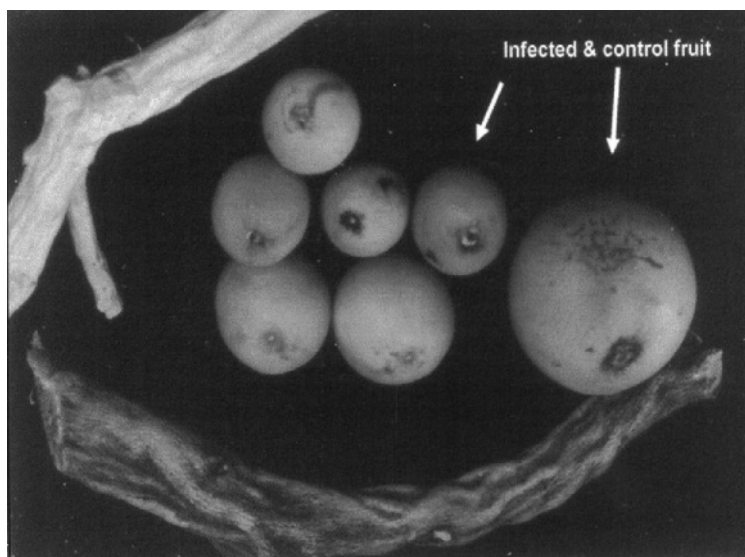
Figure 1: Tristeza stem pitting and small size (infected) and large (healthy) grapefruit (South Africa)

interior citrus flushes, shaded resets, and dooryard trees.

The natural enemies of the BrCA in Florida are hoverflies, ladybeetles, and lacewings. In addition, there are effective fungal pathogens such as fungi *Verticillium* and *Beauveria*. The best predators are the ladybeetles, with the larvae and adults of many species readily attacking both large and small colonies of BrCA.

4.1.4 Steps to manage tristeza

The following steps are required to manage CTV and its vectors in an area that is not yet

invaded by the virus and/or the vector(s). 1) Preventing the introduction of BrCA. Vulnerability will be via movement of infested plant materials from other areas. This greatly depends on the role of the regulatory agencies in a state or country that prevents illegal entry of citrus plant material. 2) Education of the general public concerning the potential impact of the BrCA/CTV to help minimize risk of introduction of either problem. 3) Accelerate surveys for detecting BrCA and severe CTV strains in dooryard citrus. 4) Accelerate the development of a budwood certification program for disease management. 5) Increase emphasis on testing, selection and making available CTV tolerant rootstocks as alternatives to sour orange. However, it is not advisable to switch until an alternative rootstock is found and proven to be good in the existing soil, water and climatic conditions. 6) Explore feasibility of cross protection with mild strain CTV isolates against severe CTV strains, if needed. A collection facility at Beltsville, MD facilitates comparison of CTV isolates world wide.

4.2 Citrus tatter leaf virus

Citrus tatter leaf virus (CTLV) was first discovered in California (Wallace and Drake 1962) on Meyer lemon trees introduced from China. In the 1960s and 70s this disease was also reported from other places (da Graca 1977, Friend 1954, Garnsey 1970, Ke and Wu 1991, Zhang *et al.,* 1988). In Texas, (Timmer 1975) first reported the presence of CTLV-like symptoms in indicator plants inoculated with tissue from the Meyer lemon. Most citrus species and commercial cultivars are symptomless carriers of this virus. A bud-union crease, with or without fluting of the stem may develop when infected scions are grafted to a trifoliate orange or its hybrids (Miyakawa and Tsuji 1988, Rouse and Wutscher 1985). When the bud-union crease is severe, the tops may shear off at the union in high winds. Tatter leaf virus is an important disease that should be taken into consideration if sour orange is replaced with rootstocks of trifoliate orange or its hybrids. Presence of CTLV is generally detected based on reactions of indicator plants such as *Citrus excelsa* and citranges. The common foliar symptoms of CTLV infection are the tattering of young leaves, chlorosis, and asymmetric leaf distortion (Garnsey 1964) (Fig.2). Shoot-tip grafting and thermotherapy procedure are used to eliminate this virus (Koizumi 1984).

4.3 Citrus psorosis virus

Psorosis disease of citrus has been known for the past 105 years and its viral etiology for approximately 70 years (Roistacher 1993). The term "psorosis" has been used to describe several graft-transmissible diseases that produce leaf flecking in indicator plants; however, some have been characterized as different viruses (da Graca *et al.,* 1991, Derrick *et al.,* 1988). It is the oldest virus that is known, characterized, and historically led to the establishment of a virus-free budwood program in many citrus-producing areas. The disease is caused by *Citrus psorosis virus* (CpsV), with the genus name *Ophiovirus*. The disease produces characteristic bark scaling that is different from that caused by fungus *Phytophthora* or Rio Grande gummosis diseases (Fig. 3). Moreover, this disease may cause ringspots on leaves and fruit; however, many trees may grow in

the field as symptomless carriers.

Reports from Argentina and Texas on the increase in the incidence of psorosis symptoms suggest a possible natural transmission of this virus (Benatena and Portillo 1984, Timmer and Garnsey 1980). Specifically, in Texas, the incidence of psorosis symptoms in nucellar, virus-free orchards increased from 0-11 trees over a period of seven years from 1971 to 1978 (Timmer and Garnsey 1980). The percentage of psorosis disease increased from 0.7 to 2.0 in five orchards totaling approximately 3,400 trees. Vector transmission of psorosis in Texas (Skaria *et al.,* 2001) has been suspected but not yet confirmed.

4.4 Citrus viroids

Figure 2: Citrus tatter leaf virus foliar symptoms (Texas)

4.4.1 Exocortis

Exocortis is a rootstock disease is caused by a viroid called the *Citrus exocortis viroid* (CEV). It is present in all citrus producing areas. Field symptoms are rare among commercial citrus trees that are grown on tolerant rootstock such as sour orange. However, commercial citrus on tolerant rootstock can be stunted to some degree. Susceptible rootstocks that show field symptoms are: the trifoliate orange and its hybrids. These rootstocks show bark scaling and tree dwarfing. Etrog citron is an indicator plant for CEV. The symptom expression of CEV in Etrog citron vary from leaf epinasty, splitting

and necrosis of the mid vein, necrosis on the petiole, moderate to mild leaf or petiole twisting and wrinkling. The earliest symptom may be an initial leaf epinasty and browning of the mid vein. This may be followed by severe epinasty and vein necrosis. The symptoms may appear within several days to months after inoculation. CEV inoculated indicator plants show the best symptom expression when the plants are kept under higher temperature and light intensity conditions. However, with longer incubation period, more plants may show symptom expression.

4.4.2 Cachexia

Cachexia (synonym, xyloporosis) is another viroid disease of citrus. Unlike exocortis, it affects the scion part of mandarins, tangelos, and Palestine sweet lime trees. This viroid

Figure 3: Citrus psorosis virus showing scaling and gumming on grapefruit trunk, Texas.

is present in several citrus producing areas; however, the disease symptom is more prevalent in some of the Mediterranean countries. Cachexia produces wood pitting and gumming in the bark. Mature, infected trees are looked distorted.

4.5 Nematodes

Though more than three dozen nematodes are known to be associated with citrus roots, the economic damage is caused by only a couple of nematodes. The two important problems associated with nematode infestation are: the slow decline and the spreading

nematode.

4.5.1 Slow decline

Citrus nematode, *Tylenchulus semipenetrans,* is a serious pest in many citrus producing areas and causes slow decline of trees. This nematode feeds on young root tissue, using the spear or stylet protruding from the 'head' (Cohen 1965). Feeding by an inconceivably large number of nematodes often results in general decline of tree health and decreased fruit number and size (Duncan *et al.,* 1994). Affected trees do not die from citrus nematode infection alone. The effect of citrus nematode is often referred to as slow decline. Sour orange rootstock is susceptible to the citrus nematode attack. The greatest concentration of nematodes is in the upper foot of soil.
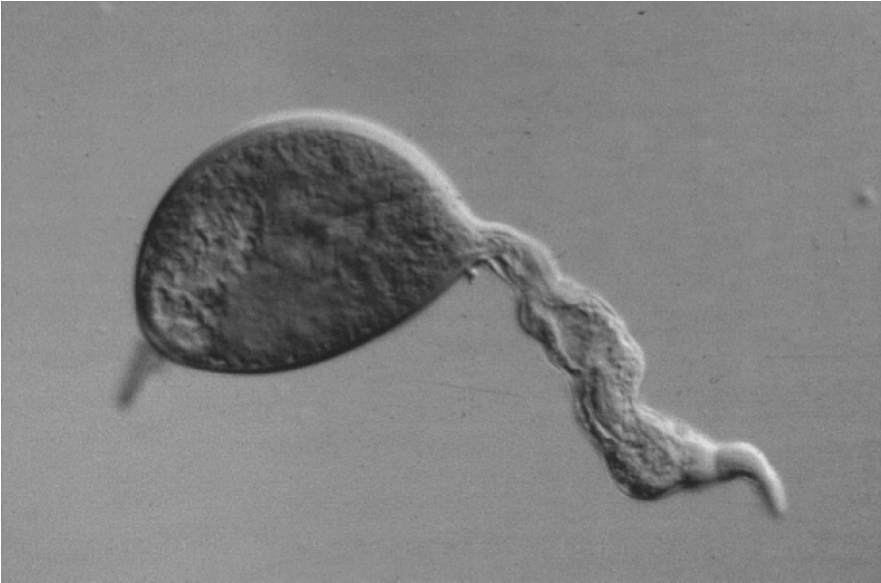
Figure 4: Citrus nematode, adult female

The typical life cycle of a citrus nematode starting from an egg to eggs can be one to two months. Eggs that are destined to be males do not develop a stylet and therefore, cannot feed on root tissue. Female juvenile larvae feed on root surface cells, often embedding a quarter of its anterior body in root tissue (this is about 4 to 5 cells deep), whereas, adult females penetrate deep into roots (Fig. 4). Thus, a typical feeding nematode becomes a sedentary pest and develops a comparatively larger size body (the posterior part) outside of the root tissue. A female nematode excretes gelatin and deposit many eggs into it. Factors such as climate, soil type, and root mass influence nematode population. New growth of roots is often found associated with peaks in citrus nematode population in soil and roots.

Prevention is the best strategy to control the citrus nematode. This starts with the purchase and planting of trees grown in nematode-free soil. Unfortunately, most nurseries in many citrus producing areas have no programs to eliminate or to plant trees in nematode-free nursery plots. Preplant fumigation of orchard sites are preferred in soils infested with a heavy population of nematodes. In dooryard situations, a preplant, soil solarization with plastic mulch, is useful in managing nematode populations.

4.5.2 Spreading decline

The spreading decline is caused by burrowing nematode, *Radopholus similis* (Cobb) Thorne. They feed on feeder roots and affect the activity of apical meristem. This nematode is distributed deep in soil. The affected trees may loose half of its productivity.

4.6 Postharvest problems

Postharvest diseases of citrus result in serious economic damage. We often fail to consider postharvest disease loss in several steps in the pipeline of harvested fruit. For example, disease losses in grocery stores, restaurants and in kitchens are often not well documented. Also, we normally do not consider losses in nutritional qualities of fruit and vegetables when we report postharvest disease loss. Though it is difficult to determine the actual loss, a conservative estimate is that in the U.S. approximately 25% of the harvested fruit and vegetables are lost due to postharvest problems. Worldwide, this estimate jumps to 50%. The important postharvest diseases of citrus in Texas are: green mold caused by *Penicillium digitatum* and sour rot caused by *Geotrichum candidum*. Disease loss can be prevented by minimizing injury to fruit during harvest, improved sanitary practices, and fungicide applications both before and after harvest.

4.7 Budwood program to eliminate graft-transmissible virus diseases

To obtain virus-free propagation materials, commercially important cultivars should be subjected to shoot tip grafting (STG) (Murashige *et al.,* 1972, Navarro *et al.,* 1975) and /or thermotherapy (Calavan *et al.,* 1972, Koizumi 1984) to eliminate viruses and viroids. Also, virus-free cultivars may be imported under quarantine from credible sources that distribute virus-free budwood (Skaria *et al.,* 1996). Both sources of plant materials should be then subjected to various levels of indexing procedures to assure freedom from viruses and/or viroids. Once clean materials are obtained, foundation trees can be established, from which nursery increase blocks can be developed to provide propagation materials to growers, nurserymen, and home owners.

4.7.1 Shoot-tip grafting

STG is a technique that has been used successfully to produce virus-free citrus plants (Murashige *et al.,* 1972, Navarro *et al.,* 1975). It involves a precise excision of a minute (0.17 mm) shoot tip from the tissue below, which may be contaminated with a graft-

transmissible pathogen, and its successful grafting on a decapitated rootstock, that was previously grown in sterile agar medium. The grafted rootstock is then placed in a test tube containing an artificial liquid growing medium and kept under light for several weeks for growth. The success rate would be about 15%. Plants grown this way should be transplanted into pots in a greenhouse.

4.7.2 Indexing

This step refers to a series of tests performed on plants developed by the STG technique to confirm the absence of graft-transmissible viruses and viroids in the newly-developed plants. The most reliable indexing method is a comprehensive biological indexing using several citrus indicator plants (Roistacher 1991). Inoculated plants are kept in a cool or a warm section of a greenhouse for several weeks, depending upon the disease being indexed, and symptoms are recorded (Table 3). The process is normally

Table 3: Indicator plants, symptoms, incubation period, and temperature regimes that are currently used for virus and viroid indexing in Texas.

Disease	Indicator	Temp.(^{o}C)	Incubation period
1. Tristeza (all strains)	Mexican lime	24-27	3-5 weeks
2. Tristeza seedling yellows (SY) and grapefruit stem pitting (SP)	Grapefruit	24-27	8-10 weeks
3. Tristeza SY	Sour orange	24-27	8-10 weeks
4. Tristeza orange SP	Madame Vinous	24-27	8-10 weeks
5. Greening	Madame Vinous	20-25	10-12 weeks
6. Stubborn	Madame Vinous	32-38	10-12 weeks
7. Psorosis	Madame Vinous	24-27	3-4 weeks
8. Exocortis and related viroids	Etrog citron 861-S1	32-40	4-6 months
	Tomato (exocortis only)	32-40	2-3 weeks
9. Cachexia	Parson's special mandarin	32-40	12-18 months
10. Tatterleaf	*Citrus excelsa* Wester	24-27	5-7 weeks
	Rusk, Carrizo, or Troyer citranges; Swingle citrumelo	24-27	5-7 weeks
	Cowpea; kidney beans, *Chenopodium quinoa*.	24-27	4-6 days
11. Concave gum	Dweet tangor	24-27	5-8 weeks
	Sweet orange	24-27	5-8 weeks
12. Infectious variegation	Dweet tangor	20-22	4-6 weeks
	Lemon	24-27	4-6 weeks

repeated over time to confirm the observations made. The indicator plants, the incubation period, and the temperature settings used are listed in Table 1. Herbaceous indicator plants can also be used for additional indexing for CTLV and CEV.

Other indexing methods that are used for indexing are ELISA and nucleic acid analysis by polyacrylamide gel electrophoresis (PAGE). ELISA is a technique based on an antigen-antibody reaction that is routinely used for the detection of plant and animal pathogens, including CTV (Bar-Joseph *et al.,* 1979b, Clark and Adams 1977). Numerous variations of this technique are available; however, the common practice is to use a polystyrene plate as a solid phase to trap potential virus particles in plant sap, with the use of two antibodies, making a virus sandwich (Garnsey and Cambra 1991). A variation of this technique, using a nitrocellulose paper instead of the solid plate is called dot-blot ELISA. Both techniques are used in our program. A monoclonal antibody, the McA-13 is useful for detecting certain severe type CTV isolates (Permar *et al.,* 1990). Nucleic acid analysis by PAGE allows the detection of plant viroids (Semancik 1991, Duran-Vila *et al.,* 1993). This technique is based on the property of the viroid nucleic acid to move in an electric field in a polyacrylamide gel where it is visualized as a band when it is stained with ethidium bromide or silver. Since this system can detect citrus viroids even when the indexed plants are symptomless or show mild symptoms, it is an excellent tool for confirming the absence of these pathogens in shoot tip grafted plants that show no symptoms after indexing on Etrog citron.

4.7.3 Foundation block

The virus-free plants should be planted in a foundation block and these plants will be the source of clean budwood for future multiplication. The foundation block trees should be re-indexed periodically for viruses and viroids. A nursery increase block should be planted next to the foundation block to produce certified budwood for the customers. Trees in the increase blocks should be tested annually and these plants should not be used for not more than a couple of years for clean budwood.

4.7.4 Support from the industry and government agencies

The success of a strong virus-free budwood program will depend on the quality of support it gets from the industry and the regulatory agency of the state or the country. A task force may be developed to outline management plans and risk assessment. Also, a task force can describe the role and responsibility of each agency involved with a virus-free budwood program. An education program to inform the public of the rules and regulations restricting importation of plant materials, and enlisting public help in identifying new vectors and virus sources would help. Such educational programs will help to prevent illegal movement of infected citrus material into an area free from severe viruses.

Another part of the education program will be to update all segments of the industry and extension personnel about changes in the status of vectors and pathogens. Agencies with statutory authority should take action to limit the spread of graft-transmissible viruses through regulating the movement of vectors and infected budwood.

4.8 Replant problems

In general, unsatisfactory yield experienced in young fruit trees that are grown soil where trees were grown for many years is often referred to as a replant problem. It is common to experience replant problems in apple, cherry, peach and citrus referred to as apple replant problem, cherry replant problem, peach replant problem, and citrus replant problem, respectively. Research information on precise factors responsible for citrus replant problems is not well-documented; however, several factors are commonly associated with it. They are: a) build-up of root parasites over a long period of time; b) build-up of toxic organic compounds and/or nutrients; c) lack of adequate nutrients; and d) altered physical properties of soil. Synergistic effect of several of these factors may also be associated with citrus replant problems. In citrus, among all the possible causes, the build-up of root parasites is the most likely source of replant problems.

5. Diseases spread by arthropods

5.1 Citrus greening

Greening is one of the most destructive citrus diseases, and is found mainly in Asia and Africa (da Graca 1991). Although all commercial cultivars of citrus are affected by greening, the most susceptible ones are sweet oranges, mandarins, and tangeloes. Cultivars such as grapefruit, limes, lemons, and pummeloes are less affected by this disease. The causal agent is classified as a new genus of a gram-negative bacterium named *Candidatus* Liberobacter spp. So far, the organism is not cultured; however, characterization has been achieved by sequencing the 16S ribosomal DNA and protein genes. In Asia, the bacterium is transmitted by psyllid *Diaphorina citri* and in Africa by psyllid *Trioza erytreae*. It probably was originated in China. Based on its reaction to heat, the organism is divided into two forms: a heat-tolerant Asian form called *Candidatus* Liberobacter asiaticus and a heat-sensitive African form, *Candidatus* Liberobacter africanus. The heat-sensitive form expresses symptoms on infected plants at a temperature between 20 - 25°C, whereas the heat sensitive form induces symptoms up to 35°C. Greening and greening-like symptoms are currently found in the Asian and African continents. Severe infection with greening is found in China, India, and South Africa.

Greening can affect an entire tree or just a section of it. Mature orange trees with greening disease show gradual decline and yield reduction. Tree loss to greening has been substantial in India, China, the Philippines, Thailand, Taiwan, Indonesia, Saudi Arabia, and South Africa. Symptoms of greening disease can be confused with the slow decline of citrus caused by the citrus nematodes (*Tylenchulus semipenetrans)* or tree debilitation from the stem pitting strain of citrus tristeza virus. Moreover, mineral deficiencies that are caused by a lack of iron, manganese, and zinc may also be confused with the symptoms caused by greening.

Psyllid *Diaphorina citri* is present in many citrus producing areas in South, Central, and North America without the presence of the greening disease or the bacte-

rium. The latest reports from Florida suggest that the vector is present there; however, the bacterium has not been detected so far.

Citrus propagation materials must be free from the bacterium to avoid the greening spread. This can be achieved through the production and distribution of a virus-free budwood program. Infected trees in the orchard and nurseries must be destroyed as soon as the pathogen is detected in order to minimize sources of inoculum. Sprays with insecticides and releasing of parasitic wasps are other possible ways to control the vector population.

A common mistake made which aids to the spreading of all graft-transmissible disease is using infected budwood for propagation in nurseries. Natural spread by adult psyllids takes place in orchards and nurseries; however, their efficiency as vectors is not considered very high in transmitting the bacterium. Identification of the disease is confirmed by inoculating sensitive citrus cultivars such as: sweet orange, nucleic acid probes, electron microscopy and symptoms of fruit and leaves.

5.2 Citrus canker

Citrus Canker caused by the bacterium, *Xanthomonas campestris* pv. *citri* was first reported in the state of Florida in 1910 in trifoliate orange rootstock seedlings imported from Japan, via Texas. Canker lesions are brown or tan and have a raised, corky surface. The lesions appear alone or in groups (Fig. 5). The infection causes serious damage to trees. Defoliation and dieback are common and it makes the fruit unmarketable. After spending $6.5 million ($2.5 million for labor and $4 million for lost value of plants) on tree removal efforts, canker was eradicated from Florida in 1933. In 1996 dollars, it is equivalent to $29.6 million in lost plant value and $112.5 million in labor cost. Over 258,000 commercial trees and three million nursery trees were destroyed in this eradication effort. Gottwald, *et al.,* (2002) has written a comprehensive account of citrus canker and its impact.

After more than 50 years, another bacterial disease which was later called citrus bacterial spot (CBS), was discovered in 1984. CBS is caused by *Xanthomonas campestriss* pv. *citrumelo*. The discovery of CBS resulted in a joint eradication program between the state of Florida and the U.S. federal government. Between 1984 and 1986, over 20 million citrus nursery trees from 44 nurseries were destroyed. CBS bacterium is less aggressive compared to the Asiatic citrus canker (ACC) bacterium.

In 1986, Asiatic canker was again discovered in Florida near the Gulf Coast, with grapefruit, Mexican lime and pineapple orange trees were the most severely affected cultivars. In Manatee County, citrus canker has been found in numerous orchards covering several hundreds of acres. Approximately 90,000 citrus trees were destroyed before it was considered eradicated in 1994. The cost of eradication and regulatory programs were $25 million from 1984 to 1986, which included over $12 million paid against tree destruction.

A severe form of citrus canker disease was again discovered in residential citrus in urban Miami, in September, 1995. This discovery was following the eight year eradication of ACC in the Gulf Coast of Florida. An initial survey for ACC immediately after its discovery showed that it was present in 13 -14 square miles of residential properties.

The Citrus Canker Eradication Program in Miami initiated an active eradication program removing both infected and exposed trees. Natural events such as thunderstorms, tropical storms, tornadoes, hurricanes, winds, rain, and the presence of citrus leafminer (Phyllocnistis citrella), and in addition, human activities had apparently contributed to rapid movement of the bacterium to nearby properties in Miami. As of June 1997, ACC had spread to some 98 square miles. Both infected and exposed trees have been removed by February 1998. At that time the total canker infected area in Florida increased to 144 square miles. Approximately 361 square miles was under quarantine and approximately 50,000 residential citrus trees have been destroyed. Based on infected citrus branches, it is believed that ACC was present in the Miami International Airport area for at least two to three years prior to its 1995 discovery. Public cooperation is imperative to the success of the citrus canker eradication program.
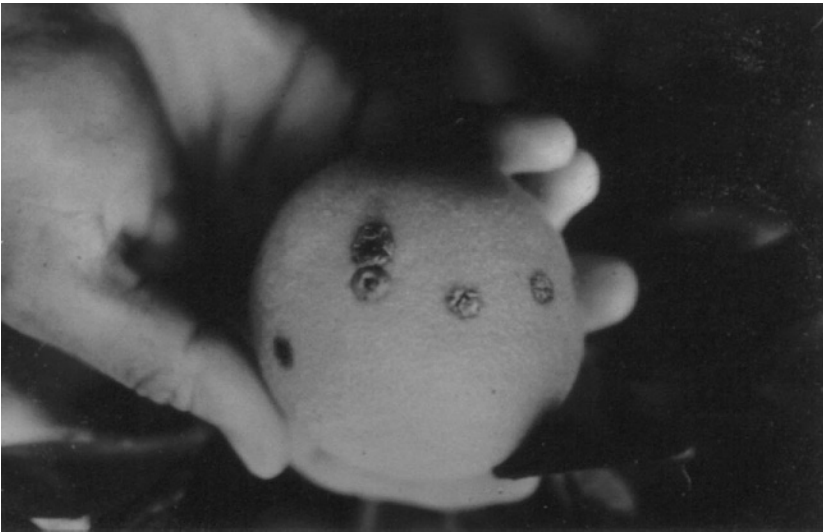
Figure 5: Citrus canker infection on sweet orange (Florida)

5.2.1 Eradication of canker

Eradication is the most important aspect of disease control strategies. This is the strategy that needs to be adopted should the canker bacterium ever be detected for the first time in a citrus growing area. However, there are several obstacles and important factors that play major roles in the success of any eradication program. These factors are: cost, personnel, and time. Eradication of well established trees is an expensive process. It requires much manpower, equipment, and time. Timing is a very important factor in controlling the disease spread. Eradication works best if the process can be done sooner and faster. The earlier the better, and cooperation among all parties involved

would make the disease control an achievable process. If cooperation is lacking, then regulatory agencies will have to use their muscle and legal resources. These take time and in the meantime, the canker bacterium multiplies and spreads, especially in the rainy season. Another important question is cost. Are funds available for more eradication efforts in the future, if needed? Proper remuneration and job satisfaction are other factors that govern the retention of quality, trained people for continuous survey, and eradication efforts.

5.2.2 Cooperation

Eradication efforts are taken to protect the citrus industry - mainly the orchards that produce fruit for the fresh fruit market. However, a major effort of canker eradication in areas like Florida involves dooryard citrus, with homeowners who do not have any special interest or attachment to the citrus industry. For them, they are being taxed and deprived of the joy of raising attractive and tasty fruit in their own backyards. Some might consider canker eradication efforts an encroachment on their rights. The first few years are going to tell us much about the status and future of the canker eradication program. If the worst comes, the state may have to adjust to living with that bacterium. Agriculture has faced several such calamities and people have overcome or adjusted to living with plant disease problems. We have survived major diseases like the potato blight and we manage fire blight in apples and pears.

5.3 Citrus leprosis virus

Citrus leprosis virus is a serious disease of citrus in South American countries, especially in Brazil. The symptoms include chlorotic lesions on the leaves, twigs, and fruit. The color of the lesions may change from brown to black. The center of the lesions on the leaves and stem may develop into a raised necrotic spot, whereas, in fruit it may be flat or depressed. The lesions may develop concentric circles. Severe infections lead to the abscission of leaves and fruit, and branches die-back. In extremely severe cases, individual spots coalesce, resulting in bark scaling similar to that induced by citrus psorosis virus. The disease is caused by the citrus leprosis virus, a member of the family *Rhabdoviridae*. It was reported to be present in Florida prior to 1926.

5.4 Citrus tristeza virus

 (*see* section 4.1)

5.5 *Phytophthora*-root weevil complex

The sugarcane root stalk borer weevil, *Diaprepes abbreviatus*, introduced into Florida in 1964, is now considered the worst long-lasting threat to the citrus industry in that state. There is no effective management program for its control and even partially effective programs cost over 250 dollars per acre per year. Feeding damage of the root system caused by the weevil larvae results in increased incidence of the fungus

Phytophthora. The tree decline as a result of *Phytophthora* infection is several folds higher when *Diaprepes* is involved. This weevil has the ability to feed on different hosts including leaves of silver buttonwood that contains a large quantity of tannins. Moreover, the reproducing capacity of *Diaprepes* is high, a female can produce thousands of offspring.Presence of *D. abbreviatus* has been confirmed in two citrus orchards and several dooryard properties in McAllen, Texas, from October 2000 to the present. It took several months of investigations to identify the cause(s) of rapid decline and death of the sweet orange trees. Affected trees first showed leaf wilt, yellowing and defoliation, followed by tree death in 4-5 weeks. Several trees were pushed out and the roots were washed with a handgun sprayer. The roots showed extensive insect feeding injury (channeling) together with severe *Phytophthora* root rot. The channels varied from 1.25 cm to more than 30 cm in length, and up to 1.25 cm wide. White larvae, subsequently identified as the blue-green citrus root weevil, *Pachnaeus opalus*, and the sugarcane root stalk borer weevil, *Diaprepes abbreviatus,* were identified in the soil. Soil and root analysis confirmed the presence of the *Phytophthora* fungus associated with the dead and declining trees. This weevil(s) and *Phytophthora* complex is the first report from Texas citrus.

5.6 Other diseases

5.6.1 Witches' broom

This disease was first detected in limes in the Sultanate of Oman and surrounding countries. It is also found in sweet lime and citron. The causal organism is characterized as *Candidates* Phytoplasma aurantifolia, the first phytoplasma to receive a binomial nomenclature. This disease is graft-transmissible and also by leafhopper, *Hishimonus phycitis* (Distant).

5.6.2 Citrus variegated chlorosis

This is commonly known as CVC and it is a severe limiting factor of sweet orange production in Brazil, first described in the state of Sao Paulo and now present in other South American countries. The causal agent is bacterium, *Xylella fastidiosa*, found in the xylem cells. This disease is graft-transmissible and also by leafhoppers

5.6.3 Stubborn

Stubborn disease is a problem in some hot, arid countries, causing stunting, nutrient deficiency-type symptoms in oranges, grapefruit, and mandarins. Fruit may be small and lopsided. The disease is caused by a mollicute, *Spiroplasma citri*. The pathogen is graft-transmitted and also by several leafhoppers.

5.6.4 Citrus chlorotic dwarf

This is a serious disease reported from Turkey beginning the 1980s (Korkmas *et al.,*

1995). It is associated with the bayberry whitefly, *Parabemesia myrica* (Kuwana). Most cultivars are found affected; however, sweet orange is the least affected. A V-shaped notch on one or both sides of young leaves is a characteristic symptom. Brlansky *et al.* (2001) have seen a flexuous filamentous virus particle associated with infected tissue.

5.6.5 Yellow mosaic

Yellow mosaic was first reported from South India in 1975 as a disease of sweet orange. Ten years later, the disease was also found affecting mandarins in North India. Now it is known to be affecting several citrus types, producing bright yellow mosaics (Ahlawat *et al.,* 1996). Field trees also show mottling and flecking along the veins of sweet orange and pummlo. This disease is caused by a bacilliform virus, a badnavirus. The disease is graft-transmissible and also by mealybug *Planococcus citri.*

6. Diseases influenced by the weather

6.1 Citrus greasy spot disease

Greasy spot, a serious disease of citrus and caused by the fungus *Mycosphaerella citri,* is becoming a severe problem in Florida and Texas. Within the last few years, trees in many orchards have been heavily defoliated and have had greasy spot-infected fruit. Greasy spot (GS) reduces tree vigor and thereby fruit size. GS infected fruit also show a tendency to re-green and such fruit are culled for the fresh fruit market.

6.1.1 Life cycle

The life cycle of GS fungus can be divided into three major stages. In the epiphytic stage, the GS fungus grows on the lower surface of leaves for a long period before it enters the stomata or air pores to initiate infection. In this stage which can last for several weeks, asexual spores or conidia are produced. The hyphal tips growing on the under surface of the leaves can function as a germ tube and initiate the infection process. The presence of water or relative humidity near 100% and a temperature between 25 - 30ºC are ideal for an ascospore germination and epiphytic growth of the fungus. While the conidia produced by the fungus on the leaf surface do grow, they are not as important as the ascospores. Ascospores are the sexual spores developed in special fruiting bodies called perithecia that are developed in decaying infected leaves on the ground.

The stage of the GS fungus which covers the development and maturation of the perithecia in decaying fallen leaves is called the saprophytic stage. Water from rain or irrigation enhances the release of ascospores into the air, and air currents carry the spores to the young growth flushes. The amount of infected leaves on the ground, humidity, temperature, insect exudations, the amount of epiphytic growth, and the physiological condition of the trees are major factors that influence greasy spot infection and disease severity.

6.1.2 Influence of irrigation practice and rainfall on disease severity

Experiments have been carried out to understand the effect of microjet irrigation on ascospore release and the reduction in epiphytic growth when the inoculum level is lowered by burning the fallen leaves.

6.2 *Ganoderma*

Four year old grapefruit and orange trees grown on sour orange rootstock were found infected by the fungus, *Ganoderma lucidum* (W. Curt.:Fr.) Karst (Skaria 1990). After a 1983 tree-killing freeze, the frozen trees were removed in some orchards by a chain saw cut made at soil level and the stumps of old trees were left intact. Infection by *Ganoderma*

Figure 6: *Ganoderma* fungus growth on soil, wrap, and inside stem of a young grapefruit tree (Texas)

has caused the death of several four-year-old Rio Red grapefruit trees grown on sour orange rootstock. In another orchard, 'Marrs' sweet orange trees on sour orange rootstock, were either killed or had reduced vigor. A hedge row of young Cleopatra mandarin, on its own root and not near any remains of older citrus wood, has been found infected with *Ganoderma*. New, young trees were then planted next to the old stumps. Excellent growth of *Ganoderma* on and around the dead stumps increased inoculum pressure on the young trees making the sour orange rootstock vulnerable to *Ganoderma* rot (Fig. 6). *G. lucidum* is a basidiomycete fungus found in many countries, including North America (Hepting 1971). While some species of *Ganoderma* are saprophytic,

several are pathogens that cause decay of roots and stems. *Ganoderma* grows on the dead or declining heartwood of trees. Usually, *Ganoderma* is not a serious problem on citrus; however, Reichert (1932) reported a root rot of citrus from Palestine caused by a *Ganoderma* spp. He found the disease only on sweet lime root. The disease was associated with the presence of wooden stakes which supported the young trees. The pathogenicity of the citrus isolate of *G. lucidum* had been proven on two rootstocks: Swingle citrummelo and Cleopatra mandarin.

6.3 Oleocellosis

Oleocellosis or rind oil spot is a nemesis induced by fruit harvesters. It is caused by the rupturing of the oil glands of fruit rind. Citrus oil released from rind burns the tissue leaving darkened spots. This type of injury is more common on early oranges harvested early in the season when fruit are still green because the oil glands are more easily ruptured than later in the season. Orange cultivars with rougher peel texture and/or with protruded oil glands have higher incidence of oleocellosis. High humidity, dew, and rain would make the oil gland more turgid, therefore, slight pressure from a harvester's fingers would result in pronounced oleocellosis.

6.4 Spread of postharvest diseases

Post-harvest diseases are caused by fungal organisms. The major post-harvest diseases of citrus are: sour rot (caused by *Geotrichum candidum*), green mold (*Penicillium digitatum*), blue mold (*P.iItalicum*), and stem-end rots (*Lasiodiplodia theobromae* and *Diaporthe citri*).

Postharvest diseases of citrus cause serious economic damage to harvested fruit. We often fail to consider postharvest disease loss in several steps in the pipeline of harvested fruit and vegetables. For example, disease losses in grocery stores, restaurants and in kitchens are often not well documented. Also, we normally do not consider losses in nutritional qualities of fruit and vegetables when we report postharvest disease loss. Though it is difficult to determine the actual loss, a conservative estimate is that in the U.S. approximately 25% of the harvested fruit and vegetables are lost due to postharvest problems. Worldwide, this estimate jumps to 50%. The important postharvest diseases of citrus in Texas are green mold and sour rot caused by *Penicillium digitatum* and *Geotrichum candidum*, respectively. Disease loss can be achieved by minimizing injury to fruit during harvest, improved sanitary practices, and fungicide applications both before and after harvest.

A postharvest fruit rot caused by *Penicillium ulaiense* (commonly known as whisker mold, because of the whisker-like synnemata they produce) was detected in the Lower Rio Grande Valley during the 1992-93 citrus harvest season, along with green mold, blue mold, and sour rot, caused by *P. digitatum, P. italicum*, and *Geotrichum candidum*, respectively. Factors that contributed to a high incidence of fruit rot during that period were: long storage periods (in packinghouses) due to a slow market and low prices, young trees, and a tree-killing freeze in 1989.

6. 5 Melanose

Melanose is a fungal disease of citrus caused by *Diaporthe citri* (also known as, *Phomopsis citri*). It produces pustules of various sizes on the fruit. A light infection of fruit produces small, discrete pustules. In a heavy infestation, the pustules are larger and may coalesce often causing the tissue to crack and produce a stage called mudcake melanose. Melanose disease is directly associated with the presence of dead twigs, especially on recently killed ones. Dead twigs often harbor the fungus, which produces several dark, ovoid structures called pycnidia that contain numerous spores. The spores are released from these structures when wetted. Rain water washes the spores onto the young fruit, leaves, and twigs. The spores that land on young tissue initiate an infection process. Grapefruit is most sensitive to melanose infection. Though infected fruit lose fresh fruit marketability, the internal quality is not affected. Melanose symptoms can be found on leaves, stems, and fruit. When rubbed with fingers those pustules give a sandpaper effect, the size and the number of pustules on fruit may vary with the stage and age. The pustules may be small or large; a few in number or many: discreet or coalesced, the fruit may be cracked and/or coalesced. If fruit get washed with spore-laden water, the effect will show as tear streaks. Melanose symptoms may be confused with a blemish caused by rust mites; however, rust mites cause smoother blemishes compared to rough pustules of melanose. Generally, grapefruit trees show more symptoms compared to other cultivars. The melanose fungus can also cause a serious post-harvest disease called *Phomopsis* stem-end rot. Both orange and grapefruit cultivars are susceptible to this disease, in which the infected tissue at the stem-end part of the fruit shrinks. A clear demarcation line that is visible between the infected and the non-infected part of the fruit is a prominent feature of *Phomopsis* stem-end rot. In contrast, another type of postharvest stem-end rot caused by the fungus, *Diplodia*, develops a characteristic finger-like projection of diseased tissue.

6.6 *Phytophthora* diseases

The fungus *Phytophthora* spp. causes three major diseases in mature citrus trees. They are: (i). foot rot and gummosis, (ii). feeder root rot, and (iii). brown rot of fruit, which is a postharvest problem (Graham *et al.,* 1998, Whiteside 1970). It can also cause damping-off, which is infection and eventual death of emerging seedlings in nurseries. In the field, foot rot and associated gummosis are the most important disease of *Phytophthora*. Foot rot is a disease of the bark of the main trunk or roots at the ground surface. The fungus *Phytophthora* is normally present in natural soil, mainly in the form of resting spores called the oospores and chlamydospores, and they have characteristic thick walls. The thick walls allow these propagules to survive in dry soils for a long period of time. *Phytophthora* has been recovered from soil that has been air dried for over two months and also after 3 to 4 months storage at -5 bars. The resting spores germinate in moist soil, producing germ tubes that may end up in a structure called sporangium, which is the next level of structural development in the distribution of *Phytophthora* in the field. The sporangium and its spores (sporangiospores) are less resistant to adverse environmental conditions. However, it can also survive some de-

gree of heat, cold and dryness. The sporangiospores get distributed in the water, especially in flood irrigated orchards. Each zoospore can initiate an infection process, especially in young tissue and under favorable conditions. Infection occurs through wounds or natural cracks on the bark. The fungus grows in the bark, killing the tissue. Infected bark shows discoloration due to the death of tissue. Though abundant gum exudation may be a symptom of foot rot, the gum may be washed off in heavy rains. Affected trees may look pale with yellow veins and leaves. The infection may extend downward into the crown root and can completely girdle the tree. Once substantial damage has happened to the bark, the tree starts to wilt, shed leaves, cause twig die back, and the fruit may hang on to a dead tree for some time.

   Feeder root rot, as the name refers, happens in the feeder roots where the bark (cortex) sloughs off. The initial symptoms are limited to the roots; however, as the
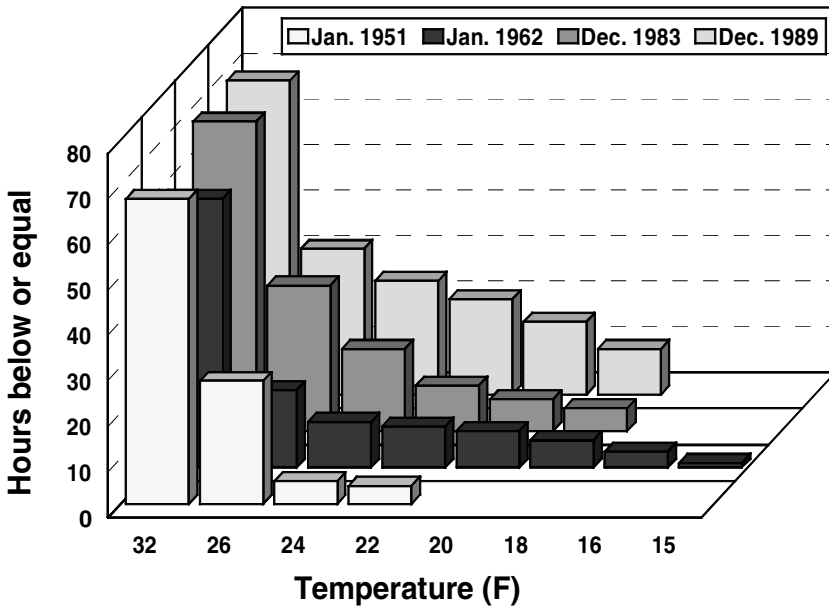
Figure 7: Citrus tree-killing freezes occurred in the Rio Grande Valley of Texas in the last 50 years.

disease progresses, affected trees show substantial decline and yield loss (Graham and Menge 1999). The trunk will not show symptoms as in foot rot. Trees that are under stress due to chemical, water, soil type, and other horticultural conditions can weaken the feeder roots and make them pre-disposed to the easy access of this fungus causing feeder root rot.

   The brown rot is a fruit disease which starts as a light brown discoloration induced by *Phytophthora*. The fruit may develop a white mycelium on the surface, under humid conditions. The disease is initiated on fruit in the branches closer to soil, through inoculum introduced via water splashing.

6.7 Tree-killing Freezes

Though citrus is a sub-tropical crop and in some sub-tropical areas such as Texas, Florida, northern Mexico, Argentina, Japan, and part of China and the Mediterranean, free-induced tree kill and/or fruit damage are serious concerns in some years. Several unpredictable freezes in Texas and Florida caused billions of dollars in crop loss. For example, in Texas alone, four tree-killing freezes occurred between 1951 and 1989 reduced the citrus acreage approximately 75% (Fig.7). Tree-killing freezes and frost ice nucleation of water occurs between 0 and -10°C; however, water droplets of diameter 10 :m or less supercools to -38.1°C. The actual freezing will be triggered by ice nucleators
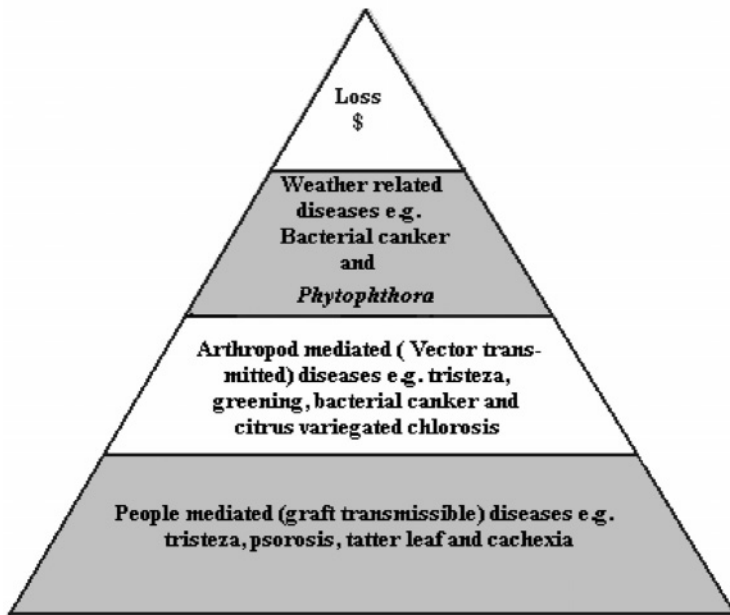
Figure 8: Relative role of people, arthropods, and weather in citrus disease spread and crop loss.

that cause water crystallization. Newly formed ice crystals can be nucleators and freezing process can continue rapidly, drawing water from plant tissue. A very significant freeze injury to plant tissue is caused by dehydration of the protoplasm as the water evaporates to the extracellular ice. As more water is lost from the cells, the concentration of solutes increases and may even become toxic.

After a1983 tree-killing freeze, Fucik (1990) studied the relationship between the number of frozen leaves and wood to subsequent fruit set in grapefruit and Valencia orange trees. In either radiational or advective type of freezes, the freezing of leaves and wood proceeds from the outer canopy to the main trunk. Therefore, surviving plant

parts would be in the center of the canopy around the scaffold limbs and trunk. There may not be a proportional relationship between the percent of leaves killed and wood damage (Fucik, 1990). A 100% increase in leaf loss may reflect 6 to 10 times mortality of branch tissue. A 50% leaf loss in Ruby Red grapefruit, one could expect 1500 to 3500 flowers per mature tree.

6.8 Hurricanes

A hurricane is a type of tropical cyclone that develops over tropical oceans in summer and move west and northwest. It is an important weather event along the U.S. coastal area. It is a counter-clockwise low pressure system with winds exceeding 64 knots (116.8 km). From late spring into summer, ocean air near the equator begins to warm and rises through atmosphere and cools, causing condensation and clouds. The condensa-

Table 4: Means of transmission of major citrus diseases compared.

Disease	People-mediated	Arthropod-mediated	Weather-mediated	Other factors
1. Tristeza	Yes	Yes	No	No
2. Tatter leaf	Yes	No	No	Tools
3. Psorosis	Yes	No	No	Fungus?
4. Viroids	Yes	No	No	Tools
5. Nematodes	Yes	No	No	No
6. Postharvest rots	Yes	Yes?	Yes	No
7. Replant problems	Yes	No	Yes Fungi	Nematodes/
8. Greening	Yes	Yes	No	No
9. Canker	Yes	Yes	Yes	Tools
10. Leprosis	Yes	Yes	No	No
11. *Phytophthora*	Yes	Yes	Yes water	Irrigation
12. Witches' broom	Yes	Yes	No	No
13. Variegated chlorosis	Yes	Yes	No	No
14. Stubborn	Yes	Yes	No	No
15. Chlorotic dwarf	Yes	Yes	No	No
16. Yellow mosaic	Yes	Yes	No	No
17. Greasy spot	No	No	Yes	Irrigation
18. *Ganoderma*	No	No	Yes	No
19. Melanose	No	No	No	Dead twigs

tion releases latent heat and lowers surface pressure, resulting in circular storm. Hurricanes in Texas have caused extensive damage to people, property, and loss and diseases of plants, including citrus. Hurricanes are named at the stage of tropical storms with well-organized cluster of thunderstorms with substantial rotary circulation and surface winds between 62.4 and 116.8 kmh. A devastating hurricane in 1900 resulted in

drowning approximately 15 % of the people in Galveston, Texas. Similar storms in the Far East are known as typhoons and in Indian Ocean and Australia as cyclones.

Hurricane Beulah of September 20, 1967 caused a total damage of $ 15 million in the Lower Rio Grande Valley of Texas. There is no accurate record of indirect tree loss as a result of increased incidence of tree-kill as a result of fungus *Phytophthora*. Winds of Beulah gusted to 218 km/hour. Rain fall in some areas of south Texas reached more than 30 inches and several areas were isolated for weeks.

Citrus trees exposed to below-freezing temperatures for hours would result in leaf drop, and twig die-back, and in a few cases, tree death. Good cultural practice is the most important strategy for both melanose and stem-end rot disease control. Remove dead twigs from the tree by pruning, hedging and/or topping. Timely application of copper fungicides in the orchard can also reduce melanose infection.

7. Conclusion

Human activity ranks number one in the transmission of citrus diseases. Arthropod-related problems such as the citrus nematode are often spread long distances through the practice of nursery propagation in contaminated soil. Failure to enforce proper quarantine formalities can result in arthropod vectors such as the brown citrus aphid that transmits tristeza virus. Exotic diseases should be prevented through proper regulatory measures and educational programs. The relative role played by people, arthropods, and weather in transmitting citrus diseases has been illustrated (Fig. 8, Table 4).

8. References

Ahlawat, Y.S., Pant, R.P., Lockhart, B.E.L., Srivastava, M., Chakrabory, N.K. and Varna, A. 1996. Association of a badnavirus with citrus mosaic disease in India. Plant Dis., 80: 590-592.

Aubert, B., Etienne,J., Cottin, R., Leclant,F., Cao Van, P., Vuillaume,C., Jaramillo,C. and Barbeau,G. 1992. Citrus tristeza disease a new threat for the Caribbean Basin. Report of a survey to Colombia, Dominican Republic, Guadeloupe, Martinique, and Trinidad. Fruits 47:393-404.

Bar-Joseph, M., Garnsey, S.M. and Gonsalves, D. 1979a. The closteroviruses: a distinct group of elongated plant viruses. Adv. Virus Res., 25: 93-168.

Bar-Joseph, M., Garnsey, S.M., Gonsalves, D., Moscovitz, M., Purcifull, D.E., Clark, M.F. and Loebenstein, G. 1979b. The use of enzyme-linked immunosorbent assay for the detection of citrus tristeza virus. Phytopath., 69:190-194.

Bar-Joseph, M., Roistacher, C.N., Garnsey, S.M. and Gumpf, D.J. 1981. A review on tristeza, an ongoing threat to citriculture. Proc. Int. Soc. Citriculture, 1: 419-423.

Bar-Joseph, M., Marcus, R. and Lee, R.F. 1989. The continuous challenge of citrus tristeza virus control. Annu. Rev. Phytopathology, 27: 291-316.

Benatena, H.N. and Portillo, M.M. 1984. Natural spread of psorosis in sweet orange seedlings. In: "Proc. 9th Conf. IOCV., IOCV, Riverside", pp. 159-164.

Bitancourt, A.A. 1940. A podridao das radicelas dos citrus na provincia de Corrientes, Argentina. O. Biologico, 285-88, 356-64.

Borbon, J.C., Abud, A.J., Millan, P.J., Asiatica, J. and Abreu, N. 1992. Presencia de la Triteza

de los citricos y *Toxoptera citricidus* (Kilkardy) en la Republica Dominicana. P. 95-101. In: "Proc. Workshop on Citrus Tristeza Virus and *Toxoptera citricidus* in Central America: Development of management strategies and use of biotechnology for control. Maracay, Venezuela", Sept. 14-19, 1992.

Brlansky, R.H., Howd, D.S., Hartung, J.S., Garnsey, S.M. and Korkmaz, S. 2001. Purification of virus-like particles from citrus chlorotic dwarf infected citrus- virus or not? In: "Proc. 15th Conf. IOCV., Paphos, Cyprus, November 11-16", p. 89 (abs.).

Burke, J.H. 1967. The commercial citrus regions of the world. In: "The Citrus Industry, Vol. I." (eds. Reuther, W., Webber, H.J. and Batchelor, L.D.). Univ. Calif., Berkeley, pp. 40-189.

Calavan, E.C., Frolich, E.F., Roistacher, C.N. and Christransen, D.W. 1964. Rapid indexing for exocortis of citrus. Phytopathology, 54: 1359-1362.

Calavan, E.C., Roistacher, C.N. and Nauer, E.M. 1972. Thermotherapy of citrus for inactivation of certain viruses. Plant Dis. Rept., 56: 976-980.

Clark, M.F. and Adams, A.M. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol., 34:475-483.

Cohen, E. 1965. On the feeding and histopathology of the citrus nematode. Nematologica, 11:47-54.

Costa, A.S. and Grant, T.J. 1951. Studies on transmission of the tristeza virus by the vector *Aphis citricidus.* Phytopath., 41:105-113.

Da Graca, J. V. 1977. Citrus tatter leaf virus in South African Meyer lemon. Citrus Subtrop. Fruit J., 529: 18.

Da Graca, J.V. 1991. Citrus greening disease. Annu. Rev. Phytopathol., 29:109-136.

Da Graca, J.V., Lee, R.F., Moreno, P., Civerolo, E.L. and Derrick, K.S. 1991. Comparison of isolates of citrus ringspot, psorosis, and other virus like agents of citrus. Plant Dis., 75:613-616.

Davis, F.S. and Albrigo, L.G. 1994. Citrus. Crop production science in horticulture 2. 254 pp. CAB International, Wallingford, UK.

Derrick, K.S., Brlansky, R.H., da Graca, J.V., Lee, R.F., Timmer L.W. and Nguyen, T.K. 1988. Partial characterization of a virus associated with citrus ringspot. Phytopathology, 78:1298-1301.

Duncan, L.W., Inserra, R.N., O'Bannon, J.H. and El-Morshedy, M.M. 1994. Reproduction of a Florida population of Tylenchulus semipenetrans on resistant citrus rootstocks. Plant Dis., 78:1067-1071.

Duran-Vila, N., Pina, J.A. and Navarro, L. 1993. Improved Indexing of Citrus Viroids. Proc. 12th Conf. IOCV pp.202-211.

Friend, W.H. 1954. History of Meyer lemon in the valley. Proc. 8th Ann. Inst. Rio Grande Valley Hort. Soc., 32-33.

Fucik, J.E. 1990. Assessing freeze damage: back to square one after 1983. Texas A&I University Citrus Center Newsletter 8 (1): 2.

Garnsey, S.M. 1964. Detection of tatter leaf virus in Florida. Proc. Fla. State Hort. Soc., 77: 106-109.

Garnsey, S. M. 1970. Viruses in Florida's Meyer lemon trees and their effects on other citrus. Proc. Fla. State Hort. Soc., 83: 66-71.

Garnsey, S.M. 1974. Mechanical transmission of a virus that produces tatter leaf symptoms in *Citrus excelsa.* In: "Proc. 6th Conf. IOCV, IOCV, Riverside, California", pp.137-140.

Garnsey, S.M., and Jones, J.W. 1967. Mechanical transmission of exocortis virus with contaminated budding tools. Plant Disease Reptr., 51: 410-413.

Garnsey, S. M. and Cambra, M. 1991. Enzyme-linked immunosorbent assay (ELISA) for citrus pathogens, In: "Graft Transmissible Diseases of Citrus". (ed. Roistacher, C. N.) , FAO, Rome, pp.193-216.

Garnsey, S.M., Civerolo, E.L., Gumpf, D.J., Yokomi, R.K. and Lee, R.F. 1991. Development of a worldwide collection of citrus tristeza virus isolates, In: "Proc. 11th Conf. IOCV., IOCV, Riverside" pp. 113-120.

Garnsey, S.M., Civerolo, E.L., Lee, R.F., Yokomi, R.K. and Behe, C.C. 1996. Using the Beltsville international CTV collection facility to determine severity of Caribbean isolates of citrus tristeza virus, Proc. 3rd Int. Workshop: Citrus Tristeza Virus and the Brown Citrus Aphid in the Caribbean Basin: Management Strategies. F.A.O., USDA-OICD, and University of Florida, Lake Alfred, FL., pp. 253-259.

Gottwald, T.R., Garnsey, S.M. and Yokomi, R.K. 1994. Potential for spread of citrus tristeza virus and its vector, the brown citrus aphid. Proc. Fla. State Hort. Soc., 106: 85-94.

Gottwald, T.R., Garnsey, S.M., Sediles-Jean, A. and Rojas-Solis, A. 1996. Co-diffusion of serologically distinct isolates of citrus tristeza virus vectored by *Toxoptera citricida* in Northern Costa Rica, In: "Proc. 13th Conf. IOCA., IOCV, Riverside, CA", pp. 112-119.

Gottwald, T.R., Graham, J.H. and Schubert, T.S. 2002. Citrus canker: The pathogen and its impact. Online. Plant Health Progress doi: 10.1094/PHP-2002-0812-01-RV.

Graham, J.H. and Menge, J.A. 1999. Root diseases. In: "Citrus Health Management". (eds. Timmer, L.W. and Duncan, L.W.) American Phytopathological Society, St. Paul, MN., pp. 126-135.

Graham, J.H., Timmer, L.W., Drouillard, D.L., and Peever, T.L. 1998. Characterization of *Phytophthora* spp. causing outbreaks of citrus brown rot in Florida. 88:724-729.

Grant, T.J., and Schneider, H. 1953. Initial evidence of the presence of tristeza or quick decline of citrus in Florida. Phytopathology, 43: 51-52.

Hardy, N. 1995. Brown citrus aphid found in Ft. Lauderdale. Citrus Ind., 76:31.

Halma, F.F., Smoyer, K.M. and Schwalm, H.W. 1944. Quick decline associated with sour rootstocks. Cali. Citrog., 29:245.

Hepting, G.H. 1971. Diseases of forest and shade trees of the United States. U.S. Dep. Agric. Handbook, 386. 658 pp.

Ke, C. and Wu, R-J. 1991. Occurrence and distribution of citrus tatter leaf in Fujian, China. In: "Proc. 11th Conf. IOCV., IOCV, Riverside", pp. 358-364.

Knorr, L. C. and DuCharme, E. P. 1951. This is tristeza - Ravager of Argentina's citrus industry. Citrus Mag., 13: 17-19.

Koizumi, M. 1984. Elimination of tatter leaf-citrange stunt virus from satsuma mandarin by shoot-tip grafting following pre-heat-treatment, In: "Proc. 9th Conf. IOCV., IOCV, Riverside", pp. 229-233.

Korkmas, S., Cinar, A., Kersting, U. and Garnsey, S.M. 1995. Citrus chlorotic dwarf: a new whitefly-transmitted virus like disease of citrus in Turkey. Plant Dis., 79:1074.

Lastra, R., Lee, R.F., Rocha-Pena, M., Niblett, C.L., Ochoa, F., Garnsey, S.M. and Yokomi, R.K. (eds.). 1992. Citrus Tristeza Virus and *Toxoptera citricidus* in Central America: Development of management strategies and use of biotechnology for control. Maracay, Venezuela. Univ. Florida, Citrus Research and Education Center, Lake Alfred, FL. 287 p.

Liu, Y., Ahmad, H., Luo, Y., Gardiner, D.T., Gunasekera, R.S., McKeehan, W.L. and Patil, B.S. 2001. Citrus pectin: Characterization and inhibitory effect on fibroblast growth factor–receptor interaction. J. Agric. Food Chem., 49: 3051-3057.

McClean, A.P.D. 1963. The tristeza virus complex: its variability in field-grown citrus in South Africa. So. African Jour.Agr.Sci., 303-32.

Mendt, R. 1992. History of CTV in Venezuela. Proc. Workshop Citrus tristeza virus and *Toxoptera citricidus* in Central America: Development of management strategies and use of biotechnology for control, pp. 137-140.

Meneghini, M. 1946. Sobre a natureza e transmissibilidade do doencia "tristeza" dos citrus. O. Biologico, 12:285-87.

Miyakawa, T. and Tsuji, M. 1988. The association of tatterleaf virus with budunion crease of trees on trifoliate orange rootstock, In: "Proc. 10th Conf. IOCV., IOCV, Riverside", pp. 360-364.

Moreira, S. 1942. Observacoes sobre a tristeza dos citrus ou podridao das radicelas. O. Biologico, 8: 269-72.

Murashige, T., Bitters, W.P., Rangan, T.S., Nauer, E.M., Roistacher, C.N. and Holliday, P.B. 1972. A technique of shoot apex grafting and its utilization toward recovering virus-free citrus clones. HortScience, 7:118-119.

Navarro, L., Roistacher, C.N. and Murashige, T. 1975. Improvement of shoot tip grafting *in vitro* for virus-free citrus. Amer. Soc. Hort. Sci., 100: 471-479.

Permar, T.A., Garnsey, S.M., Gumpf, D.J. and Lee, R.F. 1990. A monoclonal antibody that discriminates strains of citrus tristeza virus. Phytopathology, 80: 224-228.

Reichert, I. 1932. A new root rot of citrus in Palestine. Hadar, 5:254-256.

Rocha-Pena, M.A., Lee, R.F., Lastra, R., Niblett, C.L., Ochoa-Corona, F.M., Garnsey, S.M., and Yokomi, R.K. 1995. Citrus tristeza virus and its aphid vector *Toxoptera citricida.* Plant Dis., 79: 437-445.

Roistacher, C.N. (ed.) 1991. Graft-transmissible diseases of citrus. Handbook for detection and diagnosis. International organization of Citrus Virologists and Food and Agricultural of the United States, Rome. 286 p.

Roistacher, C.N. 1993. Psorosis—A review. In: "Proc. 8th Conf. IOCV., IOCV, Riverside", pp. 139-154.

Roistacher, C.N., Calavan, E.C., Blue, R.L., Navarro, L. and Gonzales, R. 1977. A new more sensitive citron indicator for detection of mild isolates of citrus exocortis viroid (CEV). Plant Disease Reptr., 61: 135-139.

Roistacher, C.N., Nauer, E.M. and Wagner, R.C. 1980. Transmissibility of cachexia, Dweet mottle, psorosis, tatterleaf and infectious variegation on knife blades and its prevention, In: "Proc. 8th Conf. IOCV., IOCV, Riverside", pp. 225-229.

Rouse, R.E. and Wutscher, H.K. 1985. Heavy soil and bud union crease with some grapefruit clones limit use of Swingle citrumelo. HortScience, 20: 259-261.

Semancik, J.S. 1991. Viroid purification and characterization, In: "Graft transmissible diseases of citrus". (ed. Roistacher, C.N.) A handbook for detection and diagnosis. FAO, pp. 233-248.

Skaria, M. 1990. A pictorial analysis of the growth and development of Ganoderma rot on young citrus in Texas. J. Rio. Grande Valley Hort. Soc., 43: 85-87.

Skaria, M., Baker, J., Kahlke, C., Solís-Gracia, N., Roistacher, C.N. and da Graça, J.V. 1996. A Virus-free Citrus Budwood Program for Texas. Proc. Int. Soc. Citriculture, 1:366-368.

Skaria, M., Miao, H. and Avila, E. 2001. Post-freeze status of Citrus psorosis virus in Texas. XV Conference of the International Organization of Citrus Virologists Meeting. Paphos, Cyprus, November 11-16, 2001, pp. 157 (abs.).

Solis-Gracia, N., Kahlke, C.J., Herron, C.M., da Graca, J.V., Essau, K.L., Miao, H.Q. and Skaria, M. 2001. Survey for Citrus tristeza virus in Texas 1991-2000. Subtropical Plant Science, 53: 4-8.

Swingle, W.T. 1909. The limitation of the satsuma-orange to trifoliate-orange stock. U.S. Dept. Agr. Bur. Plant Indus. Circ., 46:10 pp.

Tian, Qingguo, Miller, E.G., Ahmad, H., Tang, L. and Patil, B.S. 2001. Differential inhibition of human cell proliferation by citrus limonoids. Nutrition and Cancer, 40: 180-184.

Timmer, L.W. 1975. Identification of citrange stunt virus from Meyer lemon in Texas. J. Rio Grande Valley Hort. Soc., 29:65-69.

Timmer, L. W. and Garnsey, S.M. 1980. Natural spread of citrus ringspot virus in Texas and its association with psorosis-like diseases in Florida and Texas. In: "Proc. 8th Conf. IOCV., IOCV, Riverside", pp. 167-173.

Timmer, L.W., Garnsey, S.M. and Graham, J.H. (eds.). 2000. Compendium of Citrus Diseases, APS Press, St. Paul. 92 p.

Wallace, J.M. and Drake, R.J. 1962. Tatter-leaf, a previously undescribed virus effect on citrus. Plant Dis. Rept., 46: 211-212.

Webber, H.J. 1925. A comparative study of the citrus industry of South Africa. So. Africa Dept. Agr. Bul. 6: 106p.

Whiteside, J.O. 1970. Factors contributing to the restricted occurrence of citrus brown rot in Florida. Plant Dis. Rep., 54: 608-612.

Yokomi, R.K., Lastra, R., Stoetzel, M.B., Damsteegt, V.D., Lee, R.F., Garnsey, S. M., Gottwald, T. R., Rocha-Pena, M. and Niblett, C. L. 1994. Establishment of the brown citrus aphid (Homoptera: Aphididae) in central American and the Caribbean Basin and transmission of citrus tristeza virus. J. Economic Ent., 87: 1078-1085.

Zhang, T.M., Liang, X.Y. and Roistacher, C.N. 1988. Occurrence and detection of citrus tatter leaf virus (CTLV) in Huangyan, Zhejiang Province, China. Plant Dis., 72:543-545.

Diagnosis and Management of Pre and Post-harvest Diseases of Citrus fruit

S.A.M.H. Naqvi

National Research Centre for Citrus, Indian Council of Agricultural Research
PO Box 464, Amravati Road,
NAGPUR 440 010, Maharashtra, India

Abstract : Citrus is an important fruit crop grown commercially in more than 135 countries in different agro-climatic conditions for its diversified use and increasing demand world over with about 102.64 million tonnes total world production and probably stands first largest among the fruit crop. The larger part of revenue by citrus producing countries is realised through fresh fruit trade followed by its processed products and by products. The success of fresh citrus fruit export is mainly dependant on the quality of produce, phytosanitary conditions set by the importing country and WTO and the post-harvest management to minimise the decay till the consignment reaches to its destination. The citrus fruit is attacked by a number of pathogens from bloom to harvesting stage and subsequently by post-harvest pathogens that affect the production of the crop and considerably deteriorate the fruit quality. The pre-harvest pathogens like *Colletotrichum gloeosporioides, C. acutatum, Botryodiplodia theobromae, Alternaria citri, Phomopsis citri* etc. attack the fruit from fruit set till harvest and cause considerable damage to its production and quality. The incipient infection of pre-harvest pathogens subsequently also manifest in the form of post-harvest diseases besides the attack of other post-harvest wound pathogens viz. *Penicillium digitatum, P. italicum,* G*eotrichum candidum* etc. during post-harvest handling, transport, storage and marketing. The viable technologies have been developed to reduce these losses through pre-harvest management of diseases, improved handling, transport, storage, packing and marketing. The post-harvest treatments with fungicides, biocontrol agents, and wax on mechanical citrus packing line and certain physical therapies have been suggested to further minimise the post harvest decay and to extend the shelf life of fruits for successful trade of citrus in domestic and distant export market. The present scenario and future trade requirements have been discussed here.

1. Introduction

Citrus is an important fruit crop grown commercially in more than 135 countries in different agro-climatic conditions for its diversified use and increasing demand world over with about 102.64 million tonnes total world production and probably stands first largest among the fruit crops (FAOSTAT, 2002). Among the top citrus producing countries, considering production, Brazil is the leading country producing 18.39 million tonnes followed by USA (14.70), China (12.01), Mexico (6.32), Spain (5.54), and India ranks sixth producing 4.87 million tonnes (Table 1). Citrus occupies an important place in the horticultural wealth and economy of India as the third largest fruit industry after banana and mango. The larger part of revenue by citrus producing countries is

realised through fresh fruit trade followed by its processed products and by products. However, the fresh fruit trade in form of export is only 9.29 % of the total world production and only few countries viz. Spain, USA, Turkey, South Africa, Morocco, Argentina, Greece, Egypt, Italy and Israel lead in export front of fresh fruit and contribute to more than 80 % of world export of Citrus fruit (Table 2). The success of fresh citrus fruit export is mainly dependant on the quality of produce, phytosanitary conditions set by the importing country and WTO and the post-harvest management to minimise the decay till the consignment reaches to its destination.It is interesting to note that the countries producing the most are not actually leading the export trade in citrus. The

Table 1: Citrus production in some leading Citrus producing countries during 2001.

Country	Harvested area (ha)	Total Production M T
1. Brazil	9,37,074	18,392600
2. USA	4,41,065	14,701920
3. China	14,20,330	12,017000
4. Mexico	4,95,594	6,324746
5. Spain	2,83550	5,547152
6. India	2,53700	4,870000
7. Iran Rep.	2,18422	3,769996
8. Italy	1,77599	3,062650
9. Argentina	1,25533	2,706000
10. Egypt	1,35661	2,562660
11. Turkey	88,933	2,478000
WORLD	7,249,480	102,648,184

major part of citrus produce in India is being marketed as fresh fruit and less than 1 % is being exported to neighbouring countries. Thus the export potential of Indian mandarin – a loose jacket, easy peeler excellent citrus variety, is not being realised to earn the foreign exchange. The major limiting factor in successful trade of citrus in domestic and export market has been the considerable decay and quality deterioration of fruits incurred due to plant pathogens during storage and transit to distant markets.

Citrus spp. are prone to the attack of more than 100 diseases and disorders caused by fungal, viral and few bacterial pathogens right from nursery level to bearing stage resulting in incalculable losses to plantation and its produce. The citrus fruit is attacked by a number of pathogens from bloom to harvesting stage and subsequently by post-harvest pathogens that affect the production of the crop and considerably deteriorate the fruit quality. Citrus being non climacteric fruit requires about 5 - 9 month for its maturity on trees and during this long maturity period, the fruit remained expose to the attack of pre-harvest pathogens.The pre-harvest pathogens like *Colletotrichum gloeosporioides, C. acutatum, Botryodiplodia theobromae, Alternaria citri, Phomopsis citri* etc. attack the fruit from fruit set till harvest and cause considerable damage to its production and quality. The incipient infection of pre-harvest pathogens

subsequently also manifest in the form of post-harvest diseases besides the attack of other post-harvest wound pathogens viz. *Penicillium digitatum, P. italicum,* G*eotrichum candidum* etc. during post-harvest handling, transport, storage and marketing. During last two decades with manifold increase in the area under citrus cultivation, the production has increased dramatically, and yet the interest in implementing the available technologies has not gained the same momentum in protecting the fruit against post-harvest losses caused by the pathogens. It has been recognised by International organisations responsible for monitoring food resources that the most economically feasible and expedient means to increase food supply is to reduce post harvest losses (Eckert and Ogawa, 1985). The losses in fruits and vegetables have been recorded much higher due

Table 2: Export of fresh citrus fruit in world.

Exporting country	Export in 000 MT 2001-02	Percent of total production
1. Spain	3086	56.71
2. USA	1095	07.37
3. Turkey	834	46.90
4. South Africa	803	51.80
5. Morocco	440	44.76
6. Argentina	365	13.48
7. Greece	311	23.65
8. Egypt	260	10.14
9. Italy	240	07.83

to their high moisture content which make them susceptible to the attack of pathogenic fungi and bacteria between the period of harvest and consumption. Though the losses are still substantial even in production areas with most advanced technologies available in developed countries. But the situation is quite alarming especially in the conditions prevailing in India and other Southeast Asian countries, where scientific pre-harvest and post-harvest management of the pathogens, harvest, handling, transit and storage are neglected that results in spoilage of citrus fruit.

2. Plant pathogens affecting Citrus fruit

The actual loss of citrus fruits due to pre and post-harvest diseases is quite variable and depends upon the area of production, citrus variety, tree age and condition, weather conditions during the growing and harvest season, degree of injuries during harvesting operations, effectiveness of fungicide treatments and subsequent post-harvest environment. The activity of various citrus fruit pathogens is greatly influenced with the climate of the production area. In a typical summer rain-fall production area like Florida, 13 – 42 % untreated fruit developed stem end rot caused by *Diplodia natalensis* (Eckert and Brown, 1986). In central India, the citrus is grown in a typical tropical conditions where citrus fruit are exposed to extreme summer followed by rainfall and the winter during their developing stage before harvest. The Vidarbha region of Maharashtra

and adjoining area of Madhya Pradesh in India constitutes the largest commercial cultivation belt (80,000 ha area with 0.5 million tons production/ annum) of Nagpur mandarin (*Citrus reticulata* Blanco)– the chief commercial cultivar of citrus, famous for its easy peeling quality and taste. The survey for assessment of post-harvest losses in Nagpur mandarin through different commodity flow channels from farm level to Delhi market (ca 1100 km) has revealed that from harvest to Delhi market, the fruit is handled 12 to 18 times. The rough and multiple handling of fruit cause bruising and injuries to fruit which facilitated invasion of wound pathogens and subsequent decay. It was observed that the losses in market packed fruits were 20.90 % when transported by truck and 23.27 % by train while in farm house packed fruits (where handling of fruit was comparatively for less number of times), the losses were 18.17 % by truck and 20.53 % by train transport. The truck took 60 – 70 hours to reach Delhi market in comparison to 120 –130 hours by train (Table 3). The pathogen-wise contribution in the total losses revealed that Stem end rot caused by incipient infection of pre-harvest pathogens like

Table 3: Post harvest losses (%) of Nagpur mandarin from farm level to Delhi market

| Packing condition | Mode of transport (non-refrigerated) | |
	Truck (60 – 70 hr.)*	Train (120-130 hr.)*
Packed at local market	18.34 – 23.48 (Average 20.90)	21.95 – 24.59 (Average 23.27)
Packed at farm house	(15.60 – 20.74) (Average 18.17)	19.21 – 21.85 (Average 20.53)
	Average of total losses = 21.46	

* Transportation time.

C. gloeosporioides, B. theobromae, Phomopsis citri and *Alternaria citri*, contributed 21 – 26 % of the total losses. The handling injuries resulted in 38 – 45 % of the total losses in the form of soft rot due to invasion of *P. digitatum, P. italicum, G. candidum* and *Aspergillus niger*. The average 21.46 % post-harvest losses from 0.4 million tons of production amount to the loss of Rs 257.52 million / annum when fruit is priced at Rs 3.0 per Kg (Naqvi and Dass, 1994). Stem end rot followed by sour rot and anthracnose were the major post-harvest diseases of this area. In north-western part of India, the citrus is grown in typical sub tropical climate with distinct winter. Post-harvest diseases caused by *P. digitatum* (green mould), *P. italicum* (blue mould) followed by stem end rot are the major diseases of Kinnow mandarin in Punjab and Himachal Pradesh and of mandarin, sweet orange and acid lime in Rajasthan and Gujarat States of India.

Following factors were identified for high post-harvest losses in Nagpur mandarin: (i). Initially high inoculum of pathogens in the orchards in absence of adequate plant protection measure, (ii). Rough and multiple handling (12 – 18 times) of fruit in unsanitary

environment after harvest, (iii). Retention of fruit for 4 – 7 days after harvest at farm or local market for further distribution, (iv). Use of unventilated wooden packing boxes and transport carriers, (v). Almost negligible pre and post-harvest chemical treatments and vi. No refrigerated transport and storage facilities during marketing (Naqvi, 1993a, Naqvi, 1993b, Naqvi, 1996).

3. Pre-harvest diseases of citrus fruit

The production and quality of citrus fruit are affected by two types of diseases: i. Pre-harvest diseases incited by the pathogens in orchard and ii. Post-harvest diseases, the diseases develop after fruit harvest. The citrus fruit are exposed to a number of pathogens during its growing period in the orchard. These pathogens severely affect the citrus

Figure 1: Post-harvest losses in Nagpur mandarin at Delhi market.

flowering, fruit set and growth and thus directly influence the yield. Certain pre-harvest infections become quiescent during fruit development and resume their active growth after harvest resulting in post-harvest diseases.

3.1 Post bloom fruit drop

The post bloom fruit drop caused by a virulent strain of *C. gloeosporioides* (Penz.) Sacc. was first reported from Blize island causing up to 65 % crop losses (Fagan, 1979; Denham, 1979). The disease has been reported in severe form in Argentina, Brazil, Colombia, Dominica, Panama and Florida causing fruit drop in sweet orange, grapefruit

etc. (Timmer *et al.,* 1994, Timmer, 1995). In Florida, the disease is caused by *C. acutatum* (Timmer, 1995). The pathogen survives as appressoria on leaves, dead twigs and buttons. Early bloom flowers get initial infection in the form of water soaked patches on petals. One infected flower produces 10,000 to 1 million spores. Few such infected flowers are enough to cause epidemic during rainy days. Just two rains in 10 days spell can increase the infection rate from 5 to 70 % (Timmer, 1995). The disease may cause complete crop failure if not attended timely.

3.2 Pre-harvest fruit drop

The pre-harvest fruit drop is the fruit drop, which takes place 1 – 2 month before harvest. The severity of the drop depends on weather conditions and varies from season to season. The latent infection of *B.theobromae*, *C. gloeosporioides* and *Phomopsis citri* occasionally becomes active and develop necrosis on stem end, which results in fruit drop. In some cases, *Alternaria citri* and *Fusarium sp*. are also found associated with pre-harvest fruit drop. In Nagpur mandarin, up to 22 % fruit drop caused by the above pathogens have been recorded (Naqvi, 1993a). Increase of dead twigs on bearing plant proportionately increase the fruit drop because the dead twigs become the site of pathogen multiplication, spread and subsequent fresh infection.

3.3 Brown rot

Brown rot of citrus is caused by *Phytophthora* spp. in many citrus growing regions of the world especially the areas receiving late season rains. During rainy season, the zoospores of the pathogen infect low hanging fruits and develop greyish brown, firm and leathery spots with characteristic pungent odour in the orchard. The infection of *Phytophthora* may remain incipient till harvest and manifest during post-harvest operations. *P. citrophthora, P. citricola, P. nicotianae (=P.parasitica)* and *P. syringae* are some species frequently attack citrus fruits (Boccas and Laville, 1978; Naqvi, 1988, 1999).

3.4 Melanose disease

The disease is caused by *Diaporthe citri* Wolf. (conidial state: *Phomopsis citri*) is most common in high rainfall areas. The pathogen is of minor importance in drier areas. *P. citri* also causes stem end rot but the infection on rind develops small, raised reddish brown to black spots that in severe form make a tearstain or mudcake pattern on fruit surface and deteriorate the market value fruit (Whiteside, 1980).

3.5 Citrus scab

The scab is caused by *Elsinoe fawcettii* Bitanc. & Jenkins, is widely prevalent in high summer rainfall areas of Northeast states of India infecting leaves and fruits of Khasi mandarin, Darjeeling mandarin and in Coorg infecting Coorg mandarin and other citrus cultivars. The disease also occurs in severe form in Punjab and Himachal Pradesh

infecting Kinnow mandarin. Similarly, the disease is prevalent in high rainfall areas of USA like Florida and Texas but does not occur in drier areas like California, Arizona and central India (Whiteside, 1975; Naqvi, 1999). The pathogen develops irregular corky protuberant outgrowth on leaves and fruits. The scabbed area does not increase after harvest but deteriorates the fruit quality and its market value considerably.

3.6 Bacterial canker

Bacterial canker of citrus caused by *Xanthomonas axonopodis* pv. *citri* is a serious problem of acid lime (*C. aurantifolia* Swingle) in India. Initially, the small, circular, slightly raised pustules develop on leaves and fruits, which later enlarge and become corky. Bacterial canker infection considerably reduces the yield and quality of fruits for fresh fruit market. Bacterial canker has been a limiting factor in profitable cultivation of acid lime in India. Recently the citrus canker pathogen and its impact has been reviewed comprehensively (Gottwald *et al.*, 2002).

4. Post-harvest diseases

The post harvest diseases of citrus develop by two types of infections:

(i). The diseases initiated due to pre-harvest infections and (ii). The diseases develop due to post-harvest infections.

The pre-harvest latent infection of *B. theobromae, C. gloeosporioides, A. citri* and *P. citri* become active after fruit harvest and cause severe losses whereas post-harvest infection caused by *Geotrichum candidum, P. digitatum, P. italicum* and *Aspergillus niger* through wound and/ or injuries inflicted during harvesting and handling of fruit. Insect damage to the fruit also facilitates invasion of these pathogens.

4.1 The post-harvest disease manifestation due to pre-harvest infection

4.1.1 Stem end rots

Stem end rots of citrus fruit caused by *B.theobromae* Pat. (*Physalospora rhodina* Berk. & Curt.) Cooke) and *Phomopsis citri* Fawcett, are the major post-harvest diseases in citrus producing area receiving substantial rainfall during development period of fruit. In summer rainfall areas like Gulf states of U.S.A., West Indies and Southeast Asia, the spores of these pathogens multiply on dead twigs of trees and splashed by rain onto developing fruits where they initiate incipient infection just beneath the calyces of fruit (Brown and McCornack, 1969, Eckert and Brown, 1986). The infection may take place at any time from fruit set to harvest but pathogen mostly remains quiescent in form of appressoria due to resistant nature of immature tissue of the fruit. After harvest, the latent infection resume its activity and cause stem end rot. Diplodia rot starts from neck to stylar-end with a characteristic wavy margins of browning symptoms (Fig 2). In case of *Alternaria* rot and *Phomopsis* rot, the browning advances in circular fashion around the stem end. *Alternaria* rot develops faster in cold storage and fungus grows

internally along the central core (Fig. 3) of Nagpur mandarin (Naqvi, 1996).

       Alternaria rot was observed as serious problem of lemons in California and Valencia orange in Florida for long-term storage (Harvey, 1946; Brown and McCornack, 1972). Frost injuries in groves predisposes grapefruit to *Alternaria* stem end rot during storage (Schiffmann-Nadel *et al.,* 1975).

4.1.2 Anthracnose

It is a major problem of decay in Robinson tangerine and other tangerine hybrids in Florida when harvested early and degreened (Smoot, 1977). Nagpur mandarin are also affected severely with the disease (Naqvi, 1996). The conidia of *C. gloeosporioides* (Penz) Sacc. are produced on dead twigs of the mother plant and dispersed by rain

Figure 2: Stem end rot of Nagpur mandarin caused by *Botryodiplodia theobromae*

splashes to developing fruits. These conidia germinate on fruit surface and remain quiescent till maturity of the fruit. Ethylene treatment and / or natural colour breakdown of fruit makes it susceptible for invasion of infection hyphae from the appressoria (Brown, 1977, 1978). The lesions developed on the fruit surface remain firm brown to brownish black and in long term storage, the affected rind eventually develops soft rot.

4.2 The diseases caused by post-harvest infection

The post harvest infections are caused by wound pathogens. The injuries during harvest

and subsequent handling of fruit facilitate invasion of these pathogens. It has been observed that severity of such infections are directly related to handling operations of the fruit The fruits handled less number of times from harvest to packing are relatively less prone to such infections (Naqvi and Dass, 1994; Rackham and Grierson, 1971). Insect damage, wind scarring and injuries due to mechanical harvesting predispose fruit to post-harvest infection.

4.2.1 Sour rot

Sour rot caused by *G. candidum* Link. is the major cause of decay of Nagpur mandarin. The pathogen is widely distributed in citrus soil (Eckert, 1959; Butler *et al.,* 1965) and invades through deep injuries. The infected portion of the fruit turns into a watery

Figure 3: Stem end and core rot of Nagpur mandarin caused by *Alternaria citri*

incoherent mass due to secretion of highly active macerating extracellular enzymes by the pathogen (Fig 4). The disease develops rapidly at ambient temperature (28–30ºC) and contaminates other fruits by releasing huge number of spores from infected watery mass. Fruit temperature lower than 10ºC suppresses the disease development. Sour rot is a major disease problem on lemons that are stored for longer period, on late season oranges, grapefruit and mandarins that are not adequately refrigerated (Eckert and Brown, 1986). One sour rot affected fruit can contaminate huge lot during post-harvest treatment and packing operation. In a contaminated lot, fungicidal treatments given to suppress the *Penicillium* rot leads to rapid development of sour rot (Morris, 1982).

4.2.2 *Penicillium* rot

Green mould (*P.digitatum* Sacc.) and blue mould (*P.italicum* Wehmer) are important post-harvest pathogens of Nagpur mandarin next to sour rot. However, green and blue mould rots occur in all citrus growing areas and often constitute the predominant type of decay (Gardner *et al.,* 1986). These pathogens are important post-harvest pathogens of Kinnow mandarin in Punjab State, California and other subtropical parts of the world. A soft water soaked area is developed at the infection site in both the diseases. Coloured spore mass developed at the centre of the lesion surrounded by broad band of white mycelial growth in green mould infection (Fig. 5) whereas white mycelial growth around the spore mass of blue mould is usually not more than 2 mm wide. Both the pathogens occur frequently but green mould grows faster at moderate temperature and contaminates

Figure 4: Sour rot of Nagpur mandarin caused by *Geotrichum candidum*

the fruit lot. The spores of green mould are unable to infect healthy uninjured adjacent fruits while blue mould develops nesting onto uninjured healthy fruits and may cause serious damage.

4.2.3 *Aspergillus* rot

Aspergillus rot is an important post-harvest disease of citrus fruits in India. *A. niger* V.Tieghem, invades through wound/injuries and causes soft rot. The pathogen infects lemon, lime (Babu *et al.,* 1983: Bhargava, 1972) sweet orange (Srivastava and Tandon, 1969) and mandarin (Naqvi,1992). The disease has been reported on citrus in Americas,

South Africa, Mediterranean countries and most particularly in India (Snowdon, 1990). Pre harvest infection may also occur if the fruit is injured by thorns, in case of acid lime, during windy weather. The infected area of the fruit turns pale yellow with characteristic halo leaving the shrinked intact cuticle on the macerated peel (Fig. 6). In advanced stage infected surface is covered with black mass of spores that disseminate to contaminate the whole lot. The losses due to this fungus were recorded 4 – 6 % in certain consignment of Nagpur mandarin sent from Nagpur to New Delhi during 1991-1992. Unlike the susceptibility of citrus fruit to *C.gloeosporioides* at colour break stage or during degreening by ethylene (Brown, 1975), Nagpur mandarin was found comparatively more susceptible to *Aspergillus* rot in green stage than colour break or orange coloured fruit (Naqvi, 1992). *A. flavus* Link ex Fr., *A. nivens* and *A. variecolor* have also been reported to cause post-harvest rot of sweet orange in India (Sharma *et al.,* 1981).

Figure 5: Green mould decay of Nagpur mandarin caused by *Penicillium digitatum*

4.3 Other post-harvest diseases of citrus

Other than the above major and widely distributed post-harvest diseases of citrus fruits, some post-harvest diseases of minor significance are reported either location specific or seasonal and often associated with other diseases.

4.3.1 *Fusarium* rot

The disease is caused by *Fusarium* spp. (*F.lunulosporum, F. moniliforme, F. oxys*porum and *F. solani*) and often associated with other diseases from South Africa, Israel and

India (Snowdon, 1990).

4.3.2 Grey mould rot

The disease is caused by *Botrytinia fuckeliana* (de Bary) Whetzel, conidial state: *Botrytis cinerea* Pers., usually found to cause rot of lemons and mandarins in USA, Russia, Spain, Kenya, Korea and Australia (Snowdon, 1990).

4.3.3 *Trichoderma* rot

T. viride Pers. Ex S.F. Gray, has been reported to cause losses in some citrus growing regions like South Africa, Israel, Russia, China and India. The rot is usually associated
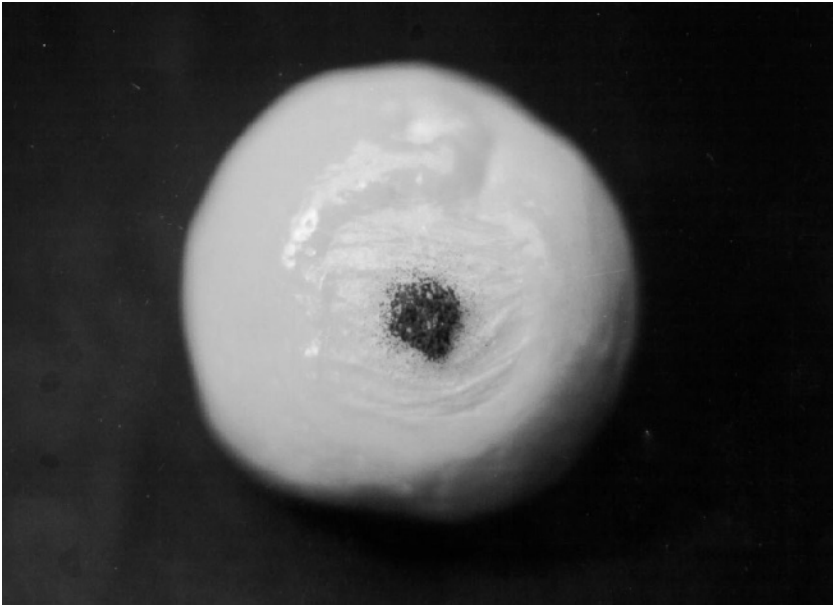
Figure 6: Soft rot of Nagpur mandarin caused by *Aspergillus niger*

with characteristic odour of coconuts (Fawcett and Weindling, 1934).

4.3.4 *Ceratocystis* rot

Ceratocystis rot caused by *C. fimbriata* Ell. & Halsted. and Pink mould rot caused by *Trichothecium roseum* have been reported from India causing decay of sweet orange (Cheema *et al.,* 1981; Singh and Chaudhary, 1974). *Pleospora* rot of Italian lemon caused by *P. herbarum* (Pers.) Rabenh has also been observed on mandarin and grapefruit (Snowdon, 1990).

5. Management of diseases of citrus fruits

The best and effective management strategies in control of citrus fruit diseases would be to avoid/ minimise the predisposing factors responsible for origin and development of the diseases through an integrated approach of disease management. These management strategies include timely use of effective fungicides, biocontrol agents, improved cultural practices, regular monitoring of the disease appearance & weather conditions, improved post-harvest handling, transport and storage conditions.

5.1 Management of pre-harvest diseases

The management of preharvest diseases of citrus fruits is most important aspect of citriculture since preharvest diseases cause losses in two ways i. The infection of fruits
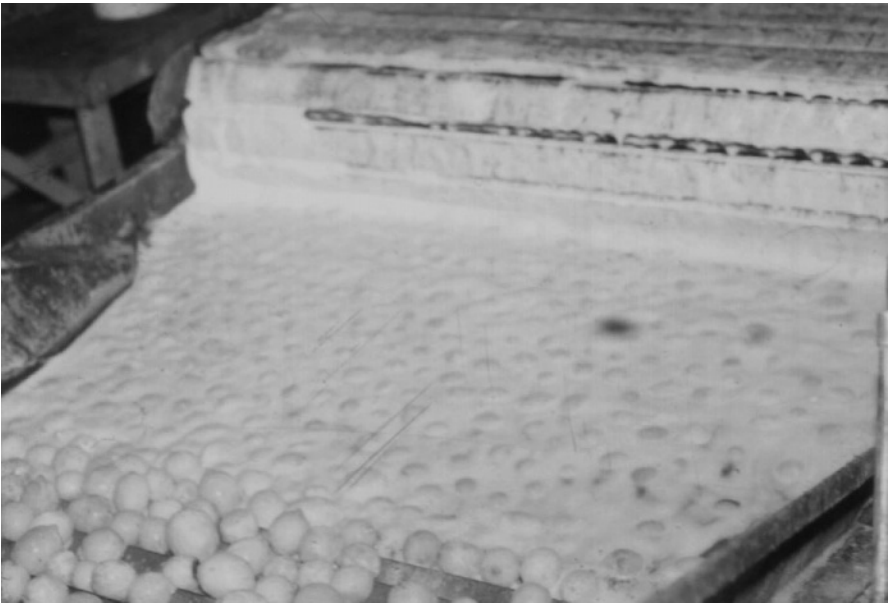
Figure 7: Washing of fruit with soad ash solution before post-harvest treaments

in groves directly affects the yield and quality of fruits and some preharvest infection manifest during post-harvest operations causing considerable post-harvest losses. Regular monitoring of fruit infection in the groves, monitoring of weather conditions conducive to disease development and timely use of effective chemical control measures in order to reduce preharvest inoculum/ infections and further contamination of fruits in the groves are the effective management strategies adopted world wide to manage the citrus fruit diseases.

The postbloom fruit drop disease may cause epidemic and complete crop failure if left unattended. A model developed at University of Florida correlating total rainfall

during the past five days and count of currently infected flowers in the grove provides effective prediction of disease development in coming four days (Timmer, 1995). The accurate information of predisposing factors makes the base of spray schedule. Properly timed applications of benzimidazole fungicides significantly control the disease and increase the fruit count. Increase in yield not only compensates the cost of applications but also substantially increases the profit.

   C. gloeosporioides, P.citri and *B. theobromae* are the major pathogens causing twig blight, anthracnose, flowers and fruit infections in mandarin orchards. The dried / dead twigs on the bearing plants act as the reservoir of inoculum for infection of developing fruits. The spores dispersed by rain splash and infect the fruits from fruit set to harvest stage leading to pre-harvest fruit drop and incipient pre-harvest infections (Brown and Albrigo, 1972; Eckert and Ogawa, 1985; Timmer, 1971 and Naqvi, 1993a,

Figure 8: Treatment with foam based wax and fungicide

1999). Pruning of dead twigs from citrus trees (Reichert and Hellinger, 1932, Naqvi, 1993a), and removing fallen fruit from the grove (Hough, 1970) have been recommended as a practice to reduce the inoculum load of preharvest pathogens. The inoculum load and preharvest infection of Nagpur mandarin can effectively be minimised by three preharvest applications of benzimidazole fungicides at 15 days interval prior to harvest. These pre-harvest sprays could reduce preharvest fruit drop of Nagpur mandarin to 9.94 % against 22.26 % in control (Naqvi, 1993a, 1993b) and also reduce > 70 % fruit decay after harvest up to three weeks of storage at ambient condition (Naqvi, 1997). The initial health of mother tree (dead wood percentage) plays an important role in pre-

harvest fruit drop, post harvest decay and efficacy of fungicides.

Melanose usually does not affect yields of citrus trees but deteriorates the market value of fresh fruits. Grapefruit is the most affected citrus cultivar followed by oranges, tangerines and tangelos. Pruning dead wood and spray of copper fungicides after petal fall provides effective control of melanose (Whiteside,1980) . Similarly treatments also provide some relief from the canker development on acid lime fruits. However, the control of scab infection on fruits requires frequent sprays of benomyl, thiophenate methyl and dithianon to prevent production of conidia on existing scab pustules on leaves especially in wet and highly humid weather conditions (Whiteside, 1981).

5.2 Management of post-harvest diseases
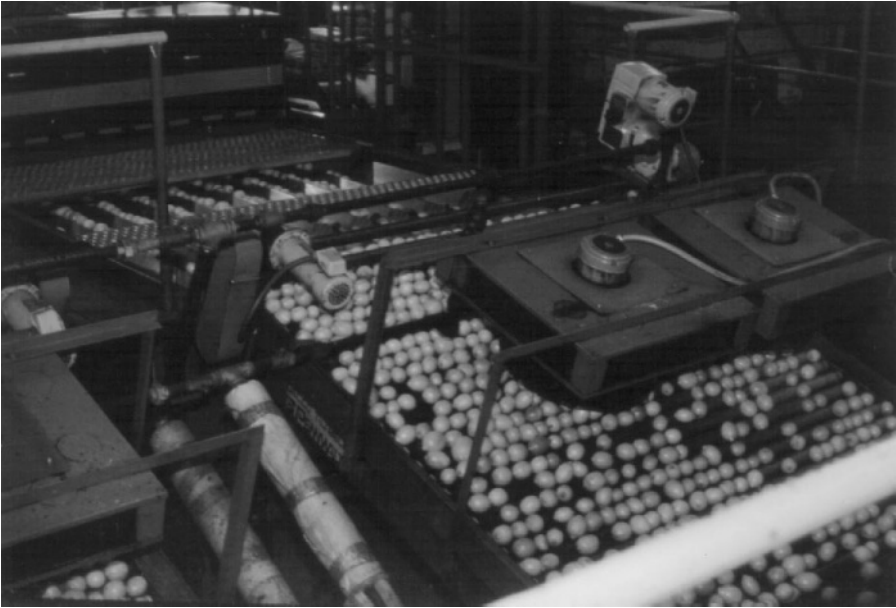
Figure 9: Drying of waxed coated fruit

The principal management strategies of post-harvest diseases of citrus fruits include inhibition of latent infections of stem end rot pathogens, inactivation of wound pathogens, protection of injuries/ wound sustained during post-harvest operations from fresh invasion of wound pathogens and to inhibit sporulation of highly sporulating pathogens like *Penicillium* to spread the diseases (Eckert and Ogawa, 1985). In spite of opposition to use chemicals in control of post-harvest diseases for their direct exposure to human health than the foliar pesticides, the post-harvest fungicides play an important role in minimising the post harvest losses and development of distant markets for citrus fruits. The incipient infection causing stem end rot can be minimised by preharvest

fungicide applications. The high post-harvest losses further can be minimised through avoiding / modifying the factors identified above for these losses. In our conditions, the most feasible and economical means are the pre and post-harvest applications of fungicides and these treatments are unavoidable even in advanced countries where refrigerated transport and storage facilities are available.

Three pre-harvest application of benzimidazole fungicides were effective in reducing > 70 % post-harvest decay of Nagpur mandarin. If such treated fruits were again given post-harvest fungicide treatment through washing, drenching and waxing either manually or on mechanical packing line, the losses up to 80 % can be curtailed for three weeks of storage/ transport/ marketing of this fruit at ambient conditions without any hazardous residual effect and quality deterioration. In other words, the losses from

Figure 10: Electronic grader for grading size, shape, colour and blemishes of fruit

18 – 24 % could be brought down to 3 – 4 % or even less when fruits were handled smoothly and packed in well cushioned adequately ventilated corrugated boxes. In refrigerated conditions, such treated fruits can be stored up to 40 – 45 days. The analysis of fruit surface residues of MBC after 24 hr of last preharvest spray of benzimidazole fungicides was 1.36 µg/g and in combination with post-harvest application less than 1 µg/g. Thus there was no significant increase in surface residue after combined treatments and the residues were with international permissible limit (10 ppm) for Citrus. Reduced post-harvest decay and enhanced shelf life fetch about 27 % additional income to the grower/ trader (Naqvi, 1993a, 1993b, 1993c, 1996, 1997). This technology can be used to exploit the export potential of the crop (Ladaniya and Naqvi, 1993, Ladaniya *et al.,* 1994).

The required steps of post-harvest treatments like washing (Fig. 7), clean water

rinse, uniform fungicide + wax application, drying, grading and packing are not feasible manually in commercial lots. The manual application would be not only cumbersome but there is always a risk of contamination and uneven application. These operations can be performed efficiently on mechanical packing line. After removal of trashes on conveyor belt, the fruits are washed within 1 – 2 days after harvest with soda ash (Sodium carbonate 4 – 6 %) to inactivate the fresh wound invasion and to reduce the spore load on fruits.

The soda ash washing cleans up to 70 % spore load of *Penicillium* from the fruit surface (Smilanick and Margosan, 1996). The fruits are further rinsed with clean water to remove the soda ash residues and excess water is removed with the help of spongy rollers. The cleaned fruits are further treated with wax + fungicides either in the form of foam (Fig. 8) or through spray. A uniform wax + fungicide coating on fruit surface can
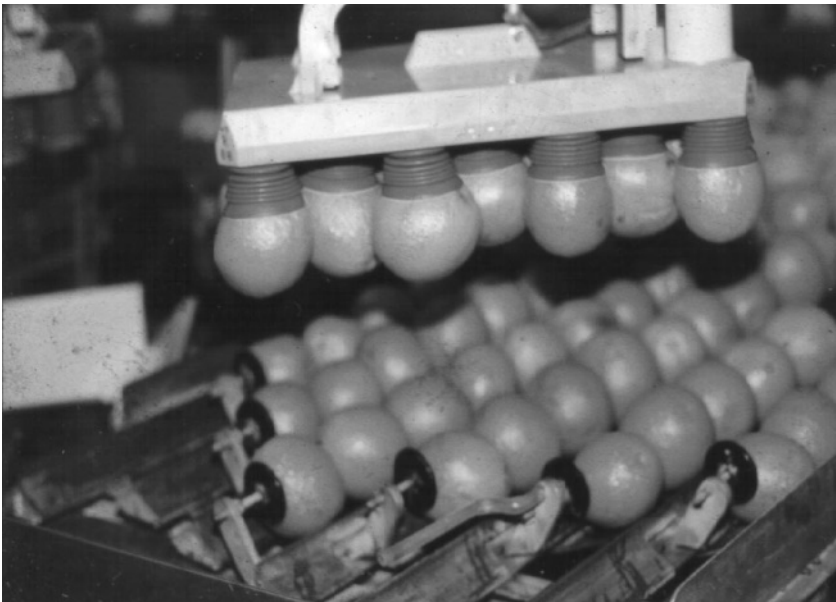
Figure 11: Mechanical auto-packing of treated fruits in boxes

easily be done through specially designed brushes on roller which helps in control of post-harvest infections and weight loss of fruits. Wax application also provides an attractive shine to the treated fruits. This coating is immediately dried in dried tunnel (Fig. 9) and then fruits are graded by mechanical or electronic graders(Fig. 10). The electronic grader can judge size, shape, colour and bruising on fruit surface. The graded fruits can be packed manually as per their grades or mechanically (Fig.11). The fruits are packed in telescopic corrugated boxes with the information about the produce printed on the boxes for distant transport / storage or marketing (Fig.12).

6. Vision for future trade of citrus fruits

The chief commercial citrus fruit grown in south-east Asia is mandarin orange – an excellent easy peeler table variety but mandarins are highly perishable in nature and more susceptible to diseases as compared to other citrus fruits. Mandarins produced in this region have no competition as table variety in domestic and export market but also requires extra management strategies. The traditional cultivation and marketing of mandarin in these countries without adequate management of pre and post harvest disease results in heavy post harvest losses in domestic market (Fig. 12) and unable to compete in export market. The adoption of available technologies in production and marketing of quality mandarin fruits is the only answer for successful and profitable trade of citrus fruits.

Figure 12: Packed treated fruit ready to transport

With the recent change in socio-economic climate and increased awareness about chemical hazards, more emphasis is being laid on chemical free and organically grown fruits and vegetable world over. Further European parliament has voted in favour of total ban on post-harvest treatment of fruits and vegetables with pesticides as soon as practice of replacement with some alternative becomes feasible (Wisniewski and Wilson, 1992). Few major importing countries have placed their conditions for such chemical treatments of citrus. The efforts are being made to develop non-chemical techniques, effective sanitation, physical therapies, improved handling operations to minimise injuries to fruits and integrated to use of biocontrol agents in control of fruit diseases. Some post-harvest bio-fungicides have already entered in commercial use in

U.S.A. Aspire – yeast-based bio-fungicides is being used in some packinghouses as a replacement of traditional fungicides (Marni Katz, 1996). The search for alternatives to fungicidal treatments is gaining momentum and recently efforts are being made to find out physical and non-toxic treatments in control of post-harvest fungal pathogens of citrus. Curing of oranges at 33°C for 65 hr significantly reduced the incidence of green mould when stored at 4°C up to two months (Plaza *et al.,* 2003), however this treatment was not effective in control of blue mould. Integrated control treatment of *Bacillus subtilis* isolate F1 along with sodium bicarbonate or treating the inoculated fruits with hot water at 45°C prior to application of *B. subtilis* isolates effectively controlled the development of *Penicillium digitatum* and *P. italicum* on Valencia and Shamouti orange (Obagwu and Korsten, 2003).

7. References

Babu, K.J., Laxminarayana, P. and Reddy, S.M. 1983. Evaluation of different volatile   compounds in the control of fruit-rot of lemon. Pesticides 17: 35,38.

Bhargava, S.N. 1972. *Aspergillus* rot on Citrus aurantifolia fruits in the market. Plant Disease Reporter 56: 64.

Boccas, B. and Lavelle, E.1978. *Les maladies a Phytophthora des agrumes*. Paris: Institut de Recherches sur les Fruits et agrumes, pp. 162.

Brown, G.E. 1975. Factors affecting post-harvest development of *Colletotrichum gloeosporioides* in citrus fruits. Phytopathology. 65: 404-409.

Brown, G.E. 1977. Ultrastructure of penetration of ethylene degreened Robinson tangerines by *Colletotrichum gloeosporioides*. Phytopathology. 67: 315-320.

Brown, G.E. 1978. Hypersensitive response of orange-colored Robinson tangerines by *Colletotrichum gloeosporioides* after ethylene treatment. Phytopathology. 68:700-706.

Brown, G.E. and Albrigo, L.G. 1972. Groove application of benomyl and its persistence in orange fruit. Phytopath. 62: 1434-1438.

Brown, G.E. and McCornack, A.A. 1969. Benlate, an experimental preharvest fungicide for control of post harvest citrus fruit decay. Proc. Fla. State Horic. Soc. 82: 39-43.

Brown, G.E. and McCornack, A.A. 1972. Decay caused by *Alternaria* citri in Florida citrus fruit. Plant Dis. Rep. 56: 909-912.

Butler, E.E., Webster, R.K. and Eckert, J.W. 1965. Taxonomy, pathogenicity and physiological properties of the fungus causing sour rot of citrus. Phytopathology. 55: 1262- 1268.

Cheema, S.S., Munshi, G.D. and Sharma, B.D. 1981. Laboratory evaluation of fungicides for the control of *Trichothecium roseum* Link. , a new fruit rot pathogen of sweet orange. Hindustan Antibiotics Bulletin. 23: 27-29.

Denham, T.G. 1979. Citrus production and premature fruit drop disease in Belize. PANS 25 (1): 30 – 36.

Eckert, J.W. 1959. Lemon sour rot. Calif. Citrogr. 45: 30-31,35-36.

Eckert, J.W. and Brown, G.E. 1986. Post harvest Citrus diseases and their control. In: Fresh Citrus fruits. AVI Publishing Co.pp. 315 – 360.

Eckert, J.W. and Ogawa, J.M. 1985. The chemical control of post harvest diseases: subtropical and tropical fruits. Annual Rev. Phytopathol. 23: 421-454.

Fagan, H.J. 1979. Postbloom fruit drop, a new disease of citrus associated with a form of *Colletotrichum gloeosporioides.* Ann. Appl. Biol. 91: 13-20.

FAOSTAT 2002. Data base results 2002, Food and Agricultural Organization of the United Nations, http;//fao.org.

Fawcett, H.S. and Weindling, H. 1934. Types of *Trichoderma* rot of lemons and oranges. Phytopathology. 24: 1144.

Gardner,P.D., Eckert, J.W., Baritelle, J.L. and Bancroft, M.N. 1986. Management strategies for control of *Penicillium* decay in lemon packing-houses: economic benefits. Crop Protection. 5: 26-32.

Gottwald, T.R., Graham, J.H., and Schubert, T.S. 2002. Citrus canker: The pathogen and its impact. Online. Plant Health Progress doi: 10.1094/PHP-2002-0812-01-RV.

Harvey, E.M. 1946. Changes in lemons during storage as affected by air circulation and ventilation. USDA Tech. Bull. 908.

Hough, A. 1970. Control of green mould by orchard sanitation. S. African Citrus J. 442: 11,13,15.

Ladaniya, M.S. and Naqvi, S.A.M.H. 1993. Postharvest handling of Nagpur mandarin. Indian Horticulture. 24-29.

Ladaniya, M.S., Naqvi, S.A.M.H. and Dass, H.C. 1994. Packing line operations and storage of Nagpur mandarin. Indian J. Hort. 51: 251-218.

Marni Katz. 1996. Bio-fungicides at Post-harvest. Citrograph. 3-5.

Morris, S.C. 1982. Synergism of *Geotrichum candidum* and *Penicillium digitatum* in infected citrus fruit. Phytopathology.72:1336-1339.

Naqvi, S.A.M.H. 1988. Prevalence of *Phytophthora* spp. pathogenic to citrus in orange groves of Vidarbha, Maharashtra. Indian J. Mycol. Pl.Path. 16(3): 274-276.

Naqvi, S.A.M.H. 1992. Chemical control of *Aspergillus* rot of Nagpur mandarin. Plant Dis. Res. 9(1): 95 – 97.

Naqvi, S.A.M.H. 1993a. Influence of pre and post-harvest factors on export oriented production of Nagpur mandarin. In: "Proc. Of Vasantrao Naik Memorial National Seminar on Agricultural Science - Export oriented horticultural Production", Nagpur, India. pp. 181-185.

Naqvi, S.A.M.H. 1993b. Pre harvest application of fungicides in Nagpur mandarin orchards to control post harvest storage decay. Indian Phytopath. 46: 190-193.

Naqvi, S.A.M.H. 1993c. Benzimidazole fungicides in control of post-harvest diseases of Nagpur mandarin. Plant Disease Research. 8: 19 – 24.

Naqvi, S.A.M.H. 1996. Managing post-harvest diseases of Nagpur mandarin. Indian Horticulture. 46-49.

Naqvi, S.A.M.H. 1997. Role of pre and post harvest applications of benzimidazole fungicides in control of post-harvest decay of Nagpur mandarin. Plant Disease Research. 12: 6 – 10.

Naqvi, S.A.M.H. 1999. Post-harvest losses and their management in Nagpur mandarin. Proc. nat. Symp. on citriculture. pp. 4481-486.

Naqvi, S.A.M.H. 1999. Integrated management of fungal diseases of citrus. In: "IPM system in Agriculture – Cash crops". (eds. Rajeev K.Upadhyay, K.G. Mukerji and O.P.Dubey), Aditya Books Pvt.Ltd. New Delhi, vol. 6, pp. 489-503.

Naqvi, S.A.M.H. and Dass, H.C. 1994. Assessment of post-harvest losses in Nagpur mandarin – A pathological perspective. Plant Disease Research. 9: 215-218.

Obagwu, Joseph and Korsten, L. 2003. Integrated control of citrus green and blue moulds using *Bacillus subtilis* in combination with sodium bicarbonate or hot water. Postharvest Biology and Technology.28: 187-194.

Plaza, P., Usall, J., Torres, R., Lamarca, N., Asensio, A. and Vinas, I. 2003. Control of green and blue mould by curing on oranges during ambient and cold storage. Postharvest Biology and Technology. 28: 195-198.

Rackham, R.L. and Grierson, W. 1971. Effect of mechanical harvesting on keeping quality of Florida citrus fruit for the fresh fruit market. HortScience. 6: 163-165.

Reichert, I. and Hellinger, E. 1932. Further experiments on the control of Diplodia stem end rot of citrus by pruning and spraying. Hadar. 5: 142-143.

Schiffmann-Nadel, M., Waks, J. and Chalutz. E. 1975. Frost injury predisposes grapefruit to

storage rots. Phytopathology. 65: 630.

Sharma, R.B., Monga, A., Roy, A.N. and Gupta, M.N. 1981. Pathophysiological studies on three new postharvest fruit rot diseases of *Citrus sinensis*. Indian J. Mycol. & Pl. Path. 11: 309-310.

Singh, A.K. and Basu Chaudhary, K.C. 1974. Ceratocystis soft rot of sweet orange. Curr. Sci. 43: 726-727.

Smilanick, J.L. and Margosan, D.A. 1996. Soda ash to control post-harvest green mold. Citrograph. 9,10,17.

Smoot, J.J. 1977. Factors affecting market diseases of Florida citrus fruits. In: Proc. Int. Soc. Citriculture. Pp. 250 – 254.

Snowdon, A.L. 1990. A colour atlas of Post-harvest diseases and disorders of fruits and vegetables. Vol. I. CRC Press, Inc. Florida. 54 – 90.

Srivastava, M.P. and Tandon, R.N. 1969. Some storage diseases of orange. Indian Phytopath. 22: 282-284.

Timmer, L.W. 1971. Effectiveness of pre harvest and post-harvest application of benomyl for control of post-harvest decay of oranges. Rio Grande Hort. Soc. J. 25: 26-30.

Timmer, L.W. 1995. Postbloom fruit drop : the model and guidelines for control. Citrus & Vegetable Magzine, 20 – 25.

Timmer, L. W., Agostini, J. P., Zitko, S. E. and Zulfiqar, M. 1994. Postbloom fruit drop, an increasingly prevalent disease of citrus in the Americas. Plant Dis. 78: 329-334.

Whiteside, J .O. 1980.Timing of fungicide spray treatments for citrus melanose control. Proc. Fla. State Hortic. Soc. 93: 21-24.

Whiteside, J. O. 1981. Evaluation of current methods for citrus scab control. Proc. Fla. State Hortic. Soc. 94: 5-8.

Whiteside, J.O. 1988. Timings of fungicide spray treatments for citrus melanose control. Proc. Fla. State Hortic. Soc. 93: 21-24.

Wisniewski, M.E. and Wilson, C.L. 1992. Biological control of post-harvest diseases of fruits and vegetable: Recent advances. HortScience. 27:94-98.

Preimmunization: Applications and Perspectives in Virus Disease control

Gerd W. Muller[1] and Jorge A. M. Rezende[2]

[1] *Centro de Citricultura Sylvio Moreira, Instituto Agronômico, 13490-970 Cordeirópolis, SP, Brazil.* [2] *Departamento de Entomologia, Fitopatologia e Zoologia Agrícola, ESALQ/USP, 13418-900 Piracicaba, SP, Brazil.*

Abstract: Preimmunization or widely also known as 'cross protection' is the technique used to control the damages caused by the most severe strain of virus or complex of the same virus or other virus or virus like diseases in the susceptible host. In preimmunization, the mild and rapidly multiplying strain of virus is used to protect the host. The technique has been widely used and demonstrated as an alternative for management of virus diseases in various crops. The chapter focuses on the pre-requisite conditions for using this technique, mandatory steps involved in employing the preimmunization programme, like various techniques available or being employed in obtaining the mild strain, examples of preimmunization in various crops/ viruses and the experiences in various countries in employing this technique in order to protect the susceptible crops from devastating virus diseases. The success stories of preimmunization programme adopted in various parts of world in order to protect various crops or its failure or breakdown of immunization have also been discussed in this chapter, besides the risks and advantages of this technology.

1. Introduction

Preimmunization (cross protection), the activity of a virus in a plant preventing the expression of a subsequent challenge virus (Dodds, 1982), was first reported by Wingard (1928), for *Tobacco ringspot virus* and McKinney (1929), for *Tobacco mosaic virus*. Although cross protection is the universal name, this phenomenon has also been called acquired immunity, acquired tolerance, cross immunization, mutual antagonism and premunity (Bennett, 1953). However, the term preimmunization has been widely adopted by several investigators in Brazil, Australia, South Africa and Japan.

Utilization of preimmunization as an alternative for virus disease control was first proposed by Salaman (1937) and Johnson (1937), on two independent works. Yet, more than sixty years later, preimmunization has been experimentally demonstrated for several viruses, but it has been used for controlling only a few plant virus diseases in the field.

Preimmunization has not only been observed to occur between strains of the same virus, Koizume and Sasaki (1980) reported protection against *Citrus tristeza virus*

(CTV) from *Citrus vein enation virus* (CVEV). It was also reported to occur between viroids (Niblett *et al.*, 1978). Satellite RNA *Cucumber mosaic virus* (CMV), that ameliorate symptoms caused by the virus, can also be used for preimmunization (Tien *et al.*, 1987). As any significant progress has not been achieved in the last 10 years on the mechanisms of preimmunization, information on this subject can be obtained in the reviews by Fulton (1986), Sherwood (1987) and Urban *et al.*, (1989).

To a better understanding of the studies on mild strain protection, the necessity exists to mention the meanings of some words normally employed in the literature in relation to this phenomenon. It is also necessary to standardize the use of some of them. The word isolate refers to a virus that was obtained from an infected plant, being in some cases associated to a given region. An isolate of a virus species might be defined simple as a collection of strains with similar structural and biological properties. For the purpose of preimmunization a strain is selected based mainly on biological properties, such as absence of symptoms on infected host, negligible damage to plant development and yield and protection against infection with severe strains of the virus. It is also important to define that the inoculation of the mild strain of the virus in the plant is called as protective inoculation or preimmunization. That of the second strain, which is called challenger, is denominated challenging inoculation. A break down in protection occurs when the plant initially infected with the protective strain is superinfected and may exhibit symptoms of the second strain used as challenger.

2. Pre-requirements for the employment of preimmunization

The diseases induced by virus, like those caused by other pathogens, are the result of an interaction of the host-pathogen and the environment. Furthermore, in the case of plant viruses, the vectors also perform a role of great relevancy in the disease epidemiology. Therefore, the decision about the most adequate manner to investigate the problem sighting at the development of control measures for a given virus needs to be evaluated with enough criteria, in such a manner that the application of the method reaches the objectives in a satisfactory and lasting form. The same care has to be taken in account in the case the decision to study the viability of the preimmunization technique. According to Posnette and Todd (1955), the utilization of mild strains to protect the plants against the normal complex shall only be implemented when the following pre-requirements can be fulfilled: (a). the disease is endemic and, due to any reason, political or economical, impossible to be eradicated; (b). the disease disseminates rapidly, putting in risk the new plantings, even when the older plantings that are infected have already been eliminated; (c). the losses with the disease are too big that even a small reduction caused by the infection with the mild strains is the preferable alternative and (d). as *sine qua non* condition, enough evidence exists that the mild strain will efficiently protect the preimmunized plants, without inducing great losses itself. Besides these, Fletcher (1978) adds other pre-requisite conditions that shall be considered in a later step of commercial implementation of the method. They are: (a). the mild strain shall be easily transmitted, in such a manner that large-scale inoculations are efficient and of low cost, (b). there shall be availability of enough amount of the mild strain inoculum (c). the mild strain shall be easily purified and stored in the case in which great

number of plants of annual crops shall be inoculated, (d). if the crop is vegetatively propagated, a great number of mother plants already infected with the mild strain shall be available to the growers, and (e). in the case in which the preimmunization inoculation is carried out by means of aerosol under pressure, this should not offer risk to the workman. Furthermore, there are some traits, which a mild strain should have to be a candidate for preimmunization: i). the strain has to be mild in all cultivar combinations it is thought for, ii). it should be stable and not prone to change or mutation, iii). the strain must be well distributed in all parts of the plant and must move quickly through new growth, iv). the mild strain should be easily graft transmissible for plants propagated on this way, and v). subinoculations from the original mild strain shall remain stable.

3. Steps for a preimmunization program

A research program that aims the establishment of preimmunization as control measure of phytoviruses may be divided into five linked steps: (a). selection of mild strains, (b). assay of the protective value of the mild strains under greenhouse conditions, (c). assay of the protective value through field pilot experiments; (d). evaluation of the stability of the mild strains and its effect upon the development of the plants, yield and quality of the product, and (e). integration of the preimmunization technique into the system of crop management. All these steps obey logical sequence and each step should be carefully evaluated before the investigation of the next step.

The selection of stable mild strains is the crucial step, and apparently the most difficult, in such a program. The concept of mild virus strain is relative and shall be evaluated for each species and/or variety to be preimmunized. Müller and Costa (1977) and Costa and Müller (1980) verified that plants of Galego (Mexican) lime (*Citrus aurantifolia* (Christm.) Swing) inoculated with mild strains of CTV collected from Pera sweet orange (*C. sinensis* Osb.) showed a more severe reaction than when inoculated with mild strains obtained from Galego lime. In the same manner, the best mild strains for Pera sweet orange were collected in plants of this variety. The same specificity was reported for mild strains of this virus evaluated in a preimmunization program (Van Vuuren *et al.*, 1993). Furthermore, the use of the term "mild" as found in CTV literature may be misleading. For example, the mild Nartia strain until recently used universally for preimmunization in South Africa causes severe seedling yellows, decline on Sour orange (*C. aurantium* L.) rootstock and moderate stem pitting in grapefruit (*C. paradisi* Macf.). Nartia, which is a "mild strain" in South Africa, is more severe than any strain yet found in Florida, USA. Mild CTV strains selected in Florida have proven to be the mildest upon comparison with other mild strains from other countries (Lee *et al.*, 1995). The same judgement is applied for the Pera mild strain used extensively in São Paulo, Brazil. Yeh and Gonsalves (1994) also related that the severity of the ringspot symptoms in fruits of papaya inoculated with a mild strain of *Papaya ringspot virus – type P* (PRSV-P) varied in function of the inoculated variety. In the case of two mild strains of *Papaya ringspot virus – type W* (PRSV-W), obtained from blisters on mosaic leaves of Zucchini squash (*Cucurbita pepo* L. cv. Caserta), no variation of symptoms was observed when they were inoculated in other species of cucurbitaceae (Rezende *et al.*, 1999, Dias and Rezende, 2000, 2001).

The selection of mild strains can be carried out by means of different methods, being that some of them may be used singly or in combinations with the aim of increasing the chance of obtaining the desired strains. Some of these methods are presented below:

3.1 Selection of outstanding plants in severely affected crop

Outstanding plants found in crops where most of the individuals are severely affected by a virus are, in some instances, infected with mild strains or strain complexes that are naturally protecting them against the continuous challenge of the normal severe complex existing in the rest of the planting. The fact that the mild strains present in such outstanding plants are apparently protecting them for a long time, suggests that those strains have great potential to be used almost immediately in preimmunization works. Although some authors consider this process empiric, due to the lack of knowledge on the mild strain, the search for outstanding plants in the condition described above, shall be the preferred process for the beginning of a preimmunization program.

According to Müller and Costa (1987), results of work carried out in Brasil, South Africa, Australia, Japan and Reunion Island indicated that this was the best manner to find mild strains of CTV, for the use in the control by preimmunization. Other examples in which the search for outstanding plants gave satisfactory results in the obtention of mild strains of protective value can be found in the works carried out with the *Cocoa swollen shoot virus* (CSSV) in the Gold Coast (presently Ghana) (Posnette and Todd, 1955), with the *Passionfruit woodiness virus* (PWV), in Australia (Simmonds, 1959), with the *Apple mosaic virus* (ApMV) in England and New Zealand (Posnette and Cropley, 1956; Chamberlain *et al.,* 1964), with the PRSV-P, in Brazil (Rezende *et al.*, 1981) and with the *Vanilla necrosis virus* (VanMV), in Tonga (Liefting *et al.,* 1992).

3.2 Selection of mild strains from atypical areas on leaves with severe systemic symptoms

Plants systemically infected with a virus frequently show atypical areas of tissue that may contain strains different from the predominating one of the normal complex. These areas may be different parts of a mosaic leaf, as for example, the dark green islands that occur in leaves of several plants infected with virus inducing mosaic, or simple small necrotic or chlorotic spots in systemically infected leaves. It can be also the blisters that appear in virus in mosaic leaves of some species, which are elevations of dark green color in pronounced contrast with the surrounding region, which presents itself yellow green.

The possibility of selecting mild strains from inoculum extracted from blisters on mosaic leaves was initially tested with success by Rezende *et al.,* (1982b), for PRSV-P on infected papaya plants. Later, Rezende *et al.,* (1992) also succeeded using this technique for the obtention of mild strains of PRSV-W from infected Zucchini squash plants.

3.3 Selection of mild or attenuated strains from plants submitted to thermal treatments

Johnson (1926) seems to be the first to demonstrate the possibility of selecting mild attenuated strains of a virus, submitting plants infected with TMV to a temperature of approximately 35º C, during 15 days. The same technique was also used by Kunkel (1934), for the obtention of mild strains of TMV, and by Oshima *et al.,* (1965), cited by Oshima (1981), for the selection of mild strains of *Tomato mosaic virus* (ToMV), in Japan. The obtention of heat attenuated strains also gave positive results in the works of Desjardins *et al.*, (1959) and Müller and Costa (1973), with CTV.

Low temperatures may also be utilized for the isolation of mild strains of virus. Kosaka and Fukunishi (1997) reported the obtention of mild strains of *Soybean mosaic virus* after exposing plants infected with the normal complex to temperature of 15ºC, for 14 or 30 days.

3.4 Selection of strains by filtration (passage) through alternative hosts

When a virus maintained in an apparently stable state in a host species is inoculated into another species and subsequently returns to the original host, a change of the dominating strain of the complex may occur. A possible explanation for this fact would be that certain strains could replicate and move more rapidly in certain species and prevent the establishment of other strains of the complex. The possibility also exists that the alternative species is only susceptible to some strains of the viral complex.

An old example of selection of an attenuated virus strain by means of an alternative host was obtained by the passage of Beet *curly - top virus* through plants of (*Chenopodium murale* L.) (Carsner and Stahl, 1924), which are resistant to this virus. Johnson (1947) verified that the passage of a severe type of TMV through *Eryngium aquaticum* L. resulted in a strain that caused mild symptoms in tobacco. He concluded that this plant worked as a type of "filter", since only the mild strain moved systemically in the plant. The severe strain remained restricted to the inoculated leaf. More recently, Yeh and Cheng (1989) also reported the obtention of mild strains of PRSV-P by means of passage of the normal complex treated with nitrous acid, through plants of *Cucumis metuliferus* (Naud) Mey. PI 292190. This specie is a source of resistance for the normal complex of this virus, however showed to be susceptible to infection with mild strains of the same virus. Roistacher and Bar-Joseph (1987) selected two mild strains of CTV (Code 37 and 40) by passage of severe isolates of the virus through *Passiflora* species and back to citrus by Aphis *gossypii.* Both have remained mild and protective ever since (Bederski and Roistacher, 2001).

A method a little more practical for the selection of mild strains is through the utilization of hosts that react with local lesions on leaves mechanically inoculated. It is an well-accepted fact that local lesions caused by the majority of the virus are result of the infection started by a single virus particle (Matthews, 1991). In this way, when the inoculum extracted from several lesions are transferred individually to plants that react with systemic symptoms, a segregation of the normal complex of the virus may occur. This segregation might originate strains that cause the most variable symptoms, in-

cluding those that induce mild symptoms in the plants. It is believed that the mild strains obtained in this manner are pure, as compared with those selected from outstanding plants or from atypical leaf areas with systemic symptoms.

This technique is very much used for the selection of mild strains, specially in the cases in which the normal complex of the virus is submitted to some kind of pretreatment to promote the appearance of mild forms. The selection of attenuated strains of some virus, through thermal treatment discussed previously, requires a local lesion host to promote the segregation of the viral complex. The same happens when the normal complex of the virus is submitted to a previously treatment with a mutagenic agent, as it will be described under 3.6.

3.5 Selection of mild strains by means of vectors

As broached previously, one of the manners of obtaining mild strains is through the search of outstanding plants in the field. In the case of virus transmitted by vectors, one could say that the natural infection of outstanding plants, carriers of mild strains, has been achieved through the feeding of such virus carrying agents. Therefore, it is perfectly normal to imagine that such segregation of the normal complex of the virus by the vector can also be reproduced under experimental conditions, with the aim of selecting mild strains.

Müller and Costa (1973) suggested that the obtention of mild strains of CTV could be achieved through the selection of outstanding plants of young nucellar progenies of CTV sensitive varieties, submitted to three successive inoculations with populations of the vector. In this case, the first inoculation would be carried out with a reduced number of vectors (5-10/plant) to provoke the segregation of mild and severe strains. For later inoculations, carried out after a period of time enough for the systemic infection of the plants, a bigger number of vectors would be utilized and they would serve to test the protective effect provided by any mild strain already present in some plants.

Matthews (1991) pointed out three other ways of trying to obtain mild strains by means of vectors: (a). viruliferous vectors submitted to a short feeding periods on the test-plants, only one strains out of a mixture may be transmitted, (b). transmission through different vector species, when the virus is transmitted by more than one species; and (c). inoculation of the vector with diluted inoculum, followed by repeated process of selection of plants infected with the desired type of symptoms.

3.6 Selection of mild strains through the use of mutagenic agents

The utilization of mutagenic agents to induce the appearance of mild strains, starting with the treatment of the normal complex of the virus, is an alternative that should only be recommended when the previous methods have been exhaustively tested without satisfactory results.

The most commonly employed mutagenic agent for the induction of mild strains is the nitrous acid, which causes the de-amination of the nucleic acid bases of the virus. Examples of induction of mild strains by means of nitrous acid treatment can be found

in the works of Rast (1972), with ToMV, Tomlinson and Sheperd (1978), with *Cauliflower mosaic virus* (CaMV), Yeh and Gonsalves (1984), with PRSV-P, Wang and Gonsalves (1992), with *Tomato spotted wilt virus*, among others. According to Yeh and Gonsalves (1994), the use of nitrous acid for the induction of mild strains of a virus is a laborious and tedious procedure and that sometimes depends on luck to achieve the desired objective.

3.7 Construction of attenuated strains through the technique of DNA recombination

The obtention of infectious RNA starting from transcription of complementary DNA clones (cDNA) of plant virus constituted of RNA was already reported for some virus (Dawson *et al.*, 1986; Heaton *et al.*, 1989). The possibility of induction of mutations in pre-determined sites in the cDNA has allowed the study of the function of the plant genes (French and Ahlquist, 1988; Atreya *et al.*, 1990; Gal-On, 2000). A TMV strain that induces mild symptoms in tobacco plants was molecularly cloned and, through the exchange of nucleic acid fragments deriving from the cDNA of the severe strain (U1), it turned possible to localize the region of the viral genome responsible for the attenuation of the symptoms (Holt *et al.*, 1990). The results showed a new approach for the obtention of mild strains of virus, through recombination, deletions or insertions of bases that can be carried out in the nucleic acid, in such a manner to promote exchanges in the severity of the symptoms induced by the virus. According to Yeh and Gonsalves (1994), the utilization of the technique of recombinant DNA might allow the obtention of pre-determined mild strains, which can have greater chance to be stable for preimmunization.

3.8 Evaluation of the protective effect of a virus

After selection and perfect characterization of the mild strains of a virus, the necessity exists to carry out preliminary tests to evaluate the protective effect against the normal complex of the virus. In these tests, the plants are initially inoculated with the mild strains and, at pre-determined intervals of time, sufficient to allow a systemic invasion of the mild strains, they are challenged with strains inducing severe symptoms. The use of repetitions and appropriate checks are essential in these evaluations. The challenge inoculation or superinoculation may be carried out mechanically, by means of tissue union (grafting) or by viruliferous vectors. Tissue union is considered a drastic challenge, since the receptor plant infected with the mild strain stays continuously exposed to the severe virus strain produced in the grafted tissue. The basic objective of this type of challenge inoculation is to localize mild strains that will protect under high inoculum pressure. The fact that no protection may occur in the cases in which the challenge is done by means of grafting, or even by means of mechanical transmission, does not invalidate the continuity of the investigations, since the decisive test always will be that closest to the way the virus is transmitted in nature. Exception occurs for the virus that is transmitted in nature only through mechanical processes.

Once the protective effect is evaluated under screenhouse conditions, one pro-

ceeds to the execution of pilot experiments in the field. In these experiments, a certain number of initially healthy plants and plants inoculated with the mild strains are exposed to the infection by the strains of the normal complex present in the region. This test has the objective to evaluate the effectiveness of preimmunization for protecting plants against the effect of the virus in the field. At the same time in which the evaluation of the protective effect of the mild strains is carried out, observation about the stability of the induced symptoms, as well as its effect on plant development and yield (weight and quality) shall also be observed. For the pilot tests the plants are generally prepared under screenhouse to avoid undesirable infections, and afterwards are transplanted preferably to areas where high incidence of the virus and adequate populations of vectors exist. In the same manner as in the protection tests under screenhouse conditions, these experiments shall have appropriate repetitions and controls. Also they shall be carried out the closest possible to the manner as the species is commercially cultivated by the growers. Whenever possible, they shall be carried out taking advantage of commercial plantings, managed by the growers, where the researcher may evaluate only the effectiveness of preimmunization.

Once the practical and economical viability of preimmunization is established as adequate and safe for control of the virus disease, adequate forms of multiplication and maintenance of the mild strains inoculum shall be studied, as well as efficient and economical methods for mass inoculation of plants, in such a way that the demand for this technology can be satisfactorily attended. For the case of stable virus, which are easily transmitted mechanically, the mild strains can be preserved for long periods in infected and dehydrated tissue, at $-20°$ C. Whenever necessary, the inoculum prepared with infected dehydrated tissue is inoculated in a great number of plants to increase the preimmunizing strain for further mass inoculation of seedlings. In the case of virus that are not easily transmitted by mechanical means or not transmitted by this procedure at all, the necessity exists, to preserve the mild strains in plants kept in places protected against contamination. Whenever there is necessity for a great amount of inoculum, the original source of mild strain is graft inoculated in adequate hosts for multiplication and posterior use as source of inoculum for the production of preimmunized nursery plants.

The inoculation of preimmunization may be carried out by two manners: for plants propagated through seeds and that will be mechanically inoculated with a mild strain, the inoculation is generally carried out a few days after germination. When a great number of plants will be preimmunized, it is recommended the application of the inoculum, mixed with a abrasive, through pulverization under air pressure, as described by Rast (1975), Yeh *et al.*, (1988), Rezende and Pacheco (1998) and Yarden *et al.*, (2000). In the case of vegetatively propagated plants, initially mild strains are inoculated in a few plants knowingly healthy, which will serve as mother plants. These will be utilized as inoculum source of the mild strain or multiplied directly for the production of the preimmunized nursery plants

4. Examples of preimmunization

4.1 *Cocoa swollen shoot virus*

The pioneer example on the efficiency of preimmunization with mild strains for the

control of virus disease was reported by Crowdy and Posnette (1947) and Posnette and Todd (1955), in Africa, in experiments for the control of cocoa swollen shoot. This disease is caused by a species of genus *Badnavirus*, transmitted by a mealybug and it is considered the most important disease affecting cocoa (*Theobroma cacao* L.) production in the African continent. In Ghana, yield loss caused by this virus is very high and all attempts for control of this disease have been done mainly by systematic eradication of diseased trees. There is estimation that, from 1946 to 1988, more than 190 million infected trees were cut off, and other 10 million were already infected in 1990 (Ollennu and Owusu, 1989; Hughes and Ollennu, 1994).

Posnette and Todd (1955) selected mild strains of *Cocoa swollen shoot virus* (CSSV) from outstanding trees present in severely affected crops and showed that plants infected with those mild strains became protected against infection with common strains of the virus. Over a 5-year period of study, 76% of unprotected trees developed severe symptoms as a result of natural spread from infected guard rows, compared with only 14% of mild strain protected trees.

In spite of this experimental success, the program for cocoa preimmunization was interrupted for several years, for unknown reasons. In late 80's, however, due to the magnitude of the problem caused by CSSV, especially in Ghana, studies on mild strain protection were reconsidered. Although some mild strains have already been selected, practical application of preimmunization on cocoa crops is still dependent on better protective mild strain and efficient procedure for mass inoculation of cocoa seedlings (Ollennu and Owusu, 1989; Hughes and Ollennu, 1994). Presently, no preimmunized cocoa plant is commercially grown in Ghana (L.A.A. Ollennu, personal information).

4.2. *Passion fruit woodiness virus*

The second pioneer work on the efficiency of preimmunization for plant virus disease control was developed by Simmonds (1959), in Australia, for the control *of Passion fruit woodiness virus* (PWV). PWV is a species of the genus *Potyvirus*, transmitted efficiently by several species of aphids in a non-persistent manner. Simmonds (1959) showed that mild strains of PWV protected passionflower plants against infection with more severe strains under field conditions, during a period of 3 years. Vines carrying a mild strain of the virus showed a marked superiority over those carrying a severe strain in number, weight and size of fruit free from woody symptoms.

In the 1970's, an accreditation scheme based on mild strain protection was established in New South Wales, Australia, to prevent losses on commercial passionfruit hybrid (*Passiflora edulis/P. edulis* f. *flavicarpa* Deg) due to severe strains of the virus (Peasley and Fitzell, 1981; Pares *et al.*, 1985). In spite of the apparent success of the program, problems associated with the occurrence of a more severe strain of PWV, able do overcome mild strain protection and an apparent synergistic effect with Cucumber *mosaic virus* (CMV) infection, which caused tip necrosis disease were reported (Peasley and Fitzell, 1981; Pares *et al.*, 1985; Fitzell and Pares, 1985).

4.3 *Tomato mosaic virus*

Tomato mosaic virus (ToMV), a species of the genus *Tobamovirus*, causes very severe

disease on several Solanaceous crops, including tomato (*Lycopersicon esculentum* Mill.). The virus is present on tomato seeds and infection of seedlings occurs during transplanting. Because of the persistent and infectious nature of ToMV, the virus is readily transmitted by human activities, such as plant tying, removal of plant suckers and fruit harvest. Broadbent (1964) proposed the use of preimmunization for control of ToMV on tomato.

Although several attempts were made in order to select mild strains for protecting tomato plants (Fletcher, 1978), wide use of this technology in Europe was only possible after selection of mild strain MII-16 by Rast (1972, 1975) in the Netherlands. MII-16 was obtained by nitrous acid mutagenesis. Average yield per square meter of protected tomato in the Netherland increased from 9.0 kg in 1971, to 10.8 kg in 1972 and to 11.3 kg in 1973 (Rast, 1975).

MII-16 was introduced to England and protected tomato plants showed an average increase on yield of 6 to 9% during 1971 to 1973. In France, the same mild strain was commercially used from 1972 to 1983, providing high degree of protection against severe strains of ToMV. More than 13 million tomato plants were protected with MII-16 in 1981. However, the risk of synergism with other viruses, specially CMV, and other drawbacks, limited the use of mild strain protection for controlling ToMV on tomato crop in France (Lecoq, 1998). Preimmunization against ToMV has also been used in Canada (Hiruki, 1979), Japan (Oshima, 1975) and California, U.S.A. (Ahoonmanesh and Shalla, 1981).

This technology was very efficient and popular for the control of ToMV on tomato in several European countries in the 1970s, but it was discontinued due to development of resistant cultivars of good agronomic quality. However, as stated by Channon *et al.,* (1978), mild strain protection may still be useful for the tomato industry in the future, if strains able to overcome resistant genes evolve from the virus population.

4.4 *Citrus tristeza virus*

Citrus varieties were cultivated for almost two millennia on their own roots in the Near East and the Mediterranean basin. In the nineteenth century, an epidemic root rot caused by *Phytophthora* spp. destroyed the sweet orange (*Citrus sinensis* Osb.) trees and forced the adaptation of the Phytophthora-tolerant Sour orange as a rootstock (Bar-Joseph et al., 1989, Klotz, 1978). Attempts to use the Sour orange rootstock in Australia, Java and South Africa were unsuccessful. The failure was due to the presence of *Citrus tristeza virus* - CTV (Bennett and Costa, 1949) an aphid-borne species of the genus *Closterovirus* (Meneghini, 1946; Bar-Joseph *et al.*, 1979; Van Regenmortel *et al.*, 2000), to whom the Sour orange proved to be intolerant. From 1890 onward, citriculture expanded worldwide and large quantities of citrus plants were shipped from areas where CTV was endemic to CTV-free areas (Tanaka, 1932). CTV was introduced along with its most efficient vector, the brown citrus aphid *Toxoptera citricidus* Kirk. from South Africa into the Atlantic coast of South America early last century. During the 1930´s and 40´s, it practically wiped out the citrus industries of Argentine, Brazil and Uruguay soon followed in the 50´s by Colombia and Peru, which were based on Sour

orange rootstock. As a result some 20,000,000 trees were destroyed (Bennett and Costa, 1949; Costa 1956; Segura, 1952). In the beginning of the 1980´s another similar disaster occurred, this time in Venezuela where some 6,000,000 trees on Sour orange rootstock perished (Mendt, 1992). Presently, in Jamaica all the orchards on Sour orange are being devastated. (Roistacher, 1999).

The fortunate fact that certain rootstocks could be used to grow citrus successfully in the presence of CTV allowed the replanting and rehabilitation of citrus industries. New rootstocks were tested and found to be tolerant to the bud union phloem necrosis induced by the virus, and the Sour orange was replaced as the primary rootstock where tristeza epidemics had occurred. However, there are strains of CTV, which can attack the scion directly, regardless of the rootstock. In spite of not killing the stionic combination, these strains may induce stunting of the tree, severe stem pitting in trunks and branches in limes (*C. aurantifolia* Christm. (Swingle), grapefruits (*C. paradisi* Macf.), sweet oranges and other sensitive citrus types resulting in the production of small fruit. This sequence of events may take 15-25 years to reach completion (Müller, 1980). Since the scions and even rootstocks may be severely pitted, changing to a new rootstock has little or no effect.

One possible control method for the losses induced by tristeza on the above mentioned citrus types is the preimmunization of virus-free clones with mild strains or complexes that would protect the plants against further infection with the severe stem-pitting complexes, naturally disseminated by aphids, mainly *T. citricidus.* Protection between components of the tristeza virus complex has been initially reported from several sources (Costa *et al.*, 1954, Giacometti and Araujo, 1965, Grant and Costa, 1951, Grant and Higgins, 1957, Olson, 1958 and Stubbs, 1964). Following are experiences attained by some countries.

4.4.1 Australia

In the early 1940s, stem pitting disease threatened to put an end to grapefruit production in coastal new South Wales (NSW), Australia. However, vigorous and productive trees commonly occurred in orchards where most trees were in various stages of decline. These vigorous and apparently healthy trees were not free of tristeza and mild reactions were produced when they were indexed on West Indian lime seedlings. Field trials demonstrated that some of these mild strains, when grafted into virus-free Marsh grapefruit could protect against the establishment of severe stem pitting strains vectored by brown citrus aphids. The degree of protection varied with the CTV strain, aphid activity and climatic conditions at the trial sites (Cox *et al.*, 1976, Fraser *et al.*, 1968). Three strains were selected as having a high degree of protection.

The mild CTV strain that has protected grapefruit trees in the Australian Citrus Budwood Scheme against stem pitting in different climatic conditions for the last 30 years, has been characterized using restriction analysis of the coat protein gene amplified by the polymerize chain reaction. The sequences of the coat protein genes of restriction fragment length polymorphism (RFLP) group 5 were very similar (Broadbent *et al.*, 1991, 1995 and 1996).

4.4.2 Brazil

Brazil, the world largest citrus juice exporter, bases its citrus industry mostly on the 'Pera' sweet orange (*C. sinensis* Osb.), a late season variety. In the end of the 50's, many orchards of this most important variety, already growing on tolerant rootstocks, were badly damaged by CTV, showing stem pitting, small fruits and dwarfing. The situation became so bad that it was even advised to replace this variety by others more tolerant to the disease.

Previous work carried out had established the existence of mild CTV strains with protective effect. Based on this, a 5-years cooperative research project funded by US Public Law 4-80 started in 1961, aiming to control CTV injury to commercial citrus types by preimmunization. Scions of ´Pera´ sweet orange, ´Galego´(Key) limes and grape-
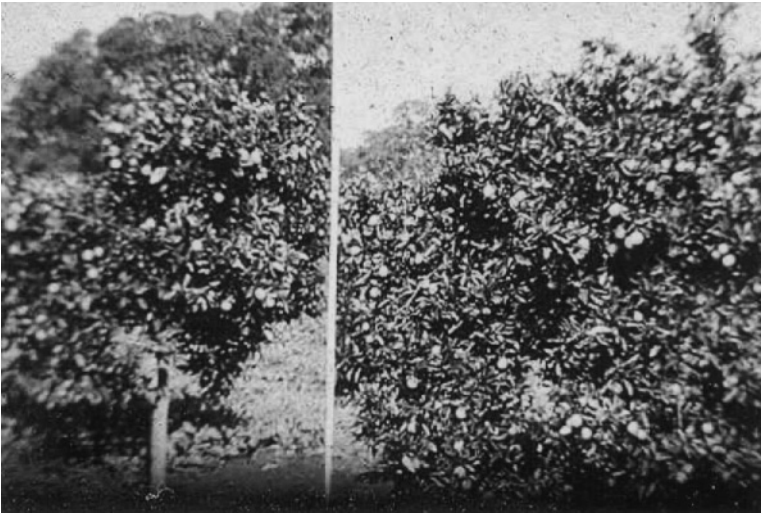
Figure 1: Preimmunized Pera sweet orange tree on right, non-preimmunized Pera sweet
            orange tree on left (Muller, 1968).

fruits, with emphases on the first one, were studied. The search for mild strains was carried out, looking for outstanding trees, sought to be carrying mild strains, in or-chards of the three varieties badly affected by CTV. Some 45 mild strains of CTV were obtained by this approach. Of these, three showed potential for satisfactory preimmunization against severe CTV strains in ´Pera´ sweet orange, two in ´Galego´ lime and one in Ruby Red grapefruit, indicating that there was a virus-tissue specificity among the CTV strains (Costa and Muller,1980) being this confirmed more recently by biomolecular studies (Souza *et al.*, 2000). Release of the best preimmunized Pera sweet orange clone, started 33 years ago, and led to its rapid increase by growers. Experiments carried out along these more than 30 years in the State of São Paulo and other states of Brazil, as well as comparisons carried out by growers, showed that the preimmunized

'Pera' clone has been superior to other existing clones (Fig.1,2) . Large-scale propagation of preimmunized 'Peras', has revealed almost no breakdown in protection in successive clone generations. Presently, some 80 millions trees descend from the original preimmunized 'Pera' material attest their satisfactorily behavior.

More recently however, in a few cases, orchards formed with budwood from preimmunized trees have a great number of plants (30%) showing severe CTV symptoms. This could be due to an exhaustion of the protective strain after all these years or due to the arising of new severe strains able to supplant it. Studies are now underway with the help of molecular biology, to investigate the reasons of the breakdown in protection and the possibility to find new mild strains that may continue to protect efficiently the 'Pera' sweet orange orchards, mainly in the Southern part of the State of São Paulo where the very severe Capão Bonito CTV complex occurs. Considering the good results obtained
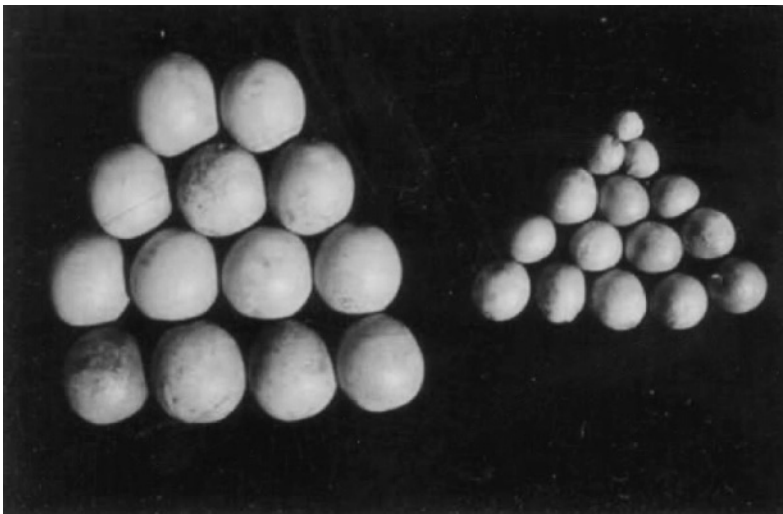
Figure 2: Fruit from preimmunized Pera sweet orange tree on left, Fruit from non-preimmunized Pera sweet orange tree on right (Muller, 1968).

along these last three decades, this is perfectly feasible (Müller *et al.*, 2000).

In the first years, there was a demand for preimmunized ´Galego´ lime (Fig.3-5), however its exchange by the ´Tahiti´ lime (*C. latifolia* Tan.) almost stopped the demand. The grapefruits since the beginning were in low demand, due the lack of interest by the Brazilian consumers. Presently, there is a demand for the preimmunized ´Star Ruby´ grapefruit, aiming some market niches (Müller and Carvalho, 2001).

4.4.3 Japan

Hassaku (*C. hassaku* Hort. ex Tan.) orchards in the Hiroshima Prefecture, Japan, were badly affected by Hassaku dwarf in 1959. Damage was so severe that it was expected that Hassaku would disappear. However, in 1979, Hassaku was still growing and its

production was increasing. This was largely due to the distribution of budwood from an old, apparently healthy Hassaku tree discovered in 1960. This tree was later found to be infected with a mild strain of CTV and vein enation virus and it was called Hassaku No. 55(HM-55) (Sasaki, 1979). However, after 19 years of mild strain preimmunization of Hassaku, the number of trees found with none to mild stem pitting was over 200, compared to 40 trees showing moderate to severe stem pitting. This indicated that despite excellent success there was some breakdown in protection (Roistacher, 2001b).

Figure 3: Non preimmunized West Indian (Galego) lime tree (Muller, 2001).

4.4.4 Peru

The commercial production of oranges, specifically the Washington navel, was virtually terminated in the coastal citrus growing areas of Peru during the 1970´s and early 80´s. This was due to the destruction of citrus by severe stem pitting strains of CTV, regardless of the rootstock chosen. The severe strains were believed to have been

introduced to Peru in the 1950´s, with the known importation of Satsuma mandarin budwood from Japan (Roistacher 1988). A search was initiated in the mid 1980´s for productive surviving or ´escape´ trees of the popular ´Washington navel´ orange (Bederski , 1990). Five promising Washington Navel budlines were found. This search also identified one highly productive Mexican (Key) lime tree that was growing under the cool Mediterranean climatic conditions of coastal Peru and showed no stem pitting. In addition, scion budsticks of ´Duncan´ grapefruit and of ´Madam Vinous´ sweet orange containing protective strains of codes 37 and 40 (Roistacher and Bar-Josehp 1987), which were introduced from California in 1990, looked promising as potential CTV – protective strains. Over the years, all of these native and introduced protective budlines have proven their ability to protect citrus from developing stem pitting symptoms under open field conditions, where adjacent trees of similar tristeza susceptible

Figure 4: Preimmunized West Indian (Galego) lime tree (Muller, 2001).

cultivars showed severe stem pitting symptoms of the disease. The native budlines of ´Washington navel´ and Mexican (Key) lime carrying these protective strains have been planted with commercial success since the early 1990´s (Bederski and Roistacher, 2001). Furthermore, promising early results have been obtained on the protective ability of some of these native and introduced budlines used to preimmunize new introductions of CTV-free plant material of early and late navel sweet orange selections and of different grapefruit cultivars. Thousand of trees of preimmunized selections have been planted with success. Washington Navel and Mexican lime production in coastal areas of Peru has increased significantly (Bederski, personal communication).

4.4.5 Reunion Island

In Reunion Island, the production of a local selection of combava (*Citrus hystrix* DC) has steadily declined since the 60´s due to severe CTV. A survey carried out in 1973 confirmed the general decline of mature combava trees, and also that new plantings grown from seed were rapidly affected by a severe stem-pitting and become unproductive. There were, however, a few exceptions like a relatively vigorous tree called A9 that was invaded by a mild CTV strain. The A9 combava line was successfully propagated in the Island (Aubert and Bove, 1984). It seems that at least until 1997, the combavas were multiplied with the mild "ouaki" strain and that at that time no breakdown in protection was observed (Christian Verniere, personal communication).

Figure 5: Fruit from preimmunized West Indian (Galego) lime tree on right and from non preimmunized tree on left (Muller, 2001).

4.4.6 South Africa

In South Africa CTV has, in the past, not caused the devastating losses that have been encountered in South American countries. The problems have mainly been slow debilitation of grapefruit trees by stem pitting forms of CTV, with the associated production of smaller fruit, and poor performance in certain young plantings in areas where the conditions appear to have resulted in severe stem pitting. In the mid 1950´s, Joubert an extension officer, discovered a 30 year old planting of grapefruit trees on

trifoliate orange (*Poncirus trifoliata* Raf), on the ´Nartia´ farm in the Western Cape, showing only mild stem pitting. Four of the best trees were initially indexed to determine the severity of the inherent CTV. One of these trees was identified as carrying a mild isolate which then became known as the ´Nartia´ mild isolate (Lee *et al.*, 1995). The mild CTV source infecting the ´Nartia´ was named GFMS-12, and was used extensively to inoculate all commercial citrus propagation for many years (von Broembsen and Lee ,1988). Presently, the GFMS-12 is kept as the protective strain for white grapefruit and pummelos, GFMS – 35 replaced GFMS –12 for all red grapefruit and, LMS – 6 a mild strain selected from acid lime replaced GFMS – 12 in all sweet oranges and mandarin types (clementines and satsumas) and limes (Roistacher, 2001a).

4.5 *Papaya ringspot virus – type P*

Papaya ringspot virus – type P (PRSV-P) is a species of the genus *Potyvirus*, transmitted by aphids in a non-persistent manner, and causes important economical losses to papaya (*Carica papaya* L.) in several tropical and subtropical countries. As an example of its destructive effect, it can be mentioned that in Brazil, the disease was responsible for the almost total disappearance of this crop from the State of São Paulo, which was the biggest papaya producer of the country in the 1970's and 1980's. The area planted with papaya in São Paulo was reduced from approximately 7,188 ha in 1977 to 234 ha in 1989, due to the rapid spread of the virus throughout the State.

The use of preimmunization for control of PRSV-P was initiated in the 1980's through independent studied in Taiwan, Brazil and USA (Lin, 1980; Rezende *et al.*, 1981; Yeh and Gonsalves, 1984). Besides many efforts toward the finding of very protective and stable mild strains of the virus, the results obtained were not consistent. In Taiwan, Lin (1980) selected some promising mild strains, that years later (Lin *et al.*, 1989) presented problems of mild symptom instability, which was expressed within 6 months after inoculation of the plants. They suggested that the instability of the symptoms was apparently associated with mutation of the mild strains. In Brazil, Rezende *et al.* (1981; 1982a) also selected some protective mild strains which remained stable for a period of 6 – 8 months after inoculation. After that, all plants showed and irreversible intensification in the severity of the symptoms. As these changes occurred in a synchronized manner for many plants, they were not interpreted as a break in protection, but due to changes of the mild strains present in the plant (Rezende and Costa, 1987).

Two mild strains obtained in the USA by Yeh and Gonsalves (1984), through treatment of the normal complex of the virus from Hawaii with nitrous acid, did not show apparent problems of stability since they were produced. One of these mild strains, designated PRSV HA 5-1, was introduced to Taiwan where more than a million papaya seedlings were preimmunized in 1986 (Yeh *et al.*, 1988; Yeh and Gonsalves, 1994). Although preimmunization helped papaya growers to produce a fruitful crop during several years in Taiwan, the measure showed several drawbacks: a). adverse effects of mild strain on papaya under cool and rainy condition; b). additional cost of inoculating and indexing the seedlings; c). propagation and preservation of the inoculum; d). possible occurrence of severe revertants; e). breakdown under severe disease pressure; and f). strains specific protection (Yeh and Gonsalves, 1994). Another mutant, designated

PRSV HA 6-1 (Yeh and Gonsalves, 1984), was later tested in Taiwan. Field trials showed that plants protected with mild strain HA 6-1 delayed the expression of severe symptoms 1 to 5 months compared with the control (Sheen *et al.*, 1998). According to these authors, presently Taiwan annually has more than 1,000 ha of papaya growing under net-houses, in order to control this disease.

In Hawaii, the selected mild strains proved to be effective both in laboratory and in pilot field tests. Extensive field trials on the Island of Oahu showed that preimmunization was very effective, as compared to Taiwan, since it allowed economic papaya production for at least 2 years. In this case, no break in protection or intensification of symptoms was observed even two and a half years after the plants were inoculated with the mild strains and set in the field. The introduction of the mild strains to Thailand and Mexico did not show promising results due apparently to the strain specific protection (Yeh and Gonsalves, 1994).

Development of transgenic resistant lines of papaya has apparently replaced mild strain protection in Hawaii (Manshardt, 1998). Field evaluation of transgenic resistant papaya is also underway in Brazil (Souza and Gonsalves, 1999).

4.6 *Papaya ringspot virus – type W*

Cucurbit virus diseases are a worldwide problem and represent one of the most liming factors for growers. Twenty different viruses have already been reported infecting species in the Cucurbitaceae family worldwide (Zitter *et al.*, 1996). The relative incidence of these viruses cannot be considered a stable pattern, since it varies from place to place and from time to time, as it has been shown in several surveys (Grogan *et al.*, 1959; Milne *et al.*, 1969; Nameth *et al.*, 1986; Perring *et al.*, 1992; Shanmugasundaram *et al.*, 1969; Ullman *et al.*, 1991; Umesh *et al.*, 1995). Among those viruses, 8 have already been reported on Cucurbitaceae in Brazil. *Papaya ringspot virus – type W* (PRSV-W), originally described as *Watermelon mosaic virus- 1* (WMV-1) (Van Regenmortel, 1971), has been considered one of the most important, due to its predominance in several cucurbit producing regions in the country and for the significant yield loss frequently associated to its occurrence (Albuquerque *et al.*, 1972; Lima *et al.*, 1980; Pavan *et al.*, 1989; Yuki *et al.*, 1991; Lima and Vieira, 1992). Recent survey on viruses affecting cucurbits in the State of São Paulo confirmed the predominance of PRSV-W (Yuki *et al.*, 2000).

Although PRSV-W infection causes damage on several species of Cucurbitaceae, yield losses are higher on sensitive species and/or cultivars, as for example Zucchini squash (*Cucurbita pepo* L.). Absence of PRSV-W resistant genes in this species, associated to the difficulties in controlling the disease by means of insecticides, which would kill the aphid vectors, or any other cultural practice, stimulated in 1991 studies to evaluate the practical and economical viability of preimmunization for the control of this mosaic disease (Rezende *et al.*, 1994). Eighteen protective mild strains of PRSV-W were selected from blisters that occur on Zucchini squash mosaic leaves. Subsequent work, carried out with the best two mild strains, named PRSV-W-1 e PRSV-W-2, showed that they effectively protected the plants against the effects of the severe strains present in the field (Fig. 6) and allowed the yield of good quality fruits (Fig. 7). (Rezende *et al.*, 1994; Rezende and Pacheco, 1998). Yield of marketable fruits harvested from protected

plants was only 10% less than that of the healthy plants in one field trial. Compared to the yield from plants infected with the severe strains, protected plants showed an increment of 633% in the weight of marketable fruits in a test in 1994 (Fig.8). In a second field trial in 1995, an increase of 344% in the weight of marketable fruits was recorded. Both PRSV-W mild strains also protected Zucchini squash plants in a field test in the State of Rio de Janeiro (Bonis *et al.*, 1998).

Due to the success of preimmunization for control of PRSV-W on Zucchini squash, Rezende *et al.* (1999) also studied the application of this technology on long neck squash *(C. moschata* Duch. ex Poir. cv Menina Brasileira), which is considered tolerant to the disease caused by this virus. One field trial showed that combination of mild strain protection and tolerance of 'Menina Brasileira' provided a better disease control, with approximately 33% increase on the average yield (weight) of marketable

Figure 6: Protected Zucchini squash plants (a) show normal growth, and unprotected plant (b) show severe mosaic, leaf malformation, and stunting after 60 days of exposure in the field (Rezende and Pacheco, 1998).

fruits (Fig. 9).

Dias and Rezende (2000) showed that preimmunization was also effective for the control of PRSV-W on hybrid squash type Tetsukabuto (*C. maxima* Duch. ex Lam x *C. moschata* 'Takayama'). In the first field trial, protected plants yielded approximately 3.1 times more fruits/plant (weight) than plants infected with common strains of the virus. Both, protected and unprotected plants yielded 83.7% and 25% of marketable fruits, respectively. In the second field trial, protected plants yielded approximately 2.3 times more fruits (weight) than plants infected with common strains of PRSV-W, with 97.1%

of marketable fruits. In this trial, only 2.1% of marketable fruits were harvested from
unprotected plants (Fig. 10 and 11).

Mass inoculation of cucurbit seedlings can be achieved with a paint spray gun
attached to an air compressor, with pressure varying from 2,8 - 7,0 kgf cm^{-2}, according to
the species (Rezende and Pacheco, 1998; Dias and Rezende, 2000) or with an automatic
equipment developed in Israel for inoculation of cucurbit seedlings with mild strain of
Zucchini yellow mosaic virus (ZYMV) (Yarden *et al.*, 2000).

Mass inoculation may not be 100% efficient in all cases. Efficiency of mass
inoculation of seedlings of hybrid squash 'Takayama' varied form 63% to 79%. In spite
of this, field survey on two commercial plantings showed that the majority of unpro-
tected plants were infected with the mild strains at the end of the crop. Transmission of
the mild strain from inoculated seedlings to healthy plants was apparently done by
aphids present in the field (Dias and Rezende, 2000).

Figure 7: Clockwise from upper left: Zucchini fruit from plants infected with common strains
of Papaya ringspot virus – type W, (PRSV-W), from healthy plants, from plants
protected by inoculation with PRSV-W-1, and from plants protected by inocula-
tion with PRSV-W-2 (Rezende and Pacheco, 1998)

Practical application of preimmunization for the control of PRSV-W on Zucchini
squash and longneck squash has occurred since 1997. The Department of Entomologia,
Fitopatologia and Zoologia Agrícola, ESALQ, University of São Paulo, Piracicaba, SP,
provides mild strains inoculum and orientation for growers interested in using this
technology. A nursery company, named Gioplanta, located in Capivari County, SP, has
also produce large-scale preimmunized seedlings of Zucchini squash and long neck
squash for growers. Studies are underway in order to establish preimmunization on
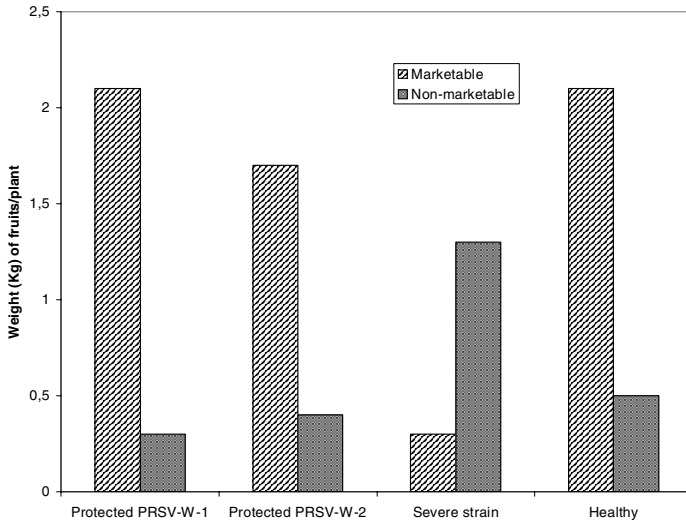
Figure 8: Yield of marketable and non-marketable fruits of Zucchini squash 'Caserta' protected with mild strains of *Papaya ringspot virus – type W* (PRSV-W), infected with severe strain and healthy.
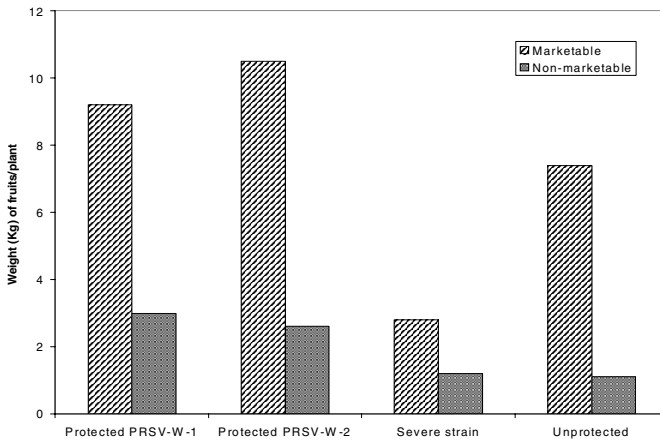
Figure 9: Yield of marketable and non-marketable fruits of longneck squash 'Menina Brasileira' protected with mild strains of *Papaya ringspot virus – type W* (PRSV-W), infected with severe strain and unprotected.

watermelon crop, which is also severely affected by PRSV-W (Dias and Rezende, 2001).

4.7 *Cucumber mosaic virus*

Cucumber mosaic virus (CMV) is the type member of the *Cucumovirus* genus. It is distributed worldwide and has the largest host range of all plant viruses, causing diseases in vegetable, fruit and ornamental crops, with severe economic losses. The virus consists of isometric particles containing a single-stranded positive sense RNA genome of three RNA segments (Palukaitis *et al.*, 1992). In addition to the viral RNAs, some strains of CMV encapsidate satellites RNAs, which are small virus-associated nucleic acids that are sequence-unrelated to, but replicatively dependent upon, the viral genome. CMV-satellite RNA, as well as satellite-RNAs from other viruses, has the property of modulating the symptoms expression caused by the helper virus, exacerbating or ameliorating the symptoms shown by infected plants.

Control of CMV using satellite-mediated protection has been investigated extensively in China (Tien *et al.*, 1987; Wu *et al.*, 1989; Tien and Wu, 1991); Japan (Yoshida *et al.*, 1985; Sayama *et al.*, 1993), the United States (Montasser *et al.*, 1991) and Italy (Gallitelli *et al.*, 1991), and it has proved useful in protecting plants from CMV infection. In spite of the efficiency of this method, it has not been widely put to practical use, except in China were satellite-mediated protection has been applied successfully in pepper crop (Tien and Wu, 1991) and in Japan, where 1.5 to 2.0 million of protected pepper seedlings were sold in 1998 by NDM company (Dr. J.M. Kaper, personal communication).

Several factors contribute to the reluctance to utilize this control technology: i). although a satellite may attenuate CMV symptoms in the target crop, it can intensify symptoms in other crops; ii). satellite RNA could change properties during serial passage in some host plants (Palukaitis and Roossinck, 1996), which may exacerbate the symptom caused by CMV; and iii). CMV strains designated from greenhouse studies as attenuated may not be as effective in the field (Sayama *et al.*, 1993).

4.8 *Zucchini yellow mosaic virus*

Zucchini yellow mosaic virus (ZYMV) is now considered one of the major pathogens of cucurbits in most regions of the world. In Brazil, it has been considered the second in incidence, as shown in a recent survey on virus disease of cucurbits in the State of São Paulo (Yuki *et al.*, 2000). ZYMV is a species of the genus *Potyvirus* and is efficiently transmitted by several species of aphids in a non-persistent manner. This virus induces yellow mosaic, severe leaf malformation, extreme reduction in the size of the leaf lamina, and severe plant stunting. Fruits are malformed and may develop knobby areas, depending on the species/cultivar. Infected plants generally cease fruit bearing 1 or 2 weeks after infection, which leads to significant yield damage and, consequently, economic loss.

Like all other aphid-transmitted virus affecting cucurbit species, including PRSV-W, ZYMV is extremely difficult to control with insecticides, reflective mulches, and mineral oil. The use of preimmunization for the control of Zucchini yellow mosaic on

Zucchini squash was apparently first proposed by Lecoq *et al.* (1991) in France. Field experiments carried out with the mild strain ZYMV-WK, which is poorly aphid transmissible, showed effective protection on Zucchini squash plants and significant increment on the yield of marketable fruit. Weight of marketable fruits from protected plants was 14.7 times higher than that harvested from unprotected plants. ZYMV-WK was introduced to Taiwan (Wang *et al.*, 1991) and UK (Walkey *et al.*, 1992) and provided a very effective protection on field tests with Zucchini squash. In Taiwan, Wang *et al.* (1991)
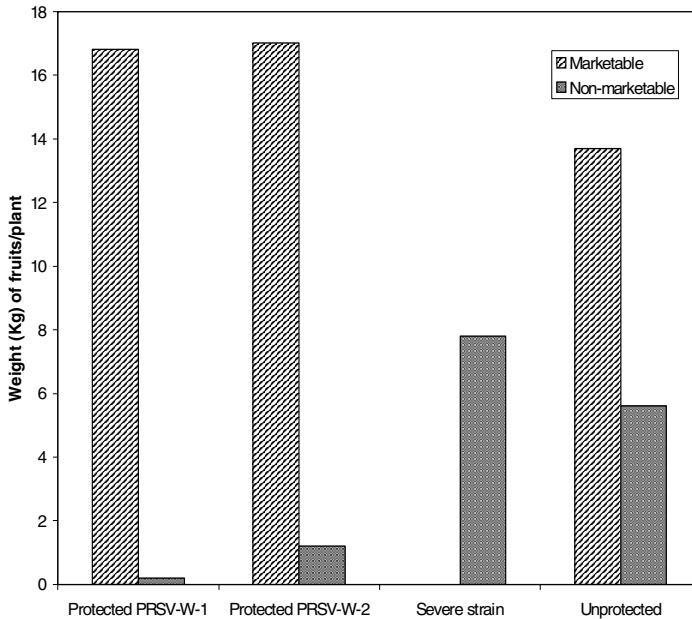
Figure 10: Yield of marketable and non-marketable fruits of hybrid squash 'Takayama' protected with mild strains of *Papaya ringspot virus – type W* (PRSV-W), infected with severe strain and unprotected.

reported increments on the yield of marketable fruits of 116% and 1256% in the first and second field trial, respectively. Preimmunization for the control of Zucchini yellow mosaic has also been experimentally tested in California, USA, with promising results (Perring *et al.*, 1995). Although ZYMV-WK have proved to be efficient in protecting plants against severe strains from several origins, laboratory experiments showed no efficient protection against severe strains of ZYMV from Reunion, Mauritius and Madagascar, which are also serologically distantly related to the type strains of this virus (Lecoq, 1998).

Preimmunization has been considered a viable alternative for the control of ZYMV Zucchini squash due to the absence of resistant genes in the germplasm collec-

tion of *C. pepo* and the lack of any other method able to provide effective and economic control of the disease (Lecoq *et al.*, 1991; Wang *et al.*, 1991; Walkey *et al.*, 1992). In addition, Lecoq *et al.* (1991) mentioned that the poor aphid transmissibility of the mild strain ZYMV-WK is an advantageous characteristic, since it reduces the risk for the mild strain to be transmitted to any other species and cause severe disease.

Commercial use of preimmunization for the control of Zucchini yellow mosaic has been reported in Hawaii (Cho *et al.*, 1992) and Israel (Yarden *et al.*, 2000). In Israel, this technology has been applied in the last 5 years for protecting Zucchini squash, squash, melon and watermelon crops. In 1999, almost 800 ha were planted with plants of these species protected with a mild strain of ZYMV.

4.9 Multiple preimmunization

Figure 11: Fruit of hybrid squash 'Takayama' from plants protected by inoculation with mild strain of Papaya ringspot virus- type W (PRSV-W-1) and from plants infected with common strains of PRSV – W-1 (Dias and Rezende, 2000).

Multiple inoculation with mild strains has been reported from experimental trials in Japan and Brazil for the control of cucurbit viruses transmitted by aphids in a non-persistent manner. In Japan, Kosaka and Fukunishi (1997) reported that multiple inoculation of cucumber (*Cucumis sativus* L.) seedlings with attenuated strains of CMV, ZYMV and WMV-2 reduced yield loss due to mix infection with virulent strains of these viruses in field experiments under sever epidemic conditions in 1994 and 1995. However, triply inoculated plants developed synergistic symptoms and 15% fewer market-able fruits compared to healthy plants. Due to this fact, multiple preimmunization has

been recommended only for summer-early autumn production, when economic damage caused by multiple incidence of CMV, ZYMV and WMV-2 is significantly greater than the damage due to the inoculation with the mild strains of these viruses.

In Brazil, double inoculation with the mild strain PRSV-W-1, and a recently selected mild strain of ZYMV, named ZYMV-LR (Rabelo and Rezende, 2001a) was successfully tested in Zucchini squash plants under greenhouse and field conditions (Rabelo and Rezende, 2001b). Other field experiments are under way in order to establish the commercial use of double preimmunization for the control of PRSV-W and ZYMV.

5. Concluding remarks

Eventhough the phenomenon of preimmunization has been known for more than 70 years, there are still very few examples in which this technology has been commercially applied for the control of virus diseases. Part of the reduced use of preimmunization may be due to the fact that, for many years, this method was only thought as viable for perennial crops or annual crops propagated by means of tubers, bulbs, etc. This method was considered time consuming and expensive for annual crops propagated by seeds, in which inoculation of seedlings would be repeated every crop season. However, the last two decades have experienced several changes on agricultural practices for several species of annual crops, including the mass production of seedlings. This practice has facilitated the use of preimmunization for the control of virus diseases on cucurbit crops, for which seedlings were not produced in the past years. As mentioned before, mass inoculation of seedlings can be easily achieved with a paint spray gun attached to an air compressor (Rezende and Pacheco, 1998; Dias and Rezende, 2000) or with automatic inoculation equipment as proposed by Yarden *et al.* (2000).

Other reasons for the reduced use of preimmunization may be related to the risks that deliberate distribution of the mild strain of a virus may cause to the protected crop and, specially, to nontarget crops. These include the stability of the mild strain in the target crop, the longevity of the protection offered by the mild strain in the target crop, the damage that a mild strain may cause to an nontarget crop and the occurrence of synergistic interaction of the mild strain with unrelated viruses or other pathogens. The stability of the mild strain has to be constantly monitored, during its successive multiplication, in order to eliminate any variant that will cause symptoms similar to those induced by common strains of the virus. Palukaitis and Roossink (1996) showed that successive passages of a CMV strain, containing a satellite RNA that attenuated the symptoms caused by the virus, produced a mutation in the satellite RNA, which exacerbated the symptom caused by CMV. The stability of mild strains PRSV-W-1 and PRSV-W-2 was evaluated by Rezende and Pacheco (1997), in the greenhouse, during 28 months, throughout 18 successive transfers to plants of Zucchini squash. PRSV-W-1 induced stable mild symptoms on all inoculated plants after each transfer, while PRSV-W-2 was stable for 22 months. After that, it induced symptoms as severe as those caused by the common strains of the virus. Mild strains PRSV-W-1 and PRSV-W-2, which was later recovered from original inoculum preserved in dehydrated tissue at –

20° C, have not shown any other change up to now (J.A.M. Rezende, data not published).

The results obtained in Brazil with citrus, have shown that one of the mild strains has been quite stable in successive propagation. As indicated before, one of the traits of a mild CTV strain is to be easily graft-transmissible, and also prone to be transmitted by aphids. One with such fulfills seems to be the mild strain used in São Paulo to protect the ´Pera´ sweet orange, that showed the same SSCP pattern, independently whether the inoculation was carried out by tissue union or aphids (Machado *et al*., 2000). Changes in the severity of the symptoms have been practically absent and the few cases recorded, could have been due to virus mixtures resulting from establishing preimmunized budwood on rootstock seedlings previously infected in the nursery with the local CTV strains (Müller and Costa, 1971). Besides a breakdown in protection, as pointed out by Fraser and Broadbent (1979) and Sasaki (1979), or preimmunization incompleteness as suggested by Bar-Joseph (1978), the above explanation could apply in a number of failures reported, since it is not clear whether the rootstocks used for the experiments or even for commercial propagation had been raised in the open. The possibility thus exists that the rootstocks were infected prior to being budded. It is known that different rootstocks might vary in their susceptibility to natural CTV infection (Müller and Costa, 1972). This hypothesis was further confirmed in South Africa, where a trial orchard of Nartia grapefruit showed a high percentage (25%) of plants showing stem pitting, likely due to a result of prior natural infection of the Rough lemon rootstocks. On the other side, monitoring mother trees propagated from Nartia preimmunized budwood on virus-free rootstocks has shown that the incidence of severe stem pitting in these trees was not higher than 10% (Marais *et al.*, 1996). This problem shall be overcome in the near future, as more and more nursery plants will be raised worldwide under screenhouses. Another explanation in those cases in which a deterioration is noticed after the trees mature, or will give origin to plants showing high percentage of severe CTV symptoms, is that some dormant buds, or even cells in the meristems, could have lost the virus and given rise to healthy unprotected growth that could be superinfected by severe strains. These, once established would progress throughout the tree, suppressing or blending with the original mild CTV strain. One such case, has been recently studied in São Paulo, Brazil, where the daughter plants showing stunting and stem-pitting, produced a CTV CP SSCP pattern that was not similar to any of the mother trees (Alesandra, 2000). Mild strains from a given type of citrus give the best protection if reintroduced into the same citrus type. Thus, in São Paulo, Brazil, the best strains for Pera sweet orange were those collected from Pera sweet orange. In South Africa, GFMS-12 (Nartia) is kept as the protective strain for white grapefruit (were it came from). Further, LMS-6 from lime affords good protection in limes, besides on other citrus types.

Experiments carried out in São Paulo, Brazil, with citrus tristeza, showed that preimmunization with mild strains works basically against the challenging severe strains inoculated by means of the vectors. On the other side, when the challenging inoculation is carried out by tissue union (budding or grafting) there is a breakdown in protection with the time. Probably the longer the effect (severe symptoms) of a challenging inoculation by tissue union with a severe CTV strain is delayed on a preimmunized

plant, the better is the protective capacity of the mild strain. Therefore, in any experiment, preimmunized checks shall be always challenged with the severe strain by tissue union, in order to give valuable information about the protective effect of the mild strain (Müller, 2001, unpublished data).

Another risk of preimmunization is that a strain selected for mildness for a particular crop may infect and cause severe symptoms in a nontarget crop. None of the examples discussed in this chapter have done any experiment or shown any evidence regarding to this risk. As mentioned before, mild strain of CTV has been widely used for 33 years and there is no evidence of infection on any other crop, besides citrus. Common strains of CTV have been reported as restricted to citrus species, although experimentally they were transmitted to *Passiflora gracilis* (Müller and Costa, 1993) and *Passiflora cerulea* (Roistacher and Bar-Joseph, 1987). Giampan and Rezende (2001) inoculated 51 species of cultivated and weed plants with the mild strain PRSV-W-1, in order to evaluate this risk for this strain to cause disease in other plants. Except *C. pepo*, which was used as control, none of the inoculated species was infected with PRSV-W-1. As known, common strains of PRSV-W cause systemic infection in cucurbit species and local lesion on inoculated leaves of *Chenopodium amaranticolor* and *C. quinoa*.

Breakdown in protection has been reported as the cause for the discontinuity of preimmunization for the control of tomato mosaic in Europe (Fulton, 1986) and papaya ringspot disease in Taiwan (Yeh and Gonsalves, 1994). Few cases of apparent breakdown in protection on preimmunized Pera sweet orange trees in Brazil are under investigation as already mentioned before.

Synergist reaction between the protecting mild strain and common strains of any other virus that can infect the plant has been mentioned, as another risk of preimmunization, but no concrete example is known. During the field experiments of preimmunization carried out in France and Taiwan, for the control of ZYMV, Lecoq *et al.*, (1991) and Wang *et al.*, (1991) reported on the incidence of other viruses that usually infect cucurbit crops, such as CMV and WMV-2, but no synergistic effect between these viruses and the mild strain ZYMV-WK was apparently observed. In Brazil, Rezende and Pacheco (1998) did not observe any synergistic effect on Zucchini squash plants protected with mild strains of PRSV-W and further inoculated with common strains of ZYMV and WMV-2, separately. Symptoms of the later viruses were predominant and as severe as those caused by each virus alone.

Although all these risks should be considered in any program of preimmunization, they should not be used as arguments to prevent studies and practical application of this technology. Preimmunization has several advantages that may overcome the risks. First, it can be included in any integrated pest management program. Second, can be applied to cultivars resistant to other diseases caused by virus or other pathogen, which were obtained by classical plant breeding or genetic transformation (transgenic). Third, preimmunization does not pollute, apparently does not represent any risk to the growers and consumers, does not interfere with cultural practices, it is simple to apply and may have a reasonable cost/benefit. Finally, as mentioned by Dodds (1999), preimmunization can fill a technological gap, while waiting for transgenic plants to arrive, and can be deployed again should new technologies fail.

6. References

Ahoonmanesh, A. and Shalla, T.A. 1981. Feasibility of cross-protection for control of tomato mosaic virus in fresh market field-grown tomatoes. Plant Disease, 65:56-58.

Albuquerque, F.C., Ikeda, H. and Costa A.S. 1972. Ocorrência do vírus do mosaico da melancia (*Citrullus vulgaris* Schrad.) em plantações de melão (*Cucumis melo* L.) na região de Belém-PA. Revista de Olericultura,12:94. (abstract).

Atreya, C.D., Raccah, B. and Pirone, T.P. 1990. A point mutation in the coat protein abolishes aphid transmissibility of a potyvirus. Virology, 178: 161-5

Aubert, B. and Bove, C. 1984. Mild and severe strains of citrus tristeza virus in Reunion Island. In: "Proc.9th Conf.IOCV" (eds. Garnsey, S. M.,Timmer, L. W. and Dodds, J. A.) Univ. Calif. Press, Riverside, CA, pp. 57-61.

Bar-Joseph, M. 1978. Cross protection incompleteness: a possible cause for natural spread of citrus tristeza virus after a prolonged lag period in Israel. Phytopathology, 68: 1110-1111.

Bar-Joseph, M., Garnsey, S.M. and Gonsalves, D. 1979. The closteroviruses a distinct group of elongated plant viruses. Advances in Virus Research , 25 93-168.

Bar-Josehp, M., Marcus, R. and Lee, R.F. 1989. The continuous challenge of citrus tristeza virus control. Ann. Rev. Phytopath. 27: 291-316.

Bederski, K. 1990. The presence of severe strains of SP-CTV in peruvian citrus orchards challenges the reponsabilities of nurserymen. In: "Proc. 3rd World Cong. ISCN" (eds. Albert Newcomb and Ian Tolley), Sidney, Australia, pp. 56-57.

Bederski, L. and Roistacher, C.N. 2001. Overcoming the endemic severe tristeza stem pitting problems of citrus in Peru by cross-protection. In: "6th Intern.Cong. Citrus Nurserymen", Ribeirão Preto, SP-Brazil. July 9-13, pp. 109-110. (Program and Abstracts).

Bennett, C.W. 1953. Interactions between viruses and virus strains. Advanced Virus Research, 1:39-67. 1953.

Bennett, C.W. and Costa, A . S. 1949. Tristeza disease of citrus. Journal of Agricultural Research, 78:207-237.

Bonis, M., Rezende, J.A.M.., Watanabe, H., Brioso, P.S.T. 1998. Premunização contra o vírus do mosaico da melancia em abobrinha no Estado do Rio de Janeiro. Fitopatologia Brasileira, 23(suplemento):316. (abstract).

Broadbent, L.H. 1964. The epidemiology of tomato mosaic. VII. The effect of TMV on tomato fruit yield and quality under glass. Annals of Applied Biology, 54:209-224.

Broadbent, P., Bevington, K.B., and Coote, B.G.1991. Control of stem pitting of grapefruit in Australia by mild strain. In: "Proc. 11th Conf. IOCV" (eds. Brlansky, R.H., Lee, R.F. and Timmer, L.W.) Univ. Calif. Press, Riverside, CA, pp. 64-70.

Broadbent, P., Dephoff, C.M., Franks, N., Gillings, M. and Indsto, J. 1995. Pre-immunization of grapefruit with a mild protective isolate of citrus tristeza in Australia. In: "Proc. of the Third International Workshop (final report) citrus tristeza and the brown citrus aphid in the Caribbean basin. Management strategies" (eds. Lee, R.F. Rocha-Peña, M., Niblett, C.L., Ochoa, F., Garnsey, S.M., Yokomi, R.K. and Lastra, R.) Univ. Fla, Lake Alfred, FL, pp. 163-168.

Broadbent, P., Brlansky, G.H. and Indsto, J. 1996. Biological characterization of Australian isolates of citrus tristeza virus and separation of sub-isolates by single aphid transmission. Plant Disease , 80: 329-333.

Carsner, E. and Stahl, C.F. 1924. The relation of *Chenopodium murale* to curly top of the sugar beet. Phytopathology, 14: 57(abstr.).

Chamberlain, E., Atkinson, J.D. and Hunter, J.A. 1964. Cross protection between strains of apple mosaic virus. New Zealand Journal of Agricultural Research, 7: 480-90.

Channon, A.G., Cheffins, N.J., Hitchon, G.M. and Barker, J. 1978. The effect of inoculation with an attenuated mutant strain of tobacco mosaic virus on the growth and yield of early

glass-house tomato crop. Annals of Applied Biology, 88:121-129.

Cho, J.J., Ullman, D.E., Wheatley, E., Holly J. and Gonsalves, D. 1992. Commercialization of ZYMV cross protection for Zucchini production in Hawaii. Phytopathology, 82:1073. (abstract).

Costa, A. S. 1956. Present status of tristeza disease of citrus in South America. FAO Plant Protection Bulletin, 4:97-105.

Costa, A.S., Grant, T.J., and Moreira, S. 1954. Behavior of various citrus rootstock-scion combinations following inoculation with mild and severe strains of tristeza. Proc. Fla. State Hort. Soc,. 67:26-30.

Costa, A. S. and Müller, G.W. 1980. Tristeza control by cross protection: A U.S. Brazil cooperative success. Plant Disease, 64:538-541.

Cox, J.E., Fraser, L.R., and Broadbent, P. 1976. Field protection by the use of mild strains, an evaluation of trials in two climatic districts. In: "Proc. 7th Conf. IOCV" (ed. Calavan, E. C.) Univ. Calif., Riverside, CA, pp. 68-70.

Crowdy, J.T. and Posnette, A. F. 1947. Virus diseases of cacoa in West Africa II. Cross immunity experiments with viruses 1A, 1B and 1C. Annals of Applied Biology, 34:403-411.

Dawson, W. O. ; Beck, D.L. ; Knorr, D. A. and Granthan, G.L. 1986. cDNA cloning of the complete genome of tobacco mosaic virus and production of infectious transcript. Proceedings of the National Academy of Science of the USA,83: 1832-6.

Desjardins, P.R., Wallace, J.M., Wollman, E.S.H. and Drake, R.J. 1959. A separation of virus strains from a tristeza-seedling-yellows complex by heat treatment of infected lime seedlings. In: "Citrus virus diseases" (ed. Wallace, J.M.), Univ. Calif., Berkeley CA, pp.91-95.

Dias P.R.P. and Rezende. J.A.M. 2000. Premunização da abóbora híbrida 'Tetsukabuto' para o controle do mosaico causado pelo *Papaya ringspot virus – type W. Summa* Phytopathologica, 26:390-398.

Dias P.R.P. and Rezende. J.A.M. 2001. Problemas na premunização de melancia para o controle do mosaico causado pelo *Papaya ringspot virus*. Fitopatologia Brasileira, 25:651-654.

Dodds, J.A.1982. Cross-protection and interference between eletrophoretically distinct strains of cucumber mosaic virus in tomato. Virology, 118:235-240.

Dodds, J.A. 1999. Cross-protection and systemic acquired resistance for control of plant diseases. In: "Handbook of Biological Control" (eds. Bellows, T.S. and Fisher, T.W.) Academic Press, San Diego, pp. 549-556.

Fitzell, R.D. and Pares, R.D. 1985. Woodiness and die back disease of passionfruit. *AGFACTS*, Deptment of Agriculture, N.S.W., Australia.

Fletcher, J.T. 1978. The use of avirulent strains to protect plants against the effects of virulent strains. Annals of Applied Biology, 89:110-114.

Fraser, L.R., Long, K. and Cox., J. 1968. Stem pitting of grapefruit – field protection by the use of mild virus strains. In: "Proc. 4th Conf. IOCV" (ed. Childs, J.F.L.) Univ. Fla. Press, Gainesville, FL, pp. 27-31.

Fraser, L. R. and Broadbent, P. 1979. Tristeza. In: "Virus and related diseases of citrus in New South Wales" (eds. Fraser, L. R. and Broadbent, P.) Biological and Chemical Research Institute Rydalmere. Department of Agriculture, New South Wales.

French, R. and Ahlquist, P. 1988. Characterization and engineering of sequences controlling *in vivo* synthesis of brome mosaic virus subgenomic RNA. Journal of Virology, 52: 2411-20.

Fulton, R.W. 1986. Practices and precautions in the use of cross protection for plant virus disease control. Annual Review Phytopathology, 24:67-81.

Gallitelli, D., Martelli, G., Montasser, M.S., Tousignant, M.E. and Kaper, J.M. 1991. Satellite-mediated protection of tomato against cucumber mosaic virus: II. Field test under natural epidemic conditions in Southern Italy. Plant Disease, 75:93-95.

Gal-On, A. 2000. A point mutation in the FRNK motif of the Potyvirus helper component-

protease gene alters symptom expression in cucurbits and elicits protection against the severe homologous virus. Phytopatholgy, 90:467-473.

Giacometti, D.C. and Araujo, C.M. 1965. Cross protection from tristeza in different species of citrus. In: "Proc. 3rd Conf. IOCV" (ed. Price, W.C.) Univ. Fla Press, Gainesville,FL, pp. 14-17.

Giampan, J.S. and Rezende, J.A.M. 2001. Transmissibilidade por afídeos e reação de diversas espécies vegetais às estirpes fracas premunizantes do PRSV-W. Summa Phytopathologica, 27:279-283.

Grant, T.J. and Costa, A.S. 1951. A mild strain of the tristeza virus of citrus. Phytopathology, 41:114-122.

Grant, T.J. and Higgins, R.P. 1957. Occurrence of mixtures of tristeza strains in citrus. Phytopathology, 47: 272-276.

Grogan, R.G., Hall, D.H. and Kimble, K.A. 1959. Cucurbit mosaic viruses in California. Phytopathology, 49:366-376.

Heaton, L. A., Carrington, J.C. and Morris, T.J. 1989.Turnip crinkle virus infection from RNA synthesized *in vitro*. Virology, 170: 214-218.

Hiruki, C. 1979. Increases in marketable fruit yield of greenhouse tomatoes as the result of cross protection against tomato mosaic. Phytopathology, 69:916. (abstract).

Holt, C.A., Hodgson, R. A., Coker, F. A., Beachy, R. N. and Nelson, R.S. 1990. Characterization of the masked strain of tobacco mosaic virus – identification of the region responsible for symptom attenuation by analysis of and infectious cDNA clone. Molecular Plant-Microbe Interaction, 3: 417-423.

Hughes, J. D'a. and Ollennu, L.A.A. 1994. Mild strain protection of cocoa in Ghana against cocoa swollen shoot virus - a review. Plant Pathology, 43:442-457.

Johnson, J. 1926. The attenuation of plant viruses and the inactivating influence of oxygen. Science, 64: 210.

Johnson, J. 1937. An acquired partial immunity to the tobacco streak disease. Trans. Wisconsin Acad. Sci. Arts Letters 30:27.

Johnson, J. 1947. Virus attenuation and the separation of strains by specific hosts. Phytopathology, 37:822-837.

Klotz, L.J. 1978. Fungal, bacterial and nonparasitic diseases and injuries originating in the seed-bed nursery and orchard. In: "The Citrus Industry" (eds. Reuther, W. Calavan, E.C. and Carman, G.E.) Vol. 4, Div. Agric. Sci. Univ. Calif. Berkeley, CA, pp.1-66.

Koizume, M. and Sasaki, A. 1980. Protection phenomeana against tristeza in trees preinoculated with vein enation virus. In: "Proc. 8th Conf. IOCV" (ed. Calavan, E.C., Garnsey, S.M. and Timmer, L. W.) Univ. of Calif., Riverside, CA, pp. 48-50.

Kosaka, Y. and Fukunishi, T. 1997. Multiple inoculation with three attenuated viruses for the control of cucumber virus disease. Plant Disease, 81:733-738.

Lee, R.F., Derrick, K.S., Niblett, C. L. and Pappu, H.R. 1995. When to use mild strain cross protection (MSCP) and problems encountered. In: "Proc. of the Third International Workshop (final report) citrus tristeza and the brown citrus aphid in the Caribbean basin. Management strategies" (eds. Lee, R.F. Rocha-Peña, M., Niblett, C.L., Ochoa, F., Garnsey, S.M., Yokomi, R.K. and Lastra, R.) Univ. Fla, Lake Alfred, FL, pp.158-162.

Lecoq, H. 1998. Control of plant virus disease by cross protection. In: "Plant Virus Disease Control" (eds. Hadidi, A., Khetarpal, R.K. and Koganezawa, H.) American Phytopathological Society, St. Paul. pp. 33-40.

Lecoq, H., Lemaire, J.M. and Wipf-Scheibel, C. 1991. Control of zucchini yellow mosaic virus in squash by cross protection. Plant Disease , 75:208-211.

Liefting, L., Pearson, M. and Pone, S. 1992. The isolation and evaluation of two naturally occurring mild strains of vanilla necrosis potyvirus for control by cross-protection. Journal

of Phytopathology, 136: 9-15.

Lima, J.A.A., Fernandes, E.R. and Mendes, M.L. 1980. Identificação sorológica de "watermelon mosaic virus 1" em cucurbitáceas cultivadas e nativas do Rio Grande do Norte. Fitopatologia Brasileira, 5: 414. (abstract).

Lima J.A.A. and Vieira, A.C. 1992. Distribuição do vírus do mosaico da abóbora em municípios cearences e gama de hospedeiros de um isolado. Fitopatologia Brasileira, 17:112-114.

Lin, C.C. 1980. Strains of papaya ringspot virus and their cross protection. National Taiwan University. Ph.D. Thesis. (abstract).

Lin, C.C., Su, H.J. and Wang, D.N. 1989. The control of papaya ringspot virus in Taiwan. Food and Fertilizer Technology Center, Taiwan, Tech. Bull. N° 114, pp.1-13.

Machado, M. A., Souza, A. A., Corat, M. A. F. and Müller, G.W. 2000. Desafio com isolado severo do vírus da tristeza dos citros (CTV) em laranja ´Pera´. Fitopatologia Brasileira, 25(suplemento):444. (abstract).

Manshardt, R.M. 1998. 'UH Rainbow' papaya. University Hawaii College Tropical Agric. Hum. Resour. Germoplasm G-1. 2p.

Marais, L.J., Marais, M.L. and Rea, M. 1996. Effect of citrus tristeza stem pitting on fruit size and yield of marsh grapefruit in Southern Africa. In: "Proc. 13th Conf. IOCV" (ed. da Graça, J.V., Moreno, P. and Yokomi, R.K.) Univ. of Calif., Riverside, CA pp. 163-167.

McKinney, H.H. 1929. Mosaic disease in the Canary Islands, West Africa, and Gilbratar. Journal of Agricultural Research, 39:557-578.

Matthews, R.E.F. 1991. Plant Virology. Academic Press, Inc., San Diego, California, USA.

Mendt, 1992. History of CTV in Venezuela. In: "Proc. of a Workshop on Citrus Tristeza Virus and Toxoptera citricidus in Central America Development of Management Strategies and use of Biotechnology for Control" (eds. Lastra, R., Lee, R..F, Rocha – Peña, M., Niblett, C., Ochoa, F., Garnsey, S.M., and Yokomi, R.K.) Maracay, Venezuela, pp. 137-142.

Meneghini, M. 1946. Sobre a natureza e transmissibilidade da doença " tristeza" dos citrus. O Biológico, 12:285-287.

Milne, K.S., Grogan, R.G. and Kimble, K.A. 1969. Identification of viruses infecting cucurbits in California. Phytopathology, 59:819-828.

Montasser, M.S., Tousignant, M.E. and Kaper, J.M. 1991. Satellite-mediated protection of tomato against cucumber mosaic virus: I. Greenhouse experiments and simulated epidemic conditions in the field. Plant Disease , 75:86-92.

Müller, G.W. 1980. Use of mild strains of citrus tristeza virus (CTV) to reestablish commercial production of ´Pera´ sweet orange in São Paulo, Brazil. Proc. Fla. State Hort. Soc., 93: 62-64.

Müller, G.W. and Costa, A. S. 1971. Estudos sobre a interação entre o virus da tristeza da copa e do porta-enxerto. In: "Anais do I Cong. Bras. de Fruticultura" (ed. Rodrigues, O.) Campinas, SP, Vol. II , pp. 463-473.

Müller, G.W. and Costa, A.S. 1972. Incidência do vírus da tristeza no porta-enxerto de citros na época de enxertia. Revista da Sociedade Brasileria de Fitopatologia, 5:157-158.

Müller, G.W. and Costa, A. S. 1973. Métodos de seleção de estirpes fracas do vírus da tristeza. II Congresso Brasileiro de Fruticultura, Viçosa. MG, pp. 287-297.

Müller, G.W. and Costa, A.S. 1977. Tristeza control in Brazil by preimmunization with mild strains. Proc. Int. Soc. Citric., 3:868-872.

Müller, G.W. and Costa, A.S. 1981. Uso da premunização com estirpes fracas do vírus da tristeza reergue a produção comercial de laranja Pêra no Estado de São Paulo. Fitopatologia Brasileira, 6:301-302.

Müller, G.W. and Costa, A.S. 1987. Search for outstanding plants in tristeza infected citrus orchards: the best approach to control the disease by preimmunization. Phytophylactica., 19:197-198

Müller, G.W. and Costa, A.S. 1993. Doenças causadas por vírus, viróides e similares em citros.

In: "Doenças dos citros causadas por algas, fungos, bactérias e vírus". (eds. Rossetti, V., Müller, G.W. and Costa, A.S.) Fundação Cargill, Campinas, pp. 55-84.

Müller, G.W., Targon, M.L.P.N. and Machado, M. A. 2000. Thirty years of preimmunized pera sweet orange in the citriculture in São Paulo, Brazil. In: "Proc. 14th Conf.IOCV" (ed. da Graça, J.V., Lee, R.F. and Yokomi, R.K.) Univ. of Calif., Riverside, CA, pp. 400-402.

Müller, G.W. and Carvalho, S.A. 2001. Trinta e três anos de controle da tristeza dos citros por premunização no Estado de São Paulo. Fitopatologia Brasileira, 26 (suplemento):241-242. (abstract).

Nameth, S.T., Dodds, J.A., Paulus, A.O. and Laemmlen, F.F. 1986. Cucurbit viruses in California: and ever-changing problem. Plant Disease , 70:8-12.

Niblett, C.L., Dickson, E., Fernow, K., Horst, R.K. and Zaitlin, M. 1978. Cross protection among four viroids. Virology, 91:198-203.

Ollennu, L.A.A. and Owusu, G.K. 1989. Isolation and study of mild strain of cocoa swollen shoot virus for possible cross protection. In: "Proceedings of the IVth International Plant Virus Epidemiology Workshop". Montpellier, France. pp.119-122.

Olson, E.O. 1958. Responses of limes and sour orange seedlings and four scion rootstock combinations to infection by strains of the tristeza virus. Phytopathology, 48: 454-459.

Oshima, N. 1975. The control of tomato mosaic virus disease with attenuated virus of tomato strain of TMV. Review of Plant Protection Research, 8:126-135.

Oshima, N. 1981. Control of tomato mosaic disease by attenuated virus. Jpn. Agric. Res. Q. 14: 222- 228.

Palukaitis, P., Roossinck, M.J., Dietzgen, R.G. and Francki, R.I.B. 1992. Cucumber mosaic virus. Advances in Virus Research, 41:281-348.

Palukaitis, P. and Roossinck, M.J. 1996. Spontaneous change of a benign satellite RNA of cucumber mosaic virus to a pathogenic variant. Nature Biotechnology, 14:1264-1268.

Pares, R.D., Martin, A.B. and Fitzell, R.D. 1985. Virus-induced tip necrosis of passionfruit (*Passiflora edulis* Sims.). Australasian Plant Pathology, 14:76-78.

Pavan, M.A., Carvalho, M.G. and Fernandes, J.J. 1989. Distribuição do vírus do mosaico da melancia (papaya ringspot virus - W), nas principais regiões produtoras de pepino (*Cucumis sativus*) e abobrinha (*Cucurbita pepo*) de Minas Gerais. Fitopatologia Brasileira, 14:84-85.

Peasley, D. and Fitzell, R.D. 1981. Passionfruit industry benefits through scion wood scheme. Agriculture Gazette, N.S.W., (Australia), 92:5-8.

Perring, T.M., Farrar, C.A., Mayberry, K. and Blua, M.J. 1992. Research reveals pattern of cucurbit virus spread. California Agriculture, 2:35-40.

Perring, T.M., Farrar, C.A., Blua, M.J., Wang, H.L. and Gonsalves, D. 1995. Cross protection of cantaloupe with a mild strain of zucchini yellow mosaic virus: effectiveness and application. Crop Protection, 14:601-606.

Posnette, A.F. and Todd, J.Mca. 1955. Virus diseases of cacao in West Africa. IX. Strain variant and interference in virus 1A. Annals of Applied Biology, 43:433-453.

Posnette, A.F. and Cropley, R. 1956. Apple mosaic viruses. Host reactions and strain interference. The Journal of Hortcultural Science, 31: 119-33.

Rabelo, L.C. and Rezende, J.A.M. 2001a. Efeito protetor de uma estirpe fraca do *Zucchini yellow mosaic virus* (ZYMV) em abobrinha-de-moita. Summa Phytopathologica, 27:116. (abstract).

Rabelo, L.C. and Rezende, J.A.M. 2001b. Dupla premunização para o controle dos mosaico comum e amarelo da abobrinha de moita. Fitopatologia Brasileira, 26 (suplemento):518. (abstract).

Rast, A.T.B. 1972. MII-16, an artificial symptomless mutant of tobacco mosaic virus for seedling inoculation on tomato crops. Netherland Journal of Plant Pathology, 28:110-112.

Rast, A.T.B. 1975. Variability of tobacco mosaic virus in relation to control of tomato mosaic in glasshouse tomato crops by resistance breeding and cross protection. Agricultural Research

Reports, (Wageningen) 834:1-76.

Rezende, J.A.M., Costa, A.S. and Soares, N.B. 1981. Ocorrência de um isolado fraco do vírus do mosaico do mamoeiro *Carica papaya* L. Fitopatologia Brasileira, 6:534 (abstract).

Rezende, J.A.M., Costa, A.S. and Soares, N.B., 1982a. Novas observações sobre um isolado fraco do vírus do mosaico do mamoeiro e seu efeito protetivo. Summa Phytopathologica, 8:5-6 (abstract).

Rezende, J. A. M.,Costa, A. S. and Veja, J. 1982b. Obtenção de isolados fracos do virus do mosaicodo mamoeiro a partir de "bolhas" das folhas com mosaico. Fitopatologia Brasileira, 7:542 (abstract).

Rezende, J.A.M. and Costa, A.S. 1987. Intensificação dos sintomas dificulta o controle do mosaico do mamoeiro por premunização. Fitopatologia Brasileira, 12:110-101.

Rezende, J. A.M., Yuki, V.A., Vega, J., Scagliusi, S.M.M. and Costa, A.S. 1992. Bolhas podem fornecer isolados fracos também do potyvirus do mosaico da abobrinha-de-moita (VMM-Me). Summa Phytopathologica, 18:11(abstract).

Rezende, J.A.M. and Pacheco, D.A. 1997. Estabilidade de isolados fracos premunizantes do vírus do mosaico do mamoeiro – estirpe melancia. Fitopatologia Brasileira, 22:64-68.

Rezende, J. A. M. and Pacheco, D. A. 1998. Control of papaya ringspot virus-type W in zucchini squash by cross protection in Brazil. Plant Disease , 82:171-175.

Rezende, J.A.M., Pacheco, D.A. and Iemma, A.F. 1999. Efeitos da premunização da abóbora 'Menina Brasileira' com as estirpes fracas do vírus do mosaico do mamoeiro. Pesquisa Agropecuária Brasileira, 34:1481-1489.

Rezende, J.A.M., Yuki, V.A., Vega, J., Scagliusi, S.M.M., Borba, L.F. and Costa, A.S. 1994. Isolados fracos do potyvirus causador do mosaico da abobrinha presentes em bolhas atuam na premunização. Fitopatologia Brasileira, 18:55-61.

Roistacher, C.N. 1988. Observations on the decline of sweet orange in coastal Peru caused by stem-pitting tristeza. FAO Plant Protection Bulletin, 36:19-26.

Roistacher , C.N. 1999. A report on a technical visit and consultancy to Jamaica. May 16 –21. FAO, Rome.

Roistacher, C.N. 2001a. http:/www.ecoport.org/EP.exe$SSPage?Show= 103&ID=2601, 2603,2607.

Roistacher, C.N. 2001b. http:/www.ecoport.org/EP.exe$SSPage?Show=103&ID=261

Roistacher, C.N. and Bar-Joseph, M. 1987. Transmission of Tristeza virus by *Aphis gossipii* and by graft inoculation to and from Passiflora spp. Phytophylactica 19: 179-182.

Roistacher, C.N., Dodds, J. A., and Bash, J. A. 1988. Cross protection against citrus tristeza seedling yellows and stem pitting viruses by protective isolates developed in greenhouse plants. In: "Proc. 10th Conf. IOCV" (ed. Timmer, L.W., Garnsey, S.M. and Navarro, L.) Univ. of Calif, Riverside, CA, pp. 91-100.

Salaman, R.N. 1937. Acquired immunity against the "Y" potato virus. Nature, 139:924-925.

Sasaki, A. 1979. Control of Hassaku dwarf by preimmunization with mild strain. Review of Plant Protection Research, 12: 80-87.

Sayama, H., Sato, T., Kominato, M. and Natsuaki, T. 1993. Field testing of a satellite containig attenuated strain of cucumber mosaic virus for tomato protection in Japan. Phytopathology, 83:405-410.

Segura, C. B. 1952. La "tristeza" de los citricos en el Peru. Informe n° 77. Centro Nacional de Investigación Y Experimentación Agricola La Molina, Lima-Peru, 14pp.

Shanmugasundaram, S., Ishii, M., Gilbert, J.C. and Nagai, H. 1969. Cucurbit virus studies in Hawaii. Plant Disease Reporter, 53:70-74.

Sheen, T.F., Wang, H.L. and Wang, D.N. 1998. Control of Papaya ringspot virus by cross protection and cultivation techniques. Journal of Japanese Society for Horticultural Science,. 67:1232-1235.

Sherwood, J.L. 1987. Mechanisms of cross-protection between plant virus strains. In: "Plant resistance to virus" (eds. Evered,V. and Harnett, S.) New York, Chichester, pp.136-150

Simmonds, J.H. 1959. Mild strain protection as a mean of reducing losses from the Queensland woodiness virus in the passion vine. Queensland Journal of Agricultural Science, 16:371-380.

Souza, A. A., Müller, G.W., Targon, M. L. P.N. and Machado, M. A. 2000. Evaluation of changes which occurred in a mild protective citrus tristeza virus isolate in pera sweet orange trees by using RFLP and SSCP analyses of the coat protein gene. In: "Proc. 14thConf. IOCV (ed. da Graça, J.V., Lee, R.F. and Yokomi, R.K.) Univ. of Calif., Riverside, CA, pp. 136-140.

Souza, A. A., Targon, M.L.P.N., Silva, F.A., Müller, G.W. and Machado, M. A. 2001. Host specificity of the protective isolate of the citrus tristeza virus. XV Conf. Int. Org. Citrus Virol., Paphos, Cyprus, Nov. 11-16, p.46 (programme & abstracts).

Souza Jr., M.T. and Gonsalves, D. 1999. Genetic engineering resistance to plant virus diseases, na effort to control Papaya ringspot virus in Brazil. Fitopatologia Brasileira, 24:485-502.

Stubbs, L.L. 1964. Transmission and protective inoculations studies with viruses of the citrus tristeza complex. Australian Journal of Agricultural Research, 15: 752-770.

Tanaka, T. 1932. Monograph on the Satsuma orange. Taihoku Imperial Univ. Formosa/ Japan, pp. 31-321

Tien, P., Zhang, X., Qui, B., Qin, B. and Wu, G. 1987. Satellite RNA for the control of plant diseases caused by cucumber mosaic virus. Annals of Applied Biology, 111:143-152.

Tien, P. and Wu, G. 1991. Satellite RNA for biological control of plant disease. Advances in Virus Research , 39:321-339.

Tomlinson, J.A. and Sheperd, R.J. 1978. Studies on mutagenesis and cross-protection of cauliflower mosaic virus. Annals of Applied Biology, 90: 223-31.

Ullman, D.E., Cho, J.J. and German, T.L. 1991. Occurrence and distribution of cucurbit viruses in the hawaiian islands. Plant Disease , 75:367-370.

Umesh, K.C., Valencia, J., Gubler, W.D. and Falk, B.W. 1995. The incidence of aphids and aphid-transmitted viruses in melon cultivars and breeding lines in California. Phytopathology, 85:1042 (abstract).

Urban, L.A., Sherwood, J.L., Rezende, J.A.M. and Melcher, U. 1989. Examination of mechanisms of cross protection with non-transgenic plants. In: "Recognition and response in plant-virus interactions" (ed. Fraser, R.S.S.) New York, Springer-Verlag, pp.415-426.

Van Regenmortel, M.H.V. 1971. Watermelon mosaic virus. CMI/AAB. Description of Plant Viruses, N° 63. 4 p.

Van Regenmortel, M.H.V., Farcquet, C.M., Bishop, D.H.C, Cartens, E.B, Ester, M.K., Lemon, S.M., Maniloff, J., Mayo, M.A., Mc Geoch, D.J., Pringle, C.R., Wickner, R.B. (eds.) 2000. Virus taxonomy: classification and nomenclature of virus. Academic Press. San Diego, USA.

Van Vuuren, S.P., Collins, R.P. and Graça, J.V. 1993. Growth and production of lime trees pre-immunized with mild citrus tristeza virus isolates. Pytophylactica, 25:39-42.

Von Broembsen, L.A. and Lee, A.T.C. 1988. South Africa´s Citrus Improvement Program. In: "Proc. 10th Conf.IOCV" (eds. Timmer, L.W., Garnsey, S.M. and Navarro, L.) Univ. of Calif., Riverside, CA, pp. 407-416.

Walkey, D.G.A., Lecoq, H., Collier, R. and Dobson, S. 1992. Studies on the control of zucchini yellow mosaic virus in courgettes by mild strain protection. Plant Pathology, 41:762-771.

Wang, H.L., Gonsalves, D., Provvidenti, R. and Lecoq, H.L. 1991. Effectiveness of cross protection by mild strain of zucchini yellow mosaic virus in cucumber, melon, and squash. Plant Disease, 75:203-207.

Wang, M. and Gonsalves, D. 1992. Artificial induction and evaluation of a mild isolate of tomato spotted wilt virus. Journal of Phytopathology, 135:233-244.

Wingard, S.A. 1928. Host and symptoms of ringspot, a virus disease of plants. Journal of

Agricultural Research, 37:127-153.

Wu, G., Kang, L. and Tien, P. 1989. The effect of satellite RNA on cross-protection among cucumber mosaic virus strains. Annals of Applied Biology, 114:489-496.

Yarden, G., Hemo, R., Livne, H., Maoz, E., Lev, E., Lecoq, H. and Raccah, B. 2000. Cross protection of cucurbitaceae from zucchini yellow mosaic potyvirus. Proceedings of 7th EUCARPIA Meeting on Cucurbit Genetics and Breeding Acta Horticulturae, n. 510, pp. 349-356.

Yeh, S.D. and Gonsalves, D. 1984. Evaluation of induced mutants of papaya ringspot virus for control by cross protection. Phytopathology, 74:1086-1091.

Yeh, S.D., Gonsalves, D., Wang, H.L., Namba, R. and Chiu, R.J. 1988. Control of papaya ringspot virus by cross protection. Plant Disease , 72:375-380.

Yeh, S.D. and Cheng, Y.H. 1989. Use of resistant *Cucumis metuliferus* for selection of nitrous-acid induced attenuated strains of papaya ringspot virus. Phytopathology, 79:1257-1261.

Yeh, S.D. and Gonsalves, D. 1994. Practices and perspective of control of papaya ringspot virus by cross protection. Advances in Disease Vector Research, 10:237-257.

Yoshida, K., Goto, T. and Iizuka, M. 1985. Attenuated isolates of cucumber mosaic virus produced by satellite RNA and cross-protection between attenuated and virulent ones. Annals of Phytopathological Society of Japan, 51:238-242.

Yuki, V.A, Costa, A.S. and Nagai, V. 1991. Avaliação de perdas induzidas pelo mosaico da abobrinha de moita, causado pelo vírus do mosaico do mamoeiro - estirpe melancia (VMM-Me). Summa Phytopathologica, 17:40. (abstract).

Yuki, V.A., Rezende, J.A.M., Kitajima, E. W., Barroso, P.A.V., Kuniyuki, H., Groppo, G.A. and Pavan, M.A. 2000. Occurrence, distribution and relative incidence of viruses infecting cucurbits in the States of São Paulo, Brazil. Plant Disease, 84:516-520.

Zitter, T. A., Hopkins, D.L. and Thomas, C.E. 1996. Compendium of Cucurbit Diseases. APS Press, American Phytopathological Society, St. Paul, Minnesota, USA.

Carrot Diseases and their Management

R.Michael Davis

Department of Plant Pathology, University of California, Davis 95616, USA

Abstract : Wherever carrots are grown, a variety of diseases reduces both the yield and the market value of the roots. Roots destined for the fresh market must be almost blemish-free; yet, at least three bacteria and twelve fungi cause lesions that reduce their value. Bunching carrots must have damage-free tops as well as roots but foliage, too, is attacked by a large number of pathogens. While tops are not an issue for bulk, cello-packed, or lightly processed carrots (*e.g.*, 'cut and peel'), healthy tops are critical for harvest since the undercut carrots are often mechanically picked up by the leaves. The presence of heavy infections causes inefficient harvesting and yield losses. Several viruses and phytoplasmas also cause damage to carrots both in the form of malformed roots and direct yield losses of plants. Because of the nature of the carrot root, damage caused by various nematodes is an important limiting factor in carrot production. Some nematodes, for example, have a 'zero tolerance' threshold, *i.e.*, the presence of nematodes in soil at the start of the season will result in some crop loss. Various pest control strategies, including cultural practices, such as irrigation management, crop rotation, the production of clean seed, and bed preparation, in addition to chemical disease control, are used to limit economic losses to diseases.

1. Introduction

A variety of diseases reduces both the yield and market value of the roots, wherever carrots are grown. Bunching carrots must have damage-free tops as well as roots. While tops are not an issue for bulk, cello-packed, or lightly processed carrots (*e.g.*, the 'cut and peel' market), healthy tops are critical for harvest since in many areas the undercut carrots are mechanically picked up by the leaves. Thus, weak tops result in inefficient harvesting. Control of insects, diseases, and weeds, therefore, is extremely important for optimum carrot culture.

Because many pathogens of carrots are seedborne, the distribution of many diseases, including some of the serious maladies, is worldwide. For example, *Alternaria* leaf blight and bacterial leaf blight, both of which can affect 100% of the acreage in particular region, are seedborne and found wherever carrots are grown. Worldwide, *Alternaria* leaf blight is considered the most economically important carrot disease. Complete crop losses from *Alternaria* epidemics have been reported on individual farms. Bacterial leaf blight, caused by *Xanthomonas campestris* pv. *carotae*, can also cause near 100 % crop losses in areas with warm and rainy weather. In dry areas such as California, however, losses to bacterial leaf blight are rare, despite the almost ubiquitous occurrence of the disease. In the United States, bacterial leaf blight is considered the fifth most economically important carrot disease. It is generally more severe in humid climates.

The root knot nematode (*Meloidogyne* spp.) is perhaps the next most serious disease pest of carrot worldwide. Carrots affected by nematodes often exhibit forking of the taproot, stubbing of the roots, and unsightly galls on the taproot and secondary roots. Because the root knot nematode has a wide host range, it is difficult to manage. Growers often use costly control measures, such as expensive fumigants and / or nematicides, to reduce losses to nematodes. Complete crop losses in carrots have been reported. Other nematodes cause local losses of carrots, but overall, losses are minimal.

In the United States, cavity spot, a soilborne disease, is considered the third most economically important carrot disease. This root malady has also been reported in Australia, Canada, France, Great Britain, Japan, and Norway. Although its occurrence is sporadic even in the areas where it causes significant economic losses, it no doubt occurs in many more areas. In the United States, it occurs on about 50% of the carrot-producing acreage in California and Washington, 25 % of the acreage in Colorado, and is sporadic in Wisconsin. Cavity spot occasionally causes complete crop losses in a field.

Pythium-induced diseases are often chronic and go unnoticed until the quality of the crop at harvest is assessed. These include root dieback, forking and stubbing, all of which result in a misshapened carrot that cannot be sold in the fresh market, and damping-off, which causes poor crop stands. As a group, the former diseases are considered the forth most serious malady of carrots in USA while damping-off is the sixth most economically important carrot disease. Although the incidence of these diseases can be as high as 100 %, generally the incidence is low. Occasionally, the majority of carrots in a field is misshapened and unsuitable for the fresh market.

In the United States, black rot is a serious local problem. In California, it causes an economically important disease of the neck area of carrots. In northern Europe and other carrot-growing regions with cool climates, black rot causes foliar blight and a postharvest decay of roots that are stored for long periods. Black rot is a seedborne disease, thus, it is found wherever carrots are grown. Areas all over the world where carrots are stored for long periods suffer losses from crater rot, violet root rot, black root rot, among other diseases.

Most diseases of carrots, like diseases of all vegetables, are weather related and their occurrence is closely tied with the climate. For example, in warm and dry climates, powdery mildew is an economically important disease like in Middle Eastern countries. In more humid areas, cottony rot and downy mildew sometimes cause significant losses.

Diseases caused by phytoplasmas and viruses occur where carrots are grown year-round or alternate hosts habour the pathogens and their vectors. In some areas, phytoplasma and / or viruses are the most important pests of carrots. For example, aster yellows is considered one of the most serious pests of carrots in Wisconsin, USA. In the state of Washington, another phytoplasma disease sometimes reaches epidemic proportions. In Australia, a newly discovered virus, carrot virus Y, threatens carrot production in that country.

2. Diseases Caused by Bacteria

2.1 Bacterial Leaf Blight

Bacterial leaf blight is a common disease of carrot worldwide and can cause near 100 % crop losses in areas with warm and rainy weather. In the United States, bacterial leaf blight is considered the fifth most economically important carrot disease. It is generally more severe in humid climates. In dry areas such as California, however, losses to bacterial leaf blight are rare, despite the almost ubiquitous occurrence of the disease.

2.1.1 Symptoms

Symptoms on leaves include irregular, brown, watersoaked lesions surrounded by yellow haloes. In age, the haloes disappear and the lesions become dry. The lesions are commonly observed on leaf margins, especially at the 'V' shaped junction of the leaflet lobes, resulting in leaf curling and distortion. In humid weather, a yellow-brown gummy exudate may be visible on infected leaves and petioles. On infected flower stalks, copious bacterial ooze exudes from elongated lesions. Infected umbels may be completely blighted.

2.1.2 Causal organism

The disease is caused by *Xanthomonas campestris* pv. *carotae* (Kendrick) Dye, an aerobic, Gram-negative rod.

2.1.3 Epidemiology

X. campestris pv. *carotae* is a common contaminant of carrot seed, which is an important source of primary inoculum as well as a means for long-distance dissemination. Both external and internal contamination is possible (Kendrick, 1934). In a study conducted in overhead-irrigated fields in the arid Central Valley of California, high levels of contamination (*i.e.*, $>10^4$ cfu/gram of seed) were necessary for the development of symptoms (Umesh *et al.*, 1998). Relatively high rates of contamination (*i.e.*, 10^7 cfu/gram of seed) were required for an epidemic to develop. However, lower levels of seed contamination could probably lead to disease development in areas with high rainfall and humidity. The bacterium persists in soil in association with carrot debris and when the debris decomposes, the bacterium is apparently unable to survive. The bacterium is spread plant-to-plant by splashing rain and/or irrigation water as well as on insects and contaminated farm implements. On leaves, *X. campestris* pv. *carotae* grows epiphytically. When bacterial populations reach certain levels (*e.g.*, $>10^6$ cfu/gram of leaf tissue), disease symptoms develop. Optimal temperatures for infection are 25 to 30ºC, but disease development can occur at warmer temperatures.

2.1.4 Management

Effective control of bacterial blight involves an integrated strategy that begins with the

planting of assayed seed. Contaminated lots should be treated with hot water (52° C for 25 minutes) and re-assayed on a semi-selective medium (Kuan *et al.,* 1985). Strategies that reduce hours of leaf wetness, such as reducing plant populations in the field, may reduce disease severity.

Copper-based bactericides are frequently used to slow the development of bacterial blight in the field, particularly if applications are initiated before infection occurs. Because *X. campestris* pv. *carotae* survives in crop debris, infected crop residue should be thoroughly incorporated to hasten decomposition. Crop rotations of 2 to 3 years should be practiced. Where feasible, the use of furrow rather than overhead irrigation can significantly reduce bacterial blight.

2.2 Scab

Scab, caused by the actinomycete *Streptomyces scabies* (Thaxter) Lambert & Loria, is usually a minor problem in carrots. However, it occasionally causes significant losses in crops that may or may not follow potatoes, a commonly infected host (Hanson and Lacy, 1990, Goyer and Beaulieu, 1997).

2.2.1 Symptoms

S. scabies infects carrot roots through wounds and natural openings, such as areas of lateral root emergence. It kills superficial cell layers and stimulates surrounding cells to form corky wound periderm. These scab lesions, which can be raised or crater-like, are typically oriented horizontally across the breadth of the root. Numerous lesions result in carrots unsuitable for the fresh market.

2.2.2 Epidemiology

In general, isolates of *S. scabies* are not host specific. In cross-inoculation studies, *S. scabies* isolates from carrot infected carrot, radish, and potato but not beets, and isolates from potato infected potato, carrot, radish, and beet (Goyer and Beaulieu, 1997). Artificial inoculation of carrots with potato isolates of *Streptomyces acidiscabies* Lambert & Loria and *S. caviscabies* Goyer, Faucher & Beaulieu also produced scab lesions (Goyer and Beaulieu, 1997, Lambert, 1991). However, these have not been reported from naturally infected carrots.

2.2.3 Management

S. scabies survives indefinitely in soil in infected plant debris. The pathogen is disseminated by wind, water, infected potato seed pieces, or infested soil on machinery. Cultural controls include acidifying the soil since growth of the bacterium is inhibited by low pH's. Disease incidence and severity are greatest in soils with a pH of 5.5 to 7.5. Incorporating sulfur into some soils effectively lowers the pH. If calcium fertilizers are necessary, gypsum should be used instead of lime since gypsum will not raise soil pH.

Because the disease is more severe in dry soil, adequate soil moisture should be maintained throughout the carrot cropping cycle. If carrots are grown with natural rainfall, supplemental irrigation may be necessary to maintain adequate soil moisture. Rotation with non-host crops, such as small grains, will reduce inoculum levels but should not be used as the sole method of control since damaging levels of inoculum may remain in the soil for many years.

2.3 Soft Rot

Soft rot is a common disease of most vegetables. In carrots, it causes disease both in the field and during storage. In the field, soft rot is often limited to low areas where water collects and its occurrence is erratic. However, severe, widespread outbreaks associated with warm temperatures and extended periods of soil saturation have been reported (Farrar *et al.,* 2000). In storage, soft rot is associated with wounds, contaminated wash water, or improper storage and transit conditions (Seagall and Dow, 1973).

2.3.1 Symptoms

Soft rot is characterized by pitting along the taproot or a soft decay of parts or all of the taproot (Fig. 1). Decay often progresses from the taproot tip to the crown. When pitting occurs, soft rot lesions are sunken and dull orange and the epidermis either rots or remains intact. The middle lamella between cells in affected tissues is dissolved by pectin degrading enzymes and the tissue often collapses into a soft mass. In some situations, a soft rot develops that leaves the epidermal tissue intact while the entire core rots (Tower and Beraha, 1976). The odor of infected tissue is generally not foul unless secondary organisms invade.

2.3.2 Causal organism

Soft rot is most commonly caused by the bacteria *Erwinia carotovora* subsp. *carotovora* (Jones) Bergey *et al*. and *E. chrysanthemi* Burkholder, McFadden & Dimock. Both species are single-celled, Gram-negative rods that are motile by peritrichous flagella. They are facultatively anaerobic, oxidase negative, and catalase positive. They ferment glucose, reduce nitrate to nitrite, produce H_2S from sodium thiosulfate, grow at 36° C, and produce deep pits on selective media containing sodium polypectate. *E. carotovora* subsp. *carotovora* is phosphatase negative, insensitive to erythromycin, does not utilize malonate, and produces acid from trehalose. In contrast, *E. chrysanthemi* is phosphatase positive, sensitive to erythromycin, utilizes malonate, and does not produce acid from trehalose. Both bacteria have very wide host ranges.

2.3.3 Epidemiology

E. carotovora subsp. *carotovora* is ubiquitous in plant tissue in soil and is found in many surface water sources. *E. chrysanthemi* is also widespread but its epidemiology is largely unknown. Both bacteria overwinter in crop residue. Bacterial cells enter the

plant through wounds and natural openings and rapidly degrade tissue under favorable temperatures (20 to 25° C for *E. carotovora* subsp. *carotovora* and 30 to 35° C for *E. chrysanthemi*). Long periods of soil saturation are necessary for field infection and symptom development. Post-harvest handling wounds, immersion in contaminated wash water, and unrefrigerated storage can increase the incidence of post-harvest soft rot.

Figure 1: Pitting of carrot caused by the bacterium *Erwinia carotovora* subsp. c*arotovora*.

2.3.4 Management

Control of soft rot in the field includes proper irrigation and avoiding wounds. Because *E. carotovora* subsp. *carotovora* and *E. chrysanthemi* are facultative anaerobes, plant tissues deprived of oxygen, a condition that occurs in saturated soil, are especially susceptible to infection. Therefore, fields should drain well and should not be over-irrigated, especially during warm weather. In general, soft rot incidence is corre-

lated with increasing durations of soil saturation and increasing temperature (Farrar *et al.,* 2000). To prevent postharvest softrots, minimize wounds during harvest, chlorinate the wash water, regularly clean all processing lines, and store carrots close to freezing with 95% relative humidity. In one study, carrots stored at 2°C for 3 days followed by incubation at 21°C reduced soft rot compared to the incidence of rot in carrots held at 21°C (Segall and Dow, 1973). Because certain isocoumarins are produced after storage at cold temperatures, but not in freshly harvested carrots, these compounds may be partly responsible for the observed increased resistance to soft rot in cold storage (Sondheimer, 1957).

3. Diseases Caused by Fungi

3.1 Foliar diseases

3.1.1 *Alternaria* leaf blight

Alternaria leaf blight is considered the most economically important carrot disease worldwide. Complete crop losses from *Alternaria* epidemics have been reported on individual farms.

3.1.1.1 Symptoms

Symptoms of the disease on leaves include dark brown to black lesions that may or may not be surrounded by yellow halos and restricted by leaf veins. Lesions are often most numerous on leaf margins of older leaves. Because these symptoms are similar to those caused by bacterial leaf blight, laboratory analysis may be necessary for an accurate diagnosis. Under warm and humid conditions, lesions coalesce and cause severe foliar blight. When about half of the leaf area is affected, the entire carrot leaf yellows, collapses, and dies. Petiole lesions are elongate and dark brown or blackish. Under optimal conditions, severe foliar epidemics develop rapidly, leading to loss of foliage and reduced yields. Leaf blight also indirectly reduces yields since roots are left in the ground when the weakened foliage breaks from the root during mechanical harvests.

3.1.1.2 Causal organism

Alternaria leaf blight, caused by *Alternaria dauci* (Kuehn) Groves & Skolko *(*synonym: *Alternaria porri* (Ell.) Neergaard f. sp. *dauci* Kühn. *Alternaria dauci* produces distinctive conidia and conidiophores. Conidia are borne singly or very rarely in chains of two. Mature conidia possess long terminal filamentous beaks that are often three times the length of the conidium body, which are ellipsoid to obclavate, brown, often minutely punctulate, and 50 to 100 μm long x 12 to 24 μm wide. They have 5 to 11 transepta and one to several longisepta per segment. The olive-brown conidiophores form singly or in small clusters. On potato-dextrose agar, a distinctive light purple

pigment diffuses from most colonies into the surrounding medium.

3.1.1.3 Epidemiology

Seed-borne inoculum in the form of spores on the surface of the seed and as dormant mycelia and conidia within the seed mericarp is important in the establishment of *Alternaria* leaf blight in new production areas (Strandberg, 1983). When damping-off results from contaminated seed, the fungus sporulates abundantly on the dead and dying seedlings. As humidity drops in the morning, spores are released and are spread by air turbulence to other plants and nearby fields (Langenberg *et al.,* 1977).

The fungus overwinters on seed, in crop residue, and on volunteer and wild carrots. Moderate to warm temperatures and prolonged leaf wetness favor infection. Incubation at 16 to 28° C in 100% relative humidity for 12 hours is required for infection of carrot leaves (Strandberg , 1988). Free water from dew, rain, or overhead irrigation prolong leaf wetness and greatly enhance disease development. Spray forecast models are available that estimate risks of infection based on temperature and duration of leaf wetness (Gillespie *et al.,* 1979).

3.1.1.4 Management

The control of *Alternaria* leaf blight is optimized with an integrated program that uses several strategies. Because *A. dauci* can be seedborne, all seed lots should be assayed for the presence of *A. dauci* on blotter paper. If contamination occurs, the seed can be soaked in a warm (30° C) fungicide suspension to reduce seed contamination (Maude, 1966, Maude, 1992). Residue from affected carrot crops should be incorporated soon after harvest since the fungus will not survive in soil in the absence of host tissue. Some carrot cultivars are fairly resistant to *Alternaria* leaf blight and a wide range of disease tolerances exists among commercial cultivars.

In the field, fungicides are frequently used to control *Alternaria* blight but as the crop matures and the leaf canopy becomes increasingly dense, good coverage is difficult to obtain. In most cases, applications of fungicides should be initiated before the disease first appears. Applications of gibberellic acid to carrot foliage may be used with or without fungicides. Gibberellic acid consistently increases the length of leaves and diameters of petioles, resulting in a more upright habit of the foliage, which may improve air movement through the canopy and thus reduce leaf wetness (Santos *et al*. 2000). Applications of gibberellic acid may provide an additional benefit by improving the harvestability of the crop since the majority of carrots destined for the fresh market are harvested by lifting the roots by their tops. Treated foliage is more robust and better withstands damage by pathogens as well as cold temperatures of winter.

3.1.2 *Cercospora* leaf blight

3.1.2.1 Symptoms

The tan leaf lesions, which are initially surrounded by a chlorotic halo, enlarge into

brown necrotic spots. Lesions on the leaf blades are circular to oval; lesions on leaf margins are elongated. Eventually, lesions coalesce, causing leaflets to die. Petiole lesions are elliptical and brown with a tan center but become grayish when the fungus sporulates. *Cercospora* blight first occurs on young foliage, in contrast to *Alternaria* blight, which first occurs on older tissue. Severe infection of *Cercospora* blight results in the death of the entire leaf, which results in yield losses since taproots break from the foliage when gripped by mechanical harvesters.

3.1.2.2 Causal organism

Cercospora leaf blight, caused by the imperfect fungus, *Cercospora carotae* (Pass) Solheim, occurs wherever carrots are grown. The mycelium of *C. carotae* is septate and hyaline to light brown. Conidiophores typically arise in clusters from a pseudostromata that develops in a substomatal cavity. Conidiophores are olivaceous brown and bear conidia successively at the tip as the conidiophore develops. Conidia are one- to multi-septate, filiform (40 to 110 μm long x 2.2 to 2.5 μm wide), and almost hyaline. Optimum growth of *C. carotae* is 19 to 28ºC.

3.1.2.3 Epidemiology

Infested seed, host debris, or wild carrot are sources of primary inoculum. Conidia are dispersed by wind, splashing rain and overhead irrigation, and on farm implements equipment and workers. Infection occurs in a minimum of 12 hours of leaf wetness at temperatures of 20 to 28ºC (Carisse and Kushalappa, 1990). A minimum of 24 hours of leaf wetness was necessary to induce severe infection. Symptoms may appear in as few as 3 to 5 days following inoculation under ideal conditions.

3.1.2.4 Management

Control measures include planting pathogen-free seed, crop rotation, and prompt incorporation of crop residue. Cultivar resistance has been identified and is available in commercial cultivars (Angell and Gabelman, 1968). Fungicides also effectively reduce *Cercospora* blight. In some areas, a fungicide spray program based on disease sampling and weather is used to optimize timing of fungicide applications (Kushalappa *et al.,*1989). In one scenario, disease incidence is determined at biweekly intervals, beginning at the five-leaf stage, by randomly sampling 50 plants in the field. Fungicidal sprays are initiated once 50% of the middle leaves exhibit symptoms. Subsequent applications should be made at 7 to 10 day intervals, provided temperatures exceed 16º C and leaf wetness durations exceed 12 hours.

3.1.3 Downy mildew

3.1.3.1 Symptoms

Symptoms are visible on the upper side of the leaves as chlorotic spots and as whitish

sporulation on the corresponding underside of the leaf. Infection first appears on young foliage. During periods of high humidity, sporangiophores emerge in groups through stomata and release airborne sporangia (19 to 22 x 16 to 18 (m in diameter), which germinate to produce motile zoospores that swim in free water on plant surfaces, eventually infecting through stomata. Sexual oospores, which are produced within the tissue, may survive the winter in crop debris or in seed.

3.1.3.2 Causal organism

Downy mildew occurs on many plants of the umbelliferae family, although strains that infect carrot only occur in Europe. The causal agent is *Plasmopara umbelliferarum* (Caspary) Schröter ex Watenw, an obligate parasite that requires living host tissue to grow and reproduce. Synonyms that occur in the literature include *Plasmopara crustosa* (Fr.:Fr.) Jorst, *Plasmopara nivea* (Unger) J. Schröt., and *Peronospora umbelliferarum* Unger. There may be more than one species of *Plasmopara* on various umbelliferous plants since morphology of the fungus varies widely with the host plant (Constatinescu, 1992).

3.1.3.3 Management

Several cultural practices can be adopted to manage downy mildew. Because the fungus may survive in seed, only pathogen-free seed should be planted. Strategies that minimize the duration of leaf wetness, such as decreasing plant density, avoiding the use of excess fertilizers, and managing irrigation and drainage may reduce the incidence of disease. Carrots should be rotated with non-umbelliferous crops to reduce the inoculum load in the environment.

3.1.4 Powdery mildew

3.1.4.1 Symptoms and causal organism

Powdery mildew occurs wherever carrots are grown. Two species of powdery mildew attack umbelliferous crops. The most common one on carrots is *Erysiphe heraclei* DC. Synonyms of *E. heraclei* that appear in the literature are *E. polygoni* DC and *E. umbelliferarum* de Bary (Braun, 1995). The asexual stage is Oidium. *E. heraclei* produces white mycelium and sporulation, which are conspicuous and often dense. All above-ground plant parts, including leaves and petioles, as well as flower stalks and bracts, are susceptible and exhibit powdery fungal growth. As spots enlarge on leaves, the foliage becomes chlorotic. Leaves can survive heavy infections, although they may senesce prematurely. The disease appears first on the older leaves and then spreads to the younger foliage. Depending on the crop, the severity of the disease, and the growth stage of the crop at disease onset, significant yield reductions can occur. Powdery mildew is particularly important in Mediterranean climates. *E. heraclei* occurs on many other umbelliferous crops, including anise, caraway, chervil, dill, parsnip, and parsley.

E. heraclei is ectophytic, *i.e.*, it grows primarily external to the plant with only haustoria penetrating the host epidermal cells. Sporulation on carrot tissue occurs 7 to 14 days after infection. The mycelium of *E. heraclei* is highly branched and produces lobed haustoria. Hyphal cells are 55 to 85 μm long and 4 to 5 μm wide. The conidiophores are moderately long (60 to 140 μm) and straight. They possess a cylindrical foot cell that measures 20 to 35 x 8 to 10 μm followed by a longer cell and one or two shorter cells. Cylindrical conidia (25 to 45 x 12 to 21 μm) are formed singly. Germ tubes, which are located at the ends of conidia, form lobed or club-shaped appressoria. Cleistothecia, the sexual fruiting structures, are 80 to 120 μm in diameter with few to numerous appendages that are basally inserted, mycelioid, and brown. These appendages are mostly as long as the cleistothecial diameter and are usually irregularly branched, resulting in a coral-like appearance. There are three to six asci per cleistothecium (rarely as few as two or as many as ten) and three to five ascospores (rarely two or six) per ascus. Ascospores are relatively large (18 to 30 x 10 to 16 μm) and ovate to elliptic.

The other powdery mildew that occurs on carrot is *Leveillula lanuginosa* Fuckel (synonym: *Erysiphe lanuginosa* (Fuck.) Golovin), which is generally limited to the Middle East, Armenia, India, Kazakhstan and other countries of Central Asia, Pakistan, and the Mediterranean regions of Europe and Africa. In addition to carrots, it infects anise, caraway, celery, coriander, dill, fennel, and parsley. It is sporadic and of minor economic importance.

Leveillula lanuginosa causes pale yellow areas on the upper leaf surface with associated whitish sporulation on the lower leaf surface. Infected areas may be limited by veins, thus giving the lesions an angular appearance. In advanced stages, sporulation also appears on the upper side of the leaf and the yellow areas turn brown. Severely affected areas eventually dry. Petioles are also infected. *L. lanuginosa* produces Oidiopsis-type conidia with mycelium that is both endophytic and external (as compared with the Oidium-type mildew, which is only external). Fungal growth is typically persistent, but is not as conspicuous as the Oidium-type mildew. The conidia of *L. lanuginosa* are cylindrical (around 40 to 80 x 13 to 20 μm) with distinctive rings near the ends. The conidiophores of *L. lanuginosa* are 200 to 250 μm long. Cleistothecia of *L. lanuginosa* are gregarious, sub-spherical, about 170 to 250 μm in diameter, and decorated with a few to numerous appendages on the lower half of the ascocarp. These appendages are typically shorter than the diameter of the cleistothecium, mycelioid, hyaline to yellowish, septate, often irregularly branched, interwoven with each other and with the mycelium, and measure about 4 to 10 μm wide. The asci are numerous (mostly more than 20 per cleistothecium), stalked, slender (75 to 100 x 25 to 35 μm), and two-spored. Ascospores are hyaline, one-celled, ovoid, and measure about 30 to 35 x 15 to 20 μm.

3.1.4.2 Epidemiology

Conidia of both *Erysiphe* and *Leveillula* are light and can be carried long distances in the air. The spores are unique among fungal pathogens in their lack of a requirement for free water for germination. High humidity and moderate temperatures favor infec-

tion and disease development. Powdery mildew is more severe under shady conditions, as sunlight damages the spores and mycelium. Crops become more susceptible as they age. In Israel, the earliest age at which carrots were affected was 50 days after sowing (Palti, 1975). Rain or sprinkler irrigations tend to reduce disease severity. In general, powdery mildews tend to be more common and severe in warm, dry climates. This is particularly true of *Leveillula*. For example, in Israel, *Leveillula* on carrot occurs only in the driest part of the country.

Cleistothecia, if formed, may survive on debris and have been reported as contaminants in seeds of carrot, fennel, parsley, and parsnip, but transmission via seed has not been documented. In the absence of cleistothecia, infection of new crops probably depends on air-borne conidia from other crops or wild umbelliferous hosts.

3.1.4.3 Management

Applications of sulfur are the most common chemical control but fungicides are not typically warranted unless the disease appears early in the growing season. Cultural controls include the use of tolerant cultivars, maintenance of good plant vigor while avoiding excess fertilization, and avoiding shady growing conditions and/or water stress. In Israel, mulches applied to carrot crops to reduce drought stress significantly reduced severity of powdery mildew (Palti, 1975).

3.1.5 Rust

3.1.5.1 Symptoms and causal organism

Rusts on umbelliferous crops are not economically important, although they are not uncommon in many areas. Initial symptoms of rust of carrot include a light green discoloration around infection sites. Later, the upper surface of the leaf becomes chlorotic around the lesion while yellow-orange pustules of spores often form on the underside of the leaf. Infected stems bend or arch and appear distorted or swollen. Severe infections may stunt plants.

Both autoecious and heteroecious rusts occur on umbelliferous plants. Autoecious rusts complete their life cycles on one host while heteroecious rusts typically produce their spermagonial and aecial stages on one host and their uredinal and telial stages on a second host, usually in a different plant family. In their natural habitat, rust fungi are obligate parasites, although a few can be grown on artificial media.

At least two rusts have been reported on carrot. *Uromyces graminis* (Niessl) Diet, which also occurs on fennel as well as other umbelliferous plants, produces aecidia on its umbelliferous hosts and uredinia and telia on *Melica* spp. It is found in Central Asia, Mediterranean regions, southern Russia, and South America. Urediniospores are globoid, golden, echinulate, and 22 to 30 µm in diameter. The pedicellate, one-celled teliospores are mostly ellipsoid or obovoid, a deep golden to clear chestnut-brown color, smooth, and 22 to 31 x 17 to 24 µm (Arthur, 1934, Wilson and Henderson, 1966). *Uromyces lineolatus* (Desm.) Schroet. (synonym: *U. scirpi*), another heteroecious rust, occurs on carrots in Bermuda, Canada, Europe, and the U.S. Aecidia occur on umbel-

liferous species and uredinia and telia occur on *Scirpus* spp. in the family Cyperaceae. Urediniospores are yellowish brown in color and 16 to 25 x 22 to 35 μm in size. The wall of the urediniospore is 1.5 to 2 (thick and minutely echinulate with three equatorial pores. Teliospores are brownish-black, clavoid, thickened apically, smooth, and 15 to 24 x 26 to 45 μm. They have persistent pedicels as long as or longer than the spore (Arthur 1934, Wilson and Henderson 1966).

3.1.5.2 Epidemiology and management

Little information is available on the epidemiology of rusts of umbelliferous crops, probably because they cause little economic damage. Because these fungi are obligate parasites, disease development depends on inoculum from alternate crops, wild hosts, and volunteers. Control measures include providing good field drainage to reduce humidity, removing nearby alternate hosts if the rust of concern is heteroecious, and the use of fungicides (systemic fungicides are reportedly more effective than protectant ones).

3.2 Root Diseases

3.2.1 Black Rot

Black rot occurs in most carrot production areas of the world. Although the disease is typically manifested as a black crown rot, it also causes seedling disease and a foliage and umbel blight. Where carrots are stored in bulk for extended periods, black rot is an important post-harvest disease.

3.2.1.1 Symptoms

A black decay of the lower petioles, which is often restricted to the petiole base and upper portion of the storage root, results in a diagnostic black ring of decay at the points of petiole attachment (Fig. 2). Decayed petioles that break during mechanical harvesting reduce yields. As a root rot, black sunken lesions develop on the taproot below the soil-line. Any stage of growth can be infected but older plants and senescent tissues are particularly susceptible.

Foliar blight symptoms begin with small necrotic spots that are often surrounded by a chlorotic margin. As lesions expand and coalesce, a black necrosis of the entire leaflet may result. Symptoms of umbel blight include necrotic lesions on the umbel stalk and on the inflorescence. Symptoms of damping-off, foliar blight, and umbel blight are similar to those of *Alternaria* leaf blight caused by *Alternaria dauci*.

As a post-harvest disease, black rot is characterized by dry, black sunken lesions on the surface of carrot taproots. Typically, margins of the lesions are distinct and clearly delineate diseased and healthy carrot tissue. Even in cold, moist conditions of storage, lesions can expand, coalesce, and decay the entire root. In bulk storage, the disease can spread from infected carrots to healthy ones.

3.2.1.2 Causal organism

Black rot is caused by the fungus *Alternaria radicina* Meier, Drechsler & Eddy (synonym: *Stemphylium radicinum* (Meier, Drechsler, & Eddy) Neergaard). Hyphae are subhyaline to olive-brown, septate, and 2.5 to 10 μm wide. The dark olive-brown conidiophores are 4 to 10 μm wide and 10 to 200 μm long. They are usually formed singly or in small clusters and are generally unbranched. One to three conidial scars

Figure 2: Necrosis of carrot crowns caused by *Alternaria radicina*, the cause of black rot.

are visible. Unlike many *Alternaria* species, conidia of *A. radicina* generally are borne singly, or occasionally in chains of two. In cultures less than 15 days old, conidia typically are dark olive-brown, broadly ellipsoid to ovoid, and 10 to 25 x 20 to 50 μm with two to five transepta and one to three longisepta in any or all segments, except the basal and apical segments, which are usually free of septa. Septa are well defined and darker than outer walls. Less frequently, conidia mature into long and narrow forms, which are broadly ellipsoid to obclavate and 15 to 20 x 50 to 65 μm with seven to eight

transepta and one to two longisepta in most of the segments. In cultures older than 15 days, an increasing proportion of conidia become subspherical and very dark brown with numerous oblique septa. No sexual state is known.

On malt or potato-dextrose agar, colonies typically are gray-black to black with dense wooly mycelia and irregular margins. Colonies typically produce a yellow pigment that diffuses throughout the medium and white dentritic crystals that form beneath the mycelial mat. These crystals are composed of the mycotoxin radicinin, a keto-lactone that also has phytotoxic properties (Grove, 1964).

3.2.1.3 Epidemiology

Alternaria radicina is a seed-borne pathogen, occurring as conidia on the seed surface and as mycelium in the inner layers of the pericarp or, occasionally, in the testa. The fungus has not been detected in the endosperm or in the embryo. The long-term consequence of planting infested seed is the introduction of the fungus into new fields and production areas. The pathogen also survives in association with crop debris and as free spores in the soil (Pryor *et al*. 1998). In long-term studies, the fungus survived eight years in soil in the absence of carrot cultivation (Maude and Shuring, 1972).

3.2.1.4 Management

Management of black rot in the field can be difficult. Because the disease often begins at the base of senescing carrot petioles or around the carrot crown, it is difficult to target fungicide applications at the base of the leaves once the crop canopy has closed. Relatively long crop rotations of 3 to 4 years are needed to effectively reduce soil-borne inoculum levels since the fungus survives for long periods in soil. To minimize reproduction of the pathogen on carrot tissue, crop residues should be incorporated into the soil promptly after harvest. Commercial carrot cultivars with resistance to black rot are available (Pryor *et al*. 2000).

The use of pathogen-free (assayed) seed is probably the most important component in the integrated management of black rot, especially in new production areas (Tylkowska, 1992). Routine treatment of seed by a hot water dip (50º C for 20 min) or a soak in a warm fungicide suspension may keep seed transmission to a minimum (Maude 1966, Pryor *et al*. 1994).

To control black rot in storage, carrots should be washed and culled prior to storage to reduce inoculum in the storage facility. Wounding and breakage should be kept to a minimum. Maintaining proper temperature and humidity control during storage (0 to 1(C and about 95% relative humidity) will prevent carrot deterioration and reduce the opportunity for disease spread.

3.2.2 Cavity Spot

Cavity spot has been reported in most carrot-producing regions of the world, including Australia, Canada, France, Great Britain, Japan, Norway, and the U.S. The disease occurs on carrots grown in both mineral and organic soils. While the disease rarely

reduces the actual yield of carrots, it can be economically important because affected roots are unsuitable for the fresh market.

3.2.2.1 Symptoms

The first symptoms of cavity spot are sunken, elliptical lesions oriented across the breadth of the root (Fig. 3). The lesions form under the intact periderm and are gray in color. Later, the periderm ruptures and dark, elongated lesions develop. These may occur randomly on the root or may be more dense on the upper half. Small vertical cracks are sometimes associated with the cavities. Cavities that are not infected by secondary organisms may become covered with callus tissue as the roots grow, leaving a clean, shallow, laterally elongated scar.

3.2.2.2 Causal organism

Cavity spot can be caused by several species of *Pythium. P. violae* Chesters & Hickman and *P. sulcatum* Pratt & Mitchell, which are slow-growing species (colony growth < 15 mm per day), are the most virulent. *P. violae* is the most important cause of cavity spot in California, Canada, France, and Great Britain, whereas *P. sulcatum* is more important in Australia and Japan. Other *Pythium* species capable of causing cavity spot symptoms are *P. intermedium* de Bary, *P. irregulare* Buisman, *P. sylvaticum* Campbell & Hendrix, and *P. ultimum* Trow.

      Before the role of *Pythium* species in cavity spot etiology was firmly established, cavity spot was attributed to several causes, including infection by anaerobic bacteria, an excess of ammonia, the feeding of fungus gnat larvae, and a deficiency of calcium (Perry and Harrison, 1979). While some or all of these factors may affect carrot root health, or interact with *Pythium* spp., *Pythium* species appear to be the primary cause (Groom and Perry, 1985, White, 1988).

3.2.2.3 Epidemiology

Little is known about the life cycle and population dynamics of *P. violae* and *P. sulcatum* in soil. These species can infect several different hosts and persist in the soil for a period of years. Germination of resting spores (oogonia and hyphal swellings) probably occurs quickly in response to root exudates. Direct infection occurs through the unwounded surface of the root. Disease incidence and severity tend to increase as the plants approach maturity.

      Intermittent heavy rains, poorly drained soils, and moderate temperatures favor disease development. Flooding of soil for a period of 24 hours increases the number of cavities; in dry soil, disease incidence is minimal. The optimum soil temperature for disease development is approximately 15°C (Vivoda *et al*. 1991).

3.2.2.4 Management

A number of cultural practices can reduce the incidence of cavity spot. Where possible,

avoid carrot production in fields with a history of cavity spot. A serological method to detect the presence of *P. violae* in soil is sometimes used in Great Britain. Because the disease increases in cool wet soils, seeding in relatively warm soils with good drainage may reduce disease. Cavity spot is sometimes associated with excessive levels of fertilizer and regular soil testing should be practiced. In Britain, cavity spot was scarce in fields with a soil pH over 8 (White, 1988). However, in a California study, disease severity was not related to soil pH, electrical conductivity, moisture-holding capacity,

Figure 3: Sunken, necrotic cavity spot lesions caused by *Pythium violae*.

organic matter, total and exchangeable calcium, particle size distribution, or planting density (Vivoda *et al.,* 1991). Timely harvests can reduce disease incidence since the disease becomes more obvious later in the season and older roots are more susceptible. Long-term cold storage has little effect on disease incidence or severity.

Differences in the susceptibility of carrot cultivars to cavity spot have been identified, but no commercially available carrots are completely resistant. Although long crop rotations of at least 3 to 4 years are recommended, many *Pythium* species

have wide host ranges. *P. violae*, for example, infects such diverse crops as alfalfa, broccoli, celery, cowpea, cucumber, sugarbeet, and wheat (Schrandt *et al.* 1994). Some of these crops may be asymptomatic. Because the pathogen has been reported on wild violet and other weeds, good weed management is also important.

The fungicide metalaxyl (or mefenoxam, one of the isomers of metalaxyl) is often used for the control of cavity spot. On sandy or loam soils it is most effective when applied more than once during the growing season. On soils with high organic matter content, a single application within six weeks of seeding provides season-long control. Apparent failures of metalaxyl to control cavity spot can result from the rapid degradation of the fungicide in soil.

3.2.3 Cottony Rot

Diseases caused by *Sclerotinia sclerotiorum* (Lib.) de Bary are known as cottony rot (of carrots), pink rot (of celery), and white mold (of many other vegetables). These diseases occur wherever umbelliferous crops are grown and are significant problems both in production and in storage. At least 408 plant species in 278 genera representing 78 families are affected. A majority of these are herbaceous, dicotyledonous plant species, but several monocotyledonous and gymnosperm species are also affected.

3.2.3.1 Symptoms

On carrots, cottony rot begins as small, watersoaked, soft lesions on crowns and roots. Subsequently, characteristic white, fluffy mycelial mats develop all over the infected tissues, leading to further softening and decaying of affected areas. Eventually, large, black sclerotia form in the rotted tissue.

3.2.3.2 Causal organism

Cottony rot is caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. In culture, the fungus produces distinctive rings of white mycelium and dark sclerotia at the growing margin of the colony. The sclerotia measure 10 to 20 x 5 to 7 mm and are black outside and white inside, although very old sclerotia are black throughout. The sclerotial rind is composed of a layer of dark-walled globose cells two to six cells thick.

3.2.3.3 Epidemiology

S. sclerotiorum can survive in soil as sclerotia for up to 10 years (Ben-Yephet *et al.*, 1993). Occasionally, it also may survive as active mycelium in living or dead plants. After a two-week period of chilling (4º C) and soil moisture near saturation, sclerotia of *S. sclerotiorum* located 2 to 3 cm below the soil surface germinate carpogenically by producing one to several white to tannish, cup-shaped apothecia (Dillard *et al.*, 1995). Asci are cylindric-clavate, up to 130 x 10 ?m, and contain eight spores. The apothecia produce and release millions of airborne ascospores, which are dispersed by wind throughout the field and to adjacent fields. Ascospores are nonseptate, uniseriate,

hyaline, and elliptical (9 to 13 x 4.6 (m). Ascospore release occurs over a period of 2 to 3 weeks. Daily release patterns during this period are cyclic; most spores are released between 10 AM and noon each morning and taper off to near nil by 2 PM. After the ascospores land on and colonize senescing or dead tissue, the fungus infects healthy tissue in the presence of free water for 48 or more hours. Mycelial infection from eruptively germinating *S. sclerotiorum* sclerotia is rare in nature.

3.2.3.4 Management

Fungicides are sometimes needed in carrot fields when conditions are cool and damp for extended periods of time. Soil fumigation is effective but is generally uneconomical in most areas (Ben-Yephet *et al.*, 1986). There are, however, a number of cultural practices important in the management of Sclerotinia diseases (Subbarao, 1998). Irrigation manipulation such as subsurface-drip provides good control by keeping the top 5 to 8 cm of soil on the planting beds dry. Where feasible, soil flooding may provide acceptable levels of control. Deep-plowing removes sclerotia from the infection court, although plowing a second time may return sclerotia to the soil surface (Merriman *et al.*, 1979). Sclerotia buried at a depth of up to 30 cm can survive for at least 15 months (Adams, 1975). Trimming carrot foliage allows greater air movement through the canopy and reduces free moisture, which may help to some degree in the management of the disease. Rotations with nonhosts, such as small grains, should be practiced. Resistance to *Sclerotinia* in most economically important crops, including carrots, is unavailable.

The key to controlling the disease in storage is to cull and clean the crop at harvest. Shipping and storage should be in sanitized containers at 0(C and near 95% relative humidity. During storage, condensation of moisture must be prevented.

3.2.4 Crown rot

Crown rot is a sporadic problem of mature carrots, although the disease occasionally manifests itself early in the season as seedling damping-off. Lesions on the carrot crown or on the taproot can result in unmarketable roots. Crown rot is more severe on muck-grown carrots and in regions that have warm weather and wet conditions near harvest (Mildenhall and Williams, 1973).

3.2.4.1 Symptoms

Above-ground symptoms include premature senescence and death of foliage, which is sometimes apparent as patches of dying plants or disease foci in the field. A rotting of the petioles and crown tissues may develop. On roots, dark brown sunken lesions or cankers are visible near the crown and occasionally further down on the root. These can be mistaken for cavity spot lesions caused by various species of *Pythium*, especially when root lesions are not accompanied by decay of crown tissue. Although crown rot is generally a dry rot, secondary invasion by bacteria can produce a soft rot. Under moist conditions or high ambient relative humidity, web-like mycelia may be visible in crown lesions.

3.2.4.2 Causal organism

Thanatephorus cucumeris (Frank) Donk (anamorph: *Rhizoctonia solani* Kühn) is a widely distributed soil inhabitant that survives by forming dark brown, undifferentiated sclerotia and by saprophytic colonization of plant debris. It produces hyphae that are relatively large, light brown, and septate. The many-branched hyphae are constricted at each point of branching. Isolates causing crown rot belong to anastomosis groups (AG) 2-2 and 4 (Grisham and Anderson, 1983, Mildenhall and Williams, 1970).

3.2.4.3 Epidemiology

Infection of the crown tissue from overwintering mycelium and sclerotia can occur at any time during the growing season if adequate moisture is available and temperatures are warm (> 18° C). Unless visible decay occurs on the petioles and crown, early infections may not be detected until the roots are harvested. Disease can be enhanced by cultural practices that place infested soil or debris in contact with the crown and petiole tissues. In some cases, the incidence of disease is associated with high levels of colonized organic matter in the soil. The pathogen can spread from plant to plant in closely spaced carrots when the canopy is fully formed, which provides a humid microclimate. Lesions on roots may continue to expand during storage and secondary colonization by other organisms, especially bacteria, is common.

3.2.4.4 Management

Cultural practices that minimize injury to the crown area and that enhance soil drainage and air circulation within the canopy are recommended. Before planting carrots, the residue from the previous crops should be allowed to decompose since the fungus colonizes fresh organic matter. Plantings of carrots following perennial crops such as alfalfa may suffer from severe crown rot infections. Late-season fungicide use may be required in situations where harvest is delayed by wet weather, although placement of the fungicide near the crown is problematic in mature carrots (Gurkin and Jenkins, 1985). Rotation of fields to small grains may help reduce inoculum levels.

3.2.5 Damping-off

3.2.5.1 Symptoms

Damping-off occurs wherever carrots are grown. The effects of damping-off are poor seed germination, root dieback due to the loss of the root apical meristem, and seedling death. Symptoms include seed decay and pre-emergence and post-emergence plant death. Infected seeds are soft and discolored and may fail to germinate. Infection of emerging seedlings may occur along any point of the plant. The infection may rapidly spread, killing the seedling before it emerges from the soil. When seedlings are infected after they emerge from the soil, infection often occurs at the soil line, girdling the stem. The infected tissue is watersoaked and often discolored reddish-brown. Plants

readily collapse. Above-ground symptoms include stunting and yellowing. The end result of damping-off is a poor stand.

3.2.5.2 Causal organism

Several *Pythium* spp., including *P. irregulare* Buisman, *P. mastophorum* Drechs., *P. ultimum* Trow, and others, cause damping-off of carrot seedlings. All are common soil inhabitants found wherever vegetables are grown. It has been observed that damping-off is more severe in soils with a history of successive carrot crops (Mildenhall *et al.*, 1971).

   Pythium species grow vegetatively by aseptate, colorless hyphae and produce thick-walled oospores and sporangia or hyphal swellings. Exudates from host plants stimulate the oospores and sporangia to germinate. In culture, *P. irregulare* seldom produces sporangia but produces globose hyphal swellings up to 25 μm in diameter. Oogonia (about 18.5 μm in diameter) are smooth or ornamented with a varying number of short, conical or finger-like projections (mostly none to five per oogonium). Oospores are apleurotic and mostly 16 μm in diameter. Antheridia are usually monoclinous.

   Rhizoctonia solani Kühn (AG-2 type 2 and to a lesser extent AG-1 and AG-4) primarily causes post-emergence damping-off (Grisham and Anderson 1983). *R. solani* causes light to dark brown stem lesions at or near the soil line. The fungus produces septated hyphae that are relatively large (5 to 10 μm in diameter) and always some shade of brown at maturity. The hyphae are constricted at each point of branching and a septum forms near the origin of each branch. Small, brown sclerotia are often formed in association with plant debris in the soil. These sclerotia consist of tight masses of hyphae that are never differentiated into a rind and medulla.

3.2.5.3 Epidemiology and management

Conditions that delay seed germination and slow seedling growth, such as cool, moist, poorly drained soils, favor seedling diseases. In some areas, damping-off caused by *R. solani* is more common in warm soils as opposed to *Pythium*, which is active in cool soils (Mildenhall and Williams, 1973). Both fungi are transported by water, contaminated soil on equipment, and movement of infected plant materials. Both have wide host ranges and exist indefinitely in most agricultural soils.

   Because damping-off is most severe when crops are grown in conditions not conducive to rapid seed germination and seedling emergence, avoid planting into cool, wet, and poorly drained soil. Fields should be prepared so that water does not stand. Although crop rotations do not eliminate the pathogens because of their wide host ranges, rotations with crops like small grains help reduce inoculum levels. Seed treatments with appropriate fungicides may provide some protection from seedling diseases.

3.2.6 *Itersonilia* Canker

3.2.6.1 Symptoms

Among umbelliferous crops, *Itersonilia* canker is a minor disease of carrots but can be a major disease of parsnip. Roots, leaves, petioles, inflorescences, and seeds may be affected. On carrot roots, cankers primarily form on the crown and shoulder. Root cankers are reddish-brown with a roughened surface that becomes black in age. Cankers generally do not extend deeply into roots.

3.2.6.2 Causal organism

Itersonilia canker is caused by *Itersonilia perplexans* Derx., a basidiomycete that has affinities with the order Tremellales (Boekhout, 1991, Channon, 1963). The fungus is characterized by dikaryotic mycelium with clamp connections at most septa and the production and discharge of binucleate, kidney-shaped ballistospores from upright, narrow sterigmata. The hyphae are usually straight, septate at 50- to 120-(m intervals, and regularly branched. Ballistospores germinate either to form a mycelium or a secondary ballistospore. Ballistospores are 6 to 10.5 x 10 to 16 (m in size. Some isolates produce golden-brown, thin to thick-walled chlamydospores singly or in terminal clusters on short lateral branches. A yeast phase forms when cultures are submerged in water.

3.2.6.3 Epidemiology

I. perplexans is widespread as a saprophyte of leaf surfaces of umbelliferous crops as well as many weeds and cultivated plants, particularly the composites. The fungus overwinters as mycelium in infected plants or as chlamydospores in soil (Smith, 1967). Spread within the field is by wind-borne ballistospores, which can infect foliage. New spores produced on the foliage fall to the ground and give rise to root infections. The fungus also infests seed.

Disease generally develops late in the growing season but may occur earlier if favorable environmental conditions occur. The fungus has an optimum temperature of 20° C. Disease development is enhanced in cool and wet weather and is limited in hot and dry conditions.

3.2.6.4 Management

Cultural management strategies include long rotations and good soil drainage. Deep plowing, which enhances the decomposition of host residues and exposes the fungus to antagonistic soil microorganisms, effectively reduces soilborne inoculum. The eradication of weeds will reduce other possible sources of inoculum. Fungicides are rarely needed to control *Itersonilia* canker in carrots. Control of the carrot rust fly is important because the larvae can predispose roots to infection.

3.2.7 *Phytophthora* Root Rot

Phytophthora root rot of carrots, also called rubbery brown rot, has been reported in Canada, France, Norway, Tasmania, and the U.S.

3.2.7.1 Symptoms

Symptoms of root rot of carrots generally occur near harvest in the spring or summer, although winter field losses have been reported. Both pre- and post-harvest losses have been associated with relatively wet soil conditions from excessive rain or irrigation. Infected portions of the taproot become dark brown to black and rubbery in consistency. The lesions may occur anywhere on the root in one or more bands. White mycelium of the pathogen is sometimes apparent on the lesions. As the lesions expand and age, a watery soft rot often permeates the root, usually in association with various bacteria and fungi.

3.2.7.2 Causal organism

Several species of *Phytophthora* have been associated with root rot, including *P. cactorum* (Lebert & Cohn) J. Schröt., *P. cryptogea* Pethybr. & Lafferty, *P. megasperma* Drechsler, and *P. porri* Foister (Dowson, 1934, Ho, 1983, Stelfox and Henry, 1978). Although generally a minor problem, *Phytophthora* root rot can cause significant damage to carrot crops grown in waterlogged soils.

3.2.7.3 Epidemiology

Although the epidemiology of *Phytophthora* root rot of carrots is largely unknown, zoospores are thought to be the principal agents of infection. Periods of prolonged water saturation and cool temperatures during carrot growth, processing, or storage generally favor production and release of zoospores. However, other means of infection may occur. For example, mycelium of *P. porri* was implicated in carrot-to-carrot spread of the disease during post-harvest storage (Stelfox and Henry, 1978).

3.2.7.4 Management

To control *Phytophthora* root rot, soil water should be carefully managed. Providing good field drainage, adequate plant bed height, and timely irrigations that avoid extremes in soil water content are important strategies for reducing losses to root rot. Carrots should be stored at temperatures near freezing without condensation on root surfaces. Strict sanitation measures should be practiced in storage facilities.

3.2.8 Root dieback

Root dieback, which results in forking and stubbing of mature taproots, occurs wherever carrots are grown. Although the incidence of the disease is sporadic and yield losses

are generally low, occasionally the majority of carrots in a field are misshapened and unsuitable for the fresh market.

3.2.8.1 Symptoms

Root dieback causes excessively branched or stubbed roots. If the root apex dies when the root is only a few millimeters long, apical dominance is removed and the taproot either fails to elongate (stubbing) or proliferates to form several functional taproots (forking) (Liddell *et al.*, 1989). In severe cases, the root may not recover and the plant dies.

3.2.8.2 Causal organism

Although forking and stubbing can be caused by any agent that damages the root apex, such as soil compaction (Strandberg and White, 1979), nematodes, and excessive water (White and Strandberg,1979), root dieback is often attributed to *Pythium* spp. (Howard *et al.*, 1978, Mildenhall *et al.*, 1971). In the United States, *P. irregulare* Buisman and *P. ultimum* Trow are the principal incitants of root dieback, although *Rhizoctonia solani* Kühn (anastomosis group 4) is occasionally associated with the disorder. Other causal fungi include *P. sylvaticum* Campbell & Hendrix and *P. sulcatum* Pratt & Mitchell, primary causal agents of dieback in carrots grown in organic soils.

3.2.8.3 Epidemiology

All of these fungi are common soil inhabitants. When a carrot seed germinates, exudates from the seedling stimulate oospores and hyphal swellings of various *Pythium* spp. to germinate and infect the young taproot. If the fungus kills the taproot less than two weeks after seed germination, it reduces root length and/or stimulates multiple root formation. The severity of the disease may be dependent on the density of *Pythium* spores in field soils, in addition to other factors, such as very wet soil conditions or large amounts of fresh residue from previous crops. An increase in populations of *P. ultimum* by saprophytic utilization of plant nutrients after soil incorporation of fresh crop residues and the importance of partly decomposed plant residues as the base for propagules of *R. solani* have been described in other cropping systems. Disease caused by these fungi substantially decreases as residues from the previous crop decompose. Crop rotation also influences the severity of seedling root infections. In research trials, the incidence of root dieback (and subsequently forking and stubbing) was increased following alfalfa relative to the incidence of dieback in a carrot crop following a fallow field or barley, carrots, cotton, or onions (Davis and Nuñez ,1999).

3.2.8.4 Management

Cultural practices such as good drainage are important in the management of root dieback. Other strategies include crop rotation to small grains, which might reduce soil populations of *R. solani* and some *Pythium* spp. Alfalfa in rotation with carrots

should be used with caution since it may harbor relatively high populations of *P. irregulare* and *P. ultimum*. It is also a host of *Pythium violae*, the cause of cavity spot. Populations of *R. solani* also may increase following alfalfa. For optimum root quality, carrots should be grown in deep, friable, well-drained soils since all carrots, especially long-rooted cultivars, are adversely affected by shallow or compacted soils.

3.2.9 Southern blight

Southern blight is a common disease of many vegetables, including carrots, grown in areas with warm climates. It derives its common name, southern blight, from its prevalence in the southeastern United States. The causal agent, *Athelia rolfsii* (Curzi) Tu & Kimbrough (anamorph: *Sclerotium rolfsii* Sacc), infects hundreds of plant species, including monocotyledons and dicotyledons.

3.2.9.1 Symptoms

On carrots, infection often begins on petioles at or near the soil surface. Infections typically arise at canopy closure because of increased humidity and foliar contact with the soil. Infected basal stems eventually brown and the entire plant may collapse. White, string-like mycelia, radiating out from the stem base, often develop on the soil surface around the infected crown region. Invaded tissues in carrot taproots are pale brown and soft but not watery, like bacterial soft rot. Infected carrots do not have a particularly unpleasant odor, unless the affected tissue is invaded by secondary organisms. The central core of carrots, held together by the woody conducting tissue, can occasionally be pulled free, leaving the outer portion of the root in the soil. In severe cases, the whole root may disappear, leaving a cavity in the soil with the sides held firmly in place by the interwoven fungal threads. Numerous spherical tan to dark brown sclerotia, about the size of a mustard seed (0.5 to 1.5 mm in diameter), develop on and in infected tissues and surrounding soil.

3.2.9.2 Epidemiology

A. rolfsii can survive for many years as sclerotia in soil and as a saprophyte on various host substrates. It produces cellulolytic and pectinolytic enzymes, which facilitate direct hyphal penetration of nonwounded tissues. Volatile compounds produced by senescent plant tissues appear to stimulate sclerotial germination. Mycelial growth develops at temperatures ranging from 8 to 40ºC, but growth is greatly inhibited at temperatures below 15ºC. In culture, optimum temperatures for sclerotia formation are 27 to 30ºC. Mycelium is killed at freezing temperatures, but sclerotia can withstand temperatures as cold as -10ºC. Moist soil conditions favor disease development, and serious outbreaks often are associated with unusually wet conditions. Southern blight is influenced by certain forms of fertilizer. Disease incidence may be reduced by the use of ammoniacal nitrogen sources and fertilizers containing plant-available calcium (Punja, 1985). The pathogen is disseminated by cultivation and tillage equipment, in irrigation and drainage water, and movement of infested soil or debris. The role of the sexual stage

in the epidemiology of southern blight has not been firmly established.

3.2.9.3 Management

The prolific growth, persistence in soil, and extensive host range of *S. rolfsii* make southern blight difficult to control, although it usually does not warrant specific control measures in cool to temperate regions. Freezing temperatures effectively destroy soilborne mycelia, limiting primary inocula in such regions to sclerotia. Although crop rotation by itself is not an effective or practical control method due to the large host range of *S. rolfsii*, rotating from carrots to a crop unaffected by the pathogen (*e.g.*, corn or small grains) may result in less disease in subsequent years. Deep plowing to bury sclerotia and infested debris reduces inoculum viability (Gurkin and Jenkins, 1985, Jenkins and Averre, 1986).

3.2.10 Violet root rot

Violet root rot has been reported in carrot production areas of Europe, North America, New Zealand, and Tasmania. The disease causes damage under field conditions and occasionally in storage.

3.2.10.1 Symptoms

Above-ground symptoms in the field include leaf chlorosis, wilting, and patches of dying or dead plants. When infected plants are pulled up, soil often clings to the decaying roots and individual, firm, dark purple-brown lesions are visible on the taproot. As the infection progresses, a dense mycelial mat forms on the root surface, eventually reaching and extending from the carrot crown onto the adjoining soil surface. The mat is pink to brown and up to 30 cm long and 15 cm wide. In age, the mat develops a purplish to dark brown color and becomes leathery in consistency. An internal soft rot of the roots occurs, and as the roots are pulled from the soil, only the external, leathery, outer layer of the root remains. The disease also develops on infected carrots in cold storage.

3.2.10.2 Causal organism

Violet root rot is caused by *Helicobasidium brebissonii* (synonym: *H. purpureum* Pat.) (anaomorph: *Rhizoctonia crocorum* (Pers.) DC). The pathogen has a very wide host range, including trees, shrubs and vegetable crops such as asparagus, bean, beet, cabbage, potato, rhubarb, sea kale, sweet potato and turnip, in addition to umbelliferous crops (Valder, 1958). It causes a serious disease of alfalfa, and also infects clover, rapeseed, and saffron crocus. It has been isolated from numerous weed species, including broadleaf weeds and grasses.

3.2.10.3 Epidemiology

Infection and disease development occur slowly. In culture, *R. crocorum* grows between 9 and 39°C, with an optimum of 26°C. Infection of carrots can occur between 5 and

30°C, with an optimum of 20°C (Whitney, 1954). While infection probably takes place in the spring, symptoms on the roots appear later in the season. Wounds are not required and any part of a carrot plant is susceptible to infection. Infection can occur from mycelium or sclerotia residing in soil or on weed hosts or other susceptible crops. The major means of spread within and between fields is by movement of infested soil on farm implements and by movement of infected plants. High soil moisture levels and low pH increase the severity of the disease.

3.2.10.4 Management

Fields with infected plants should be harvested early to prevent late-season development of the disease. Rotations with nonhosts, such as cereals, are recommended. Good drainage, proper fertilization, and liming to raise the soil pH may reduce the extent of infection. Equipment should be thoroughly cleaned to avoid movement of infested soil to clean ground. In some areas, management of violet root rot is achieved by maintaining low soil moisture levels. In one study, Chantenay-type carrots were less susceptible to violet root rot than other carrot types, but resistance could not be confirmed (Dalton *et al*. 1981, Whitney 1956).

3.3 Postharvest diseases

3.3.1 Black root rot

Black root rot is generally considered a post-harvest disease, but seedlings and occasionally mature carrots in the field are also affected.

3.3.1.1 Symptoms

The disease is usually noticed after carrots have been washed, graded, and packaged in polyethylene bags. Under conditions of high humidity and warm temperatures (25° C or higher), a blackening of wounded root surfaces develops from masses of dark brown to black chlamydospores. Spread of the pathogen to adjacent roots within the package may occur. The disease is more serious on carrots grown in muck soils than carrots grown in mineral soils.

3.3.1.2 Causal organism

Chalara elegans Naj, Raj & Kendrick (synonym: *Thielaviopsis basicola* (Berk. & Broome) Ferraris) is a dematiaceous hyphomycete that produces multicelled, thick-walled, melanized chlamydospores (aleuriospores) as well as large numbers of single-celled, rectangular-shaped, phialospores (endoconidia) produced within phialides. Both spore types are common in culture and on diseased tissues. A sexual state is unknown.

3.3.1.3 Epidemiology

C. elegans has a wide host range, including ornamentals, vegetables, and field crops,

and is found in soils worldwide. Since saprophytic growth in soil is minimal, its survival is dependent on the longevity of chlamydospores or reproduction on host roots (Gayed 1972). Inoculum levels are higher in acidic soils containing high levels of organic matter. The pathogen can be detected using carrot root discs as baits or on semi-selective media. Contact of infested soil with wound sites on the carrot roots during or after harvest results in infection (Punja *et al.,* 1992). Carrots damaged during harvest or grading and left for prolonged periods without cooling are predisposed to infection. When the disease occurs on taproots in the field, it is always associated with wounds of some type.

3.3.1.4 Management

Since infection primarily occurs after harvest and during grading, attempts to minimize wounding accompanied by rapid removal of field heat, such as dipping carrots in chlorinated hydrocooled water, are recommended (Punja *et al.,* 1992). Storage of carrots at temperatures below 10º C minimizes pathogen growth. Good disease control is achieved when harvested carrots are dipped in solutions of potassium sorbate and propionic acid (Punja and Gaye, 1993).

3.3.2 Crater rot

3.3.2.1 Symptoms

Crater rot is a postharvest disease of carrots placed in long-term storage. Often there are no visible symptoms of disease until 1 to 2 months of storage (Rader, 1948). Under typical storage conditions of high humidity and cool temperatures, sunken lesions (craters or pits) gradually form on root surfaces. White patches or aggregates of mycelium closely appressed to the root surface develop and small dark brown sclerotia may be evident. The carrots develop a dry rot, but if secondary invasion by bacteria occurs, soft rot can result.

3.3.2.2 Causal organism

The cause of crater rot is *Rhizoctonia carotae* Rader. The associated teleomorphic state, *Athelia arachnoidea* (Berk.) Jülich, has been found on decaying forest litter and does not seem to play a role in disease of carrot (Adams and Kropp, 1996). In culture, optimum growth occurs at 16 to 20º C and no growth occurs at 28º C (Punja, 1987).

3.3.2.3 Epidemiology

Incipient infections of carrots from soil inoculum adhering to roots or mycelium in crown tissue occur prior to harvest (Punja, 1987). Late-harvested carrots with senescent tissues at the crown may harbor higher infection levels. Mycelium on contaminated wooden crates used to store carrots also may initiate disease. Spread of the pathogen can occur to adjacent roots held in crates or bins. The pathogen subsequently

develops on the roots in cold storage at temperatures as low as 2 to 3° C. High humidity or a film of water on the root surface enhances disease development.

3.3.2.4 Management

Since lesions are difficult to detect on roots at harvest, the implementation of disease management strategies requires prior knowledge of the occurrence of the pathogen in the field. If disease pressure is high, carrots can be dipped in fungicides or inorganic salt solutions prior to long-term storage (Ricker and Punja, 1991). Washing roots in water also may reduce disease by removing inoculum attached to roots. Sanitary measures, including disinfestation of crates or lining them with polyethylene, minimize pathogen spread. Proper cold storage regimes that prevent temperature fluctuations and avoid moisture condensation on the root surface are essential to reduce infection and prevent dehydration. Timely removal of carrots from storage can reduce losses.

3.3.3 Licorice Rot

Licorice rot is one of the most important diseases of carrots held in cold storage. The disease is common in Europe and North America. Licorice rot affects at least 90 other hosts, including other umbelliferous crops such as caraway, dill, parsnip, and numerous ornamental plants.

3.3.3.1 Symptoms

On carrots, lesions on stored roots are small and inconspicuous for at least several weeks. The disease is most common in the crown and root-tip regions but sometimes occurs around lateral root scars. Later, lesions extend deep into the root tissue, causing an extensive, soft, watery, black decay. Unlike black rot lesions caused by *Alternaria radicina*, licorice rot lesions typically do not have discrete margins separating healthy from diseased tissue.

3.3.3.2 Causal organism

Licorice rot is caused by the imperfect fungus, *Mycocentrospora acerina* (Hartig) Deighton (synonym: *Centrospora acerina* (Hartig) Newhall), a soilborne pathogen that overwinters in soil as chlamydospores. These spores may remain viable in the soil for at least two years but the wide host range of the pathogen suggests that it may persist in production areas for longer periods. The fungus has been identified on peas, spinach, sugarbeet, and numerous weeds (Hermansen, 1992). It is possible that it may be saprophytic on decaying leaves of many plants.

3.3.3.3 Epidemiology

In areas of intensive carrot production, chlamydospores and short lengths of pigmented mycelium may be abundant in the rhizosphere of growing roots (Davies *et al.* 1981).

Infection can occur at all stages of plant growth but the fungus rarely causes disease in the field. Infection first occurs on petiole bases, taproot wounds, and at lateral root scars, but lesion expansion typically develops only after 5 to 6 weeks in storage, when tissue has begun to senesce. Conidia are not generally formed on carrots in the field but may form under conditions of high humidity during storage. The fungus is favored by cool temperatures; in culture, maximum growth occurs at 16ºC (Neergaard and Newhall, 1951). Unlike most other postharvest fungal pathogens of carrot, the spread of *M. acerina* from diseased to healthy plants is somewhat limited. Intact plant tissues are strongly resistant to the fungus; in carrot, this resistance is likely associated with the antifungal, polyacetylenic compound falcarindiol, which is present in periderm tissue at high concentrations (Davies and Lewis, 1981, Lewis *et al*., 1981).

3.3.3.4 Management

Careful handling of the produce during harvest and storage, proper storage conditions, and sanitation during storage are the most important control measures. Removing soil from the surface of carrots prior to storage reduces much of the initial inoculum. Exposing carrots to high temperatures and humidity for a short period prior to storage may reduce the incidence of disease by allowing callus to form on wounds. Maintaining temperatures near freezing and high humidity without surface free water help preserve carrot quality.

4. Diseases caused by Viruses and Phytoplasmas

4.1 Carrot Motley Dwarf

Carrot motley dwarf (CMD) is a widespread disease of carrots grown in cool climates, including Canada, Germany, Japan, New Zealand, United Kingdom, and the United States.

4.1.1 Symptoms

Symptoms of infected carrots vary with the environmental conditions and age of the plant at infection. In cool weather (15 to 20º C), plants infected young develop reddening and yellowing of leaves and overall stunting, symptoms that can be confused with nutritional disorders. Roots also may be severely stunted. Plants of very susceptible cultivars may die if infected when very young. Foliar symptoms of older-infected plants and plants growing under warmer conditions are less severe and the roots are nearly normal-sized. At temperatures above approximately 24º C, infected carrots may be symptomless.

4.1.2 Etiology and transmission

The etiology of CMD consists of two unrelated viruses, the *Polerovirus*, *Carrot redleaf virus* (CRLV) and the *Umbravirus*, *Carrot mottle virus* (CMoV). The CRLV virion is isometric, approximately 25 nm in diameter, and contains a single-stranded genomic

RNA of approximately 5.6 kb (Murant *et al.*, 1985). Virions of CMoV have not been identified. The CMoV genome, which does not encode a capsid protein, consists of a single-stranded RNA measuring approximately 4.2 kb in size.

Although each virus is capable of infecting plants alone, CMD only results from the mixed infection of CRLV and CmoV (Waterhouse and Murant, 1983). Together, both viruses are transmitted plant-to-plant in a circulative, nonpropagative manner by the willow-carrot aphid, *Cavariella aegopodii* (Scopoli). If CRLV alone is present in plants, the virus can be efficiently transmitted by its aphid vector but cannot be mechanically transmitted; however, CMoV alone in plants can be mechanically transmitted but not transmitted by the aphid. Therefore, vector transmission of CMoV requires the presence of the helper virus, CRLV. In doubly-infected plants, the CMoV single-stranded genomic RNA becomes encapsidated by CRLV capsid proteins (a process called genomic masking or transcapsidation), and thereby gains the ability to be transmitted by *C. aegopodii*.

A third virus-like RNA was identified in CMD-affected carrots from California. This CRLV-associated RNA (CRLVaRNA) is a small genomic RNA (approximately 2.8 kb) that encodes for its own RNA-dependent RNA polymerase but no capsid protein (Watson *et al.*, 1998). Like CMoV, the CRLVaRNA obtains capsids composed of CRLV capsid proteins from mixed infections, and as a result gains aphid transmission by *C. aegopodii*, along with CRLV and CMoV, to new plants. It is not known if the CRLVaRNA affects symptoms on infected plants.

CRLV and CMoV have relatively narrow host ranges. Under natural conditions, CMD appears to be limited to umbelliferous plants, including carrot, wild carrot, cilantro, cow parsley, cow parsnip, dill, and parsley. In addition to these plants, the viruses causing CMD can be transmitted experimentally by aphids to bean, chervil, crimson clover, petunia, and several *Nicotiana* and *Chenopodium* spp.

4.1.3 Epidemiology

Because most studies indicate that the CMD viruses are primarily associated with carrots, alternate crop or weed hosts do not appear to be important in the epidemiology of the disease. In addition to the narrow host ranges of the viruses, the aphid vector likewise has a narrow host range and prefers to feed and reproduce on carrot, although *C. aegopodii* populations also will increase on celery, chervil, and, to a lesser extent, on fennel and parsley. Primary inoculum sources are, therefore, most often old carrot plantings or overwintered carrots that are infected with the CMD viruses and harbor *C. aegopodii*. If new carrot plantings are established near or downwind from old, infected carrot crops, *C. aegopodii* can readily vector the CMD viruses from old to new plantings.

4.1.4 Management

CMD can be controlled in many areas by strategically selecting locations of new carrot plantings away from overwintered carrot fields and carrots grown for seed. Volunteer carrot plants should be destroyed. In certain areas, good control is achieved by

planting new fields at least a mile from overwintered carrot fields (Watson and Falk, 1994). If new plantings cannot be placed at a substantial distance from old fields, applying insecticides to the old fields may be warranted to reduce vector populations. Carrot cultivars exhibit a wide range of responses to CMD, and genetic resistance to the disease is available.

4.2 Carrot thin-leaf

Carrot thin leaf is a minor problem of carrots in California, Idaho, and Washington in the United States (Falk *et al.*, 1991). The disease is sometimes common but it generally does not affect carrot yield or quality. In a few areas, yield reductions are economically significant, especially when the virus occurs with other diseases.

4.2.1 Symptoms

Symptoms vary by carrot cultivar and growth stage when infection occurred. In general, leaflets are thread-like and twisted, giving the foliage a narrow and distorted appearance. Leaves also may exhibit faint mottling and yellow vein-banding. When plants are infected at a young age, the leaflets may be extremely thin, hence the name of the disease.

4.2.2 Causal organism

Carrot thin leaf is caused by the *Potyvirus*, *Carrot thin leaf virus* (CTLV). Polyclonal antisera have been produced against CTLV virions; in SDS-immunodiffusion and DAS-ELISA tests, CTLV was not closely related to other common potyviruses such as *Lettuce mosaic virus*, *Tobacco etch virus*, *Potato virus Y*, or *Zucchini yellow mosaic virus* (Howell and Mink, 1976). There is no information on strain variability of CTLV. Long, flexuous, rod-shaped virions typical of other potyviruses can be readily identified from extracts of CTLV-infected plants by using transmission electron microscopy. Virions measure 11 nm in width and 550 to 820 nm in length.

4.2.3 Epidemiology

The natural host range of CTLV appears to be limited to carrots**.** In laboratory studies, some *Nicotiana* spp. and other common virus indicator plants and a few commercial umbelliferous crops, such as coriander, parsley, and parsnip, have been infected with CTLV (Howell and Mink, 1976). However, because these crops are grown on such a limited acreage and infection is not known to occur naturally, they are not considered a reservoir for the virus.

  In some cases, CTLV survives in volunteer carrots, which serve as the primary source of inoculum for subsequent plantings. In greenhouse experiments, aphids such as the green peach aphid, *Myzus persicae* (Sulzer), and the willow-carrot aphid, *Cavariella aegopodii* (Scopoli), efficiently transmit the virus in a non-persistent manner. Other natural vectors are not known.

4.2.4 Management

Control strategies are not commonly implemented due to the limited economic impacts of CTLV on carrot. However, removal of volunteer carrots near newly planted carrot fields probably eliminates primary inoculum. Planting near older fields also should be avoided.

4.3 Carrot Virus Y

Carrot virus Y causes serious losses in carrots in Australia.

4.3.1 Symptoms

Disfiguration of roots of plants infected when young renders the crop unmarketable. Symptoms on foliage include chlorotic mottle, marginal necrosis or reddening, and general chlorosis. An increased subdivision of leaflets gives the top of the plant a feathery appearance. Infected plants may be slightly stunted. Carrot taproots on plants infected when young are stubby, severely distorted, and knobby. Roots on plants infected later develop limited distortion (Latham and Jones, 2000).

4.3.2 Causal organism and detection

The disease is caused by the *Potyvirus, carrot virus Y* (CVY). Flexuous filamentous virions, typical of other potyviruses, are readily identified in extracts of CVY-infected plants using electron microscopy. The virions are about 11 nm in width and 770 nm in length. Sequence analysis has revealed that CVY is distantly related to celery mosaic virus. General potyvirus monoclonal antibodies detect the virus in DAS-ELISA (Moran *et al*. 1999).

4.3.3 Epidemiology

The known natural host range of CVY is limited to carrots. Substantial CVY epidemics occur in areas where carrots are grown in sequential plantings year-round. Previous plantings and volunteer carrots infected with CVY are sources of inoculum for new plantings. In greenhouse experiments, the green peach aphid, *Myzus persicae* (Sulzer), efficiently transmits the virus in a non-persistent manner. Transmission by other aphid species and seed transmission are unknown.

4.3.4 Management

Control of this virus can be achieved by destroying volunteer carrots, planting new crops in isolation from old crops, and using an annual carrot-free period. Managing aphid vector populations with insecticides is unlikely to be effective. Manipulating sowing dates to avoid peak aphid populations when carrots are young may be beneficial.

4.4 Aster Yellows and BLTVA (Beet leafhopper-transmitted virescence agent) Yellows

Aster and BLTVA yellows affect a wide variety of wild and cultivated plants, including more than 300 species of vegetables, weeds, and ornamentals. Losses in umbelliferous crops are sporadic. Aster yellows occurs worldwide in many umbelliferous crops; BLTVA yellows has been reported in carrots only in the western U.S.

4.4.1 Symptoms

In carrots, initial symptoms of aster yellows infection include yellowing of the veins of young leaves, which are often narrower than healthy leaves. The yellowing progresses until the entire leaf is chlorotic. Dormant buds in the crown then break to form upright, chlorotic, adventitious shoots. Older leaves often turn bronze, red, or purple. These older leaves may break off, making bunching and mechanical harvesting difficult. The taproots of affected plants are long and thin with a proliferation of roots. The taproot is reduced in both size and quality. Premature flowering in the first growing season due to aster yellows infection is rare. In carrots grown for seed production, the flowers of aster yellows-infected plants exhibit virescence (greening of the flowers) and phyllody (development of leaf-like flower petals). The umbels are often stunted and chlorotic, and some infected plants may die before seed is produced.

Symptoms of BLTVA (beet leafhopper-transmitted virescence agent) include a mild chlorosis and red or purple lower leaves. The taproot is usually thin, woody, and hairy from the proliferation of roots. Many infected plants prematurely flower in the first year. The abnormal virescent and phyllodied flowers proliferate to form multiple compound leafy umbels.

4.4.2 Causal organism and detection

Aster and BLTVA yellows are caused by two genetically distinct phytoplasmas (previously known as mycoplasma-like organisms or MLO's) (Lee and Davis, 1988). Phytoplasmas are small (0.5 to 1µm in diameter) prokaryotes that reproduce by division or budding in the phloem sieve cells of host plants as well as in the bodies of their leafhopper vectors. They are pleomorphic in shape and lack a cell wall. Phytoplasmas have never been cultured *in vitro*. Because of their size, they can only be visualized by electron microscopy or fluorescent DNA staining (DAPI) techniques. Detection of phytoplasmas can be confirmed using bioassay, ELISA, PCR, or DNA hybridization methods (Kuske *et al*., 1991). Aster yellows- and BLTVA-infected carrots have been found in the same field, but the two phytoplamas have never been detected in the same plant.

4.4.3 Epidemiology

Although the aster yellows phytoplasma is vectored by many different species of leaf-

hoppers, the most important vector is the aster leafhopper, *Macrosteles fascifrons* Stål. After an incubation period, leafhoppers transmit the phytoplasma in a persistent manner and remain infective for life. During the spring in the northern Midwest of the U.S., infected aster leafhoppers migrate into carrot-growing areas on prevailing winds from the south central U.S. These leafhoppers have previously acquired the aster yellows phytoplasma by feeding on infected weeds and crops. Soon after arriving (some having already completed the transmission incubation period), the aster leafhopper transmits the phytoplasma into carrots. Leafhoppers that have overwintered locally as eggs can acquire the phytoplasma from weed hosts or infected crops, but these leafhopper populations mature weeks later and are usually less infectious. In the far western and eastern U.S., where aster leafhopper migration is not known to occur, local leafhopper vectors acquire the phytoplasma from infected crops and weed hosts (*e.g.*, dandelion, plantain, Russian thistle, sowthistle, wild lettuce, and many others) and transmit it to carrots.

BLTVA is acquired and transmitted by the beet leafhopper vector, *Circulifer tennellus* Bak. In the far western U.S., the leafhopper apparently acquires the phytoplasma from infected wild plants in the hills bordering farmlands (Golino *et al*., 1987). After their wild food source dries during the seasonal summer drought, the leafhoppers move into irrigated valleys on prevailing winds in search of green plants, including carrots. *C. tennellus* transmits the phytoplasma in a persistent manner and remains infectious for life after an incubation period. Carrot to carrot transmission of BLTVA by *C. tennellus* does occur in greenhouse studies, but it is unknown whether this occurs frequently in the field. BLTVA is not transmitted by the aster leafhopper. Neither BLTVA or the aster yellows phytoplasma is seed transmitted, nor is either transmitted from infected female leafhoppers to their offspring.

4.4.4 Management

Control measures for these diseases include removal of weed reservoirs and planting away from infected crops. Controlling the insect vector with insecticides gives some control of aster yellows. In the northern Midwest of the U.S., where aster yellows is a recurring economic problem, the infectivity of migratory and local aster leafhoppers is monitored using a bioassay of captured aster leafhoppers on China aster. Using a combination of the relative susceptibility of the carrot cultivar, the number of leafhoppers present, and the percentage of infective leafhoppers, an aster yellows index (AYI) can be calculated (Mahr *et al*., 1993).

The AYI= percent infectivity of the aster leafhopper population X number of aster leafhoppers present/100 sweeps. Insecticidal treatment is only recommended if the AYI is 50 for susceptible, 75 for intermediate, and 100 for resistant carrot cultivars. Because the infectivity of the migratory leafhoppers can vary yearly, applying insecticides only when the index value is reached can reduce the amount of sprays needed for leafhopper control. Applications of insecticides are not needed later than three weeks before harvest because three weeks are needed for symptom development. Once a plant is infected with either the aster yellows or BLTVA phytoplasma, there is no known control.

5. Diseases caused by nematodes

More than ninety species of plant-parasitic nematodes from many genera are associated with umbelliferous crop production, but only a few have been studied in detail. These include ectoparasites that do not enter host root tissues but feed on and injure roots from the rhizosphere, migratory endoparasites that feed on, move through, and destroy root cortical tissue, and sedentary endoparasites that penetrate and develop inside roots, where they initiate and maintain feeding sites without further migration.

5.1 Cyst Nematode

The carrot cyst nematode, *Heterodera carotae* Jones, is an obligate parasite of cultivated and wild carrot (*Daucus carota* L.), its wild relative, *D. pulcherrimus* (Willd.) Koch ex DC, and the umbelliferous weed, *Torilis* spp. The nematode has been found in carrot producing regions of Cyprus, former Czechoslovakia, England, France, Germany, Holland, Hungary, India, Ireland, Poland, Russia, Scotland, Sweden, Switzerland, and Michigan, U.S.

5.1.1 Symptoms

Symptoms include plant stunting and patches of weak and undersized plants in the field. Leaves often turn yellowish-red, with older parts exhibiting necrosis. Infected plant stands do not cover the planting beds as fully as healthy stands. Below ground symptoms include smaller than normal taproots and a proliferation of lateral feeder roots, giving the plants a 'bearded' appearance. Taproots of infected plants may become distorted or restricted in length and lignify prematurely. Numerous brown cysts are apparent on the root surfaces. Carrot cyst nematodes are detected in the field by examining roots of damaged plants for the presence of the pin-head sized white females and brown cysts. While cysts and egg sacs may occur on the root surface, many females remain buried within the root tissue. These females are only visible when infected roots are cut open or
teased apart.

5.1.2 Disease cycle

H. carotae has one of the narrowest host ranges of any plant-parasitic nematode. Only exudates from carrot roots act as a hatching stimulant for *H. carotae* eggs that are produced within cysts (Greco and Brandonisio 1986). *H. carotae* is spread locally on farm equipment and more widely by the movement of cysts adhering to taproots. The weed, *Torilis* spp., supports the nematode in the absence of carrot, and may serve as a source of inoculum. Eggs are deposited into a large egg sac containing a gelatinous matrix or they accumulate within the female body, which becomes the leathery cyst. Eggs in cysts may remain viable for several years. After one molt within the egg, the second-stage juvenile hatches and migrates into the soil until it locates a root tip. Juveniles may orient towards roots along gradients of attractant molecules in the rhizo-

sphere. These juveniles usually penetrate a root just behind the root cap. Once they come to the eventual feeding site, they become sedentary and initiate the formation of a feeding site called a syncytium. Comprised of several coalesced cells formed by partial cell wall degradation, the syncytium contains several nuclei from the component cells. This site acts as a transfer cell from which the nematode withdraws water and nutrients during feeding. Depending on the time of planting and seasonal temperature, one or two generations of *H. carotae* may be completed in a season. Optimum temperatures range from 15 to 20º C for hatch and development. Hatch occurs above 5º C but is inhibited at 25ºC.

5.1.3 Management

Soil fumigation and some nonfumigant nematicides lower the nematode population density in soil to or below an economic threshold. Measurable yield reduction occurs at population densities of about 80 eggs per 100 cc of soil (Greco and Brandonisio, 1980). Marketable carrots may not be obtained at densities greater than 6,400 eggs per 100 cc of soil. Crop rotation to nonhosts or a long fallow is useful for reducing soil population levels since *H. carotae* has a very narrow host range. However, the persistence of viable eggs in cysts requires nonhost rotation periods of 4 to 6 years. Adjusting planting and harvest dates can be beneficial in managing *H. carotae*. High summer temperatures delay root invasion and may limit the nematode to a single generation. No sources of resistance to *H. carotae* have been identified.

5.2 Root-knot nematode

5.2.1 Symptoms

Root-knot nematodes are obligate sedentary endoparasites of carrots and many other plants. The most conspicuous and diagnostic symptom of root-knot is round to spindle-shaped swellings (galls) on feeder roots, for which the disease is named. The galls induced by *Meloidogyne hapla* Chitwood tend to be smaller and more spherical or bead-like than galls induced by other root-knot species, which tend to produce larger galls that often coalesce along roots. White to dark brown egg masses (about 0.5 to 1 mm in diameter) are found on the surface of the galled roots. When galls are cut open, mature females, which appear as white 'pearls' no more than about 1.5 mm long, are often visible within the root tissue. Infected roots are usually short and have few lateral roots and root hairs. Galling causes a disfiguration of the carrot taproot due to the swellings, resulting in unmarketable roots. Another characteristic symptom of root-knot infection is forking of the taproot, which occurs when the developing root apex at the seedling stage is damaged. Additional symptoms, such as erratic plant stands, plant stunting, yellowing, and even wilting, may result from the loss of plant vigor. Typically, affected plant stands do not cover the planting beds as fully as healthy stands.

5.2.2 Causal organism

Meloidogyne arenaria (Neal) Chitwood, *M. chitwoodi* Golden, O'Bannon, Santo &

Finley, *M. fallax* Karssen, *M. hapla* Chitwood, *M. incognita* (Kofoid & White) Chitwood, and *M. javanica* (Treub) Chitwood have been reported as parasites of carrot. Two races of *M. arenaria*, three races of *M. chitwoodi*, and four races of *M. incognita* have been identified by differential hosts. Different races may reproduce at different rates and cause different symptoms on carrot.

5.2.3 Disease cycle

Eggs are deposited into a gelatinous matrix by the female nematode, which is partially or completely embedded in a root of the host plant. The egg mass may contain more than 1,000 eggs and may be larger than the female body. After one molt within the egg, the second-stage juvenile hatches and moves randomly within the egg mass or migrates into the soil until it locates and enters a root tip just behind the root cap. Once inside, the second-stage juveniles migrate, become sedentary, and initiate the formation of hypertrophied, multinucleate giant cells. The nematodes enlarge, undergo three additional molts, and develop into mature females entirely embedded within the root tissue. Males leave the root after the fourth molt and become adults. The proportion of males to females is increased under conditions of environmental stress. However, because the females are self-fertilizing, males are not required for completing the life cycle.

Depending on the root-knot species and seasonal temperature, one to three generations may be completed within a season. Optimum temperatures range from 15 to 25ºC for *M. chitwoodi*, *M. fallax*, and *M. hapla*, and 25 to 30ºC for *M. arenaria*, *M. incognita*, and *M. javanica*. There is very little activity by any *Meloidogyne* species above 38°C or below 5°C.

Root-knot generally is more severe in sandy-textured and muck soils than in clay soils. Apparently, this is related to the size of the pore and the greater mobility of the nematode in water in larger, aerated pore spaces. When soil moisture is maintained at an adequate level for plant growth, the nematode may have little effect on overall plant health, but in carrot the distortion of the taproot still can be severe. Damage to carrot is positively correlated to the size of the initial nematode population.

5.2.4 Management

Various soil fumigants, including the novel fumigant methyl iodide, provide effective control of root-knot (Hutchinson *et al.*, 1999, Roberts *et al.*, 1988). Some non-fumigant preplant nematicides also protect carrots from *M. hapla* infection in peat and muck soils by lowering the nematode population density to below an economic threshold. In practice, the threshold for carrot is considered to be at or below the root-knot nematode detection level in soil. This is referred to as a 'zero tolerance' threshold, *i.e.*, the presence of root-knot juveniles in soil at the start of the season will result in some crop loss. This is especially true for carrots grown in Mediterranean and sub-tropical climates in the presence of *M. arenaria*, *M. incognita*, and *M. javanica*, where multiple generations during the growing season intensify damage. For *M. hapla*, economic threshold levels have been defined at about 30 second stage juveniles per 100 cc of

soil in the Netherlands, 9 per 100 cc of soil in organic soils in Canada, and 2 per 100 cc of soil in the state of Washington, U.S. (Vrain, 1982).

Crop rotation to nonhosts or a long fallow effectively reduces nematode populations. However, the extensive host ranges of root-knot species make rotation difficult to implement. Resistant cultivars to certain species of *Meloidogyne* are available in alfalfa, common bean, cotton, cowpea, pepper, and tomato. All carrot cultivars should be considered susceptible, although several sources of genetic resistance to root-knot nematodes have been identified (Simon *et al.,* 2000).

The adjustment of planting date is an effective management approach for root-knot nematode on carrot. This tactic is based on avoiding the planting of carrots when nematode juveniles in soil are active. In California, a delay in the autumn planting until soil temperatures fall below 18ºC (the *M. incognita* activity threshold), avoids significant root infection (Roberts, 1987). On *M. hapla*-infested organic soil in Quebec, Canada, early spring plantings in May (soil temperatures of 6 to 8ºC) increased marketable yields by 20 to 50% compared to mid-June plantings (soil temperatures of 15ºC) (Belair, 1987).

6. References

Adams, G.C. and Kropp, B.R. 1996. *Athelia arachnoidea,* the sexual state of *Rhizoctonia carotae,* a pathogen of carrot in cold storage. Mycologia, 88:459-472.

Adams, P. B. 1975. Factors affecting survival of of *Sclerotinia sclerotiorum* in soil. Plant Dis. Rep., 59:599-603.

Angell, F. F. and Gabelman, W. H. 1968. Inheritance of resistance in carrot, *Daucus carotae* var. *sativa*, to the leafspot fungus, *Cercospora carotae*. J. Am. Soc. Hort. Sci., 93:434-437.

Arthur, J. C. 1934. Manual of the Rusts in United States and Canada. Purdue Research Foundation, Lafayette, IN.

Belair, G. 1987. A note on the influence of cultivar, sowing date, and density on damage to carrot caused by *Meloidogyne hapla* in organic soil. Phytoprotection 68:71-74.

Ben-Yephet, Y., Bitton, S. and Greenberger, A. 1986. Control of lettuce drop disease, caused by *Sclerotinia sclerotiorum*, with metham-sodium soil treatment and foliar applications of benomyl. Plant Path., 35:146-151.

Ben-Yephet, Y., Genizi, A. and Siti, E. 1993. Sclerotial survival and apothecial production by *Sclerotinia sclerotiorum* following outbreaks of lettuce drop. Phytopathology, 83:509-513.

Boekhout, T. 1991. Systematics of *Itersonilia*: a comparative phenetic study. Mycol. Res., 2:135-146.

Braun, U. 1995. The Powdery Mildews (Erysiphales) of Europe. Gustav Fisher Verlag, New York.

Carisse, O. and Kushalappa, A. C. 1990. Development of an infection model for *Cercospora carotae* on carrot based on temperature and leaf wetness duration. Phytopathology, 80:1233-1238.

Channon, A. G. 1963. Studies on parsnip canker. I. The causes of the disease. Ann. Appl. Biol., 51:1-15.

Constatinescu, O. 1992. The nomenclature of *Plasmopara* parasitic on Umbelliferae. Mycotaxon, 43:471-477.

Dalton, I. P., Epton, A. S. and Bradshaw, N. J. 1981. The susceptibility of modern carrot cultivars to violet root rot caused by *Helicobasidium purpureum*. J. Hort. Sci., 56:95-96.

Davies, W. P. and Lewis, B. G. 1981. Antifungal activity in carrot roots in relation to storage

infection by *Mycocentrospora acerina* (Hartig) Deighton. New Phytol., 89:109-119.

Davies, W. P., Lewis, B. G. and Day, J. R. 1981. Observations on infection of stored carrot roots by *Mycocentrospora acerina*. Trans. Br. Mycol. Soc., 77:139-151.

Davis, R. M. and Nuñez, J. J. 1999. Influence of crop rotation on the incidence of *Pythium*- and *Rhizoctonia*-induced carrot root dieback. Plant Dis., 83:146-148.

Dillard, H. R., Ludwig, J. W. and Hunter, J. E. 1995. Conditioning of sclerotia of *Sclerotinia sclerotiorum* for carpogenic germination. Plant Dis., 79:411-415.

Dowson, W. J. 1934. *Phytophthora megasperma* Drechsler in Tasmania. Trans. Brit. Mycol. Soc., 19:89-90.

Falk, B. W., Davis, R. M. and Piechocki, M. 1991. Identification of carrot thin leaf virus in California carrots. Plant Dis., 75:319.

Farrar, J. J., Nuñez, J. J. and Davis, R. M. 2000. Influence of soil saturation and temperature on *Erwinia chrysanthemi* soft rot of carrot. Plant Dis., 84:665-668.

Gayed, S. K. 1972. Host range and persistence of *Thielaviopsis basicola* in tobacco soil. Can. J. Plant Sci., 52:869-873.

Gillespie, T. J. and Sutton, J. C. 1979. A predictive scheme for timing fungicide applications to control *Alternaria* leaf blight of carrots. Can. J. Plant Pathol., 1:95:99.

Golino, D. A., Oldfield, G. N. and Gumpf, D. J. 1987. Transmission characteristics of the beet leafhopper transmitted virescence agent. Phytopathology, 77:954-957.

Goyer, C. and Beaulieu, C. 1997. Host range of Streptomycete strains causing common scab. Plant Dis., 81:901-904.

Greco, N. and Brandonisio, A. 1980. Relationship between *Heterodera carotae* and carrot yield. Nematologica, 26:497-500.

Greco, N. and Brandonisio, A. 1986. The biology of *Heterodera carotae*. Nematologica, 32:447-460.

Grisham, M. P. and Anderson, N. A. 1983. Pathogenicity and host specificity of *Rhizoctonia solani* isolated from carrots. Phytopathology, 73:1564-1569.

Groom, M. R. and Perry, D. A. 1985. Induction of cavity spot-like lesions in roots of *Daucus carota* by *Pythium violae*. Trans. Brit. Myc. Soc. 84:755-757.

Grove, J. F. 1964. Metabolic products of *Stemphylium radicinum*. Part I. Radicinin. J. Chem. Soc., 1964: 3234-3239.

Gurkin, R. S. and Jenkins, S. F. 1985. Influence of cultural practices, fungicides, and inoculum placement on southern blight and Rhizoctonia crown rot of carrot. Plant Dis., 69: 477-481.

Hanson, L. E. and Lacy, M. L. 1990. Carrot scab caused by *Streptomyces* spp. in Michigan. Plant Dis., 74:1037.

Hermansen, A. 1992. Weeds as hosts of *Mycocentrospora acerina*. Ann. Appl. Biol., 121:679-686.

Ho, H. H. 1983. *Phytophthora porri* from stored carrots in Alberta. Mycologia, 75:747-751.

Howard, R. J. Pratt, R. G., and Williams, P. H. 1978. Pathogenicity to carrots of *Pythium* species from organic soils of North America. Phytopathology, 68:1293-1296.

Howell, W. E. and Mink, G. I. 1976. Host range, purification, and properties of a flexuous rod-shaped virus isolated from carrot. Phytopathology, 66:949-953.

Hutchinson, C. M., McGiffen, M. E., Ohr, H. D. and Sims, J. J. 1999. Evaluation of methyl iodide as a soil fumigant for root-knot nematode control in carrot production. Plant Dis., 83:33-36.

Jenkins, S. F. and Averre, C. W. 1986. Problems and progress in integrated control of southern blight of vegetables. Plant Dis., 70:614-619.

Kendrick, J. B. 1934. Bacterial blight of carrot. J. Agric. Res., 49:493-510.

Kuan, T.-L., Minsavage, G. V. and Gabrielson, R. L. 1985. Detection of *Xanthomonas campestris* pv. *carotae* in carrot seed. Plant Dis., 69:758-760.

Kushalappa, A. C., Boivin, G. and Brodeur, L. 1989. Forecasting incidence thresholds of *Cercospora* blight in carrots to initiate fungicide application. Plant Dis., 73:979-983.

Kuske, C. R., Kirkpatrick, B. C., Davis, M. J. and Seemuller, E. 1991. DNA hybridization between Western aster yellows mycoplasma-like organism plasmids and extrachromosomal DNA from other plant pathogenic mycoplasma-like organisms. Mol. Plant-Microbe Interact., 4:75-80.

Lambert, D. H. 1991. First report of additional hosts for the acid scab pathogen *Streptomyces acidiscabies*. Plant Dis., 75:750.

Langenberg, W. J., Sutton, J. C. and Gillespie, T. J. 1977. Relation of weather variables and periodicities of airborne spores of *Alternaria dauci*. Phytopathology, 67:879-883.

Latham L. J. and Jones, R. A. C. 2000. Yield and quality losses in carrots infected with carrot virus Y. Proceedings of Carrot Conference Australia. E. Davison and A. McKay, eds. Perth, Western Australia.

Lee, I.-M. and Davis, R. E. 1988. Detection and investigation of genetic relatedness among aster yellows and other mycoplasma-like organisms by using cloned DNA and RNA probes. Mol. Plant Microbe Interact. 1:303-310.

Lewis, B. G., Davies, W. P. and Garrod, B. 1981. Wound healing in carrot roots in relation to infection by *Mycocentrospora acerina*. Ann. Appl. Biol. 99:35-42.

Liddell, C. M., Davis, R. M., Nuñez, J. J. and Guerard, J. P. 1989. Association of *Pythium* spp. with carrot root dieback in the San Joaquin Valley of California. Plant Dis., 73:246-249.

Mahr, S. E. R., Wyman, J. A. and Chapman, R. K. 1993. Variability in aster yellows infectivity of local populations of the aster leafhopper (Homoptera: Cicadelliadae) in Wisconsin. J. Econ. Entomol., 86:1522-1526.

Maude, R. B. 1966. Studies on the etiology of black rot, *Stemphylium radicinum* (Meier, Drechsl., & Eddy) Neerg., and leaf blight, *Alternaria dauci* (Kuhn) Groves & Skolko, on carrot crops; and on fungicide control of their seed-borne infection phases. Ann. Appl Biol., 57: 83-93.

Maude, R. B. 1992. Strategies for control of seed-borne *Alternaria dauci* (leaf blight) of carrots in priming and process engineering systems. Plant Pathol., 41:204-214.

Maude, R. B. and Shuring, C. G. 1972. Black rot of carrots, Rep. Nat. Veg. Res. Stn., 20:103.

Merriman, P. R., Miriam, P., Harrison, G. and Nancarrow, J. 1979. Survival of sclerotia of *Sclerotinia sclerotiorum* Soil Biol. Biochem., 11:567-570.

Mildenhall, J. P. and Williams, P. H. 1970. Rhizoctonia crown rot and cavity spot of muck-grown carrots. Phytopathology, 60:887-890.

Mildenhall, J. P., Pratt, R. G., Williams, P. H. and Mitchell, J. E. 1971. Pythium brown root and forking of muck-grown carrots. Plant Dis. Rep. 55:536-540.

Mildenhall, J. P. and Williams, P. H. 1973. Effect of soil temperature and host maturity on infection of carrot by *Rhizoctonia solani*. Phytopathology, 63:276-280.

Moran J., Gibbs, A., van Rijswijk, B., Mackenzie, A., Gibbs, M. and Traicevski, V. 1999. Potyviruses in the cultivated and wild Apiaceae in Australia and the implications for disease control. Australasian Plant Pathological Society Conference Handbook. 12th Biennial Conference, Canberra, Australia.

Murant, A. F., Waterhouse, P. M., Raschke, J. H. and Robinson, D. J. 1985. Carrot red leaf and carrot mottle virus: observations on the composition of the particles in single and mixed infections. J. Gen. Virol., 66:1575-1579.

Neergaard, P. and Newhall, A. G. 1951. Notes of the physiology and pathogenicity of *Centrospora acerina* (Hartig) Newhall. Phytopathology, 41:1021-1033.

Palti, J. 1975. Erysiphaceae affecting Umbelliferous crops, with special reference to carrot, in Israel. Phytopath. Medit., 14:87-93.

Pryor, B. M., Davis, R. M. and Gilbertson, R. L. 1998. Detection of soilborne *Alternaria radicina* and its occurrence in California carrot fields. Plant Dis., 82:891-895.

Pryor, B. M., Davis, R. M. and Gilbertson, R. L. 1994. Detection and eradication of *Alternaria radicina* on carrot seed. Plant Dis., 78:452-456.

Pryor, B. M., Davis, R.M. and Gilbertson, R. L. 2000. A toothpick inoculation method for evaluation of carrot cultivars for resistance to *Alternaria radicina*. HortScience 35:1099-1102.

Perry, D.A. and Harrison, J.G. 1979. Cavity spot of carrots. I. Symptomology and calcium involvement. Ann. Appl. Biol., 93:101-108.

Punja, Z. K. 1985. The biology, ecology, and control of *Sclerotium rolfsii*. Annu. Rev. Phytopathol., 23:97-127.

Punja, Z. K. 1987. Mycelial growth and pathogenesis by *Rhizoctonia carotae* on carrot. Can. J. Plant Pathol., 9:24-31.

Punja, Z. K., Chittaranjan, S. and Gaye, M. M. 1992. Development of black root rot caused by *Chalara elegans* on fresh market carrots. Can. J. Plant Pathol., 14:299-309.

Punja, Z. K. and Gaye, M. M. 1993. Influence of postharvest handling practices and dip treatments on development of black root rot on fresh market carrots. Plant Dis., 77:989-995.

Rader, W. E. 1948. *Rhizoctonia carotae* n. sp. and *Gliocladium aureum* n. sp., two new pathogens of carrots in cold storage. Phytopathology, 38:440-452.

Ricker, M. D. and Punja, Z. K. 1991. Influence of fungicide and chemical salt dip treatments on crater rot caused by *Rhizoctonia carotae* in long-term storage. Plant Dis., 75:470-474.

Roberts, P.A. 1987. The influence of planting date of carrot on *Meloidogyne incognita* reproduction and injury to roots. Nematologica, 33:335-342.

Roberts, P. A., Magyarosy, A. C., Matthews, W. C. and May, D. M. 1988. Effects of metam-sodium applied by drip irrigation on root-knot nematodes, *Pythium ultimum*, and *Fusarium* sp. in soil and on carrot and tomato roots. Plant Dis., 72:213-217.

Santos, P., Nuñez, J. J. and Davis, R. M. 2000. Influence of gibberellic acid on carrot growth and severity of *Alternaria* leaf blight. Plant Dis., 84:555-558.

Schrandt, J. K., Davis, R. M. and Nuñez. J. J. 1994. Host range and influence of nutrition, temperature, and pH on growth of *Pythium violae* from carrot. Plant Dis., 78:335-338.

Seagall, R. H. and Dow, A. T. 1973. Effects of bacterial contamination and refrigerated storage on bacterial soft rot of carrots. Plant Dis. Rep., 57:896-899.

Smith, P. R. 1967. The survival in soil of *Itersonilia pastinacae* Channon, the cause of parsnip canker. Aust. J. Biol. Sci., 20:647-660.

Sondheimer, E. 1957. The isolation and identification of 3-methyl-6-methoxy-8-hydroxy-3, 4-dihydroisocoumarin from carrots. J. Am. Chem. Soc. 79:5036-5039.

Stelfox, D. and Henry, A. W. 1978. Occurrence of rubbery brown rot of stored carrots in Alberta. Canadian Plant Disease Survey 58:87-91.

Strandberg, J. O. 1983. Infection and colonization of inflorescences and mericarps of carrot by *Alternaria dauci*. Plant Dis., 67:1351-1353.

Strandberg, J. O. 1988. Establishment of *Alternaria* leaf blight in controlled environments. Plant Dis., 72:522-526.

Strandberg, J. O. and White, J. M. 1979. Effect of soil compaction on carrot roots. J. Am. Soc. Hort. Sci. 104:344-349.

Subbarao, K. V. 1998. Progress toward integrated management of lettuce drop. Plant Dis., 82:1068-1078.

Towner, D. B. and Beraha, L. 1976. Core-rot: A bacterial disease of carrots. Plant Dis.Rep., 60:357-359.

Tylkowska, K. 1992. Carrot seed-borne diseases caused by *Alternaria* species. In: "*Alternaria* Biology, Plant Diseases and Metabolites". (eds. Chelkowski, J. and Visconti, A.), Elsevier Science Publishers, Amsterdam, pp. 337-352

Umesh, K. C., Davis, R. M. and Gilbertson, R. L. 1998. Seed contamination thresholds for

development of carrot bacterial blight caused by *Xanthomonas campestris* pv. *carotae*. Plant Dis. 82:1271-1275.

Valder, P. G. 1958. The biology of *Helicobasidium purpureum* Pat. Trans. Brit. Mycol. Soc., 41: 283-308.

Vivoda, E., Davis, R. M., Nuñez, J. J. and Guerard, J. P. 1991. Factors affecting the development of cavity spot of carrot. Plant Dis., 75:519-522.

Vrain, T. C. 1982. Relationship between *Meloidogyne hapla* density and damage to carrots in organic soils. J. Nematol., 14:50-57.

Waterhouse, P. M. and Murant, A. F. 1983. Further evidence on the nature of the dependence of carrot mottle virus on carrot red leaf virus for transmission by aphids. Ann. Appl. Biol., 103:455-464.

Watson, M. T. and Falk, B. W. 1994. Ecological and epidemiological factors affecting carrot motley dwarf development in carrots grown in the Salinas Valley of California. Plant Dis., 78:477-481.

Watson, M.T., Tian, T., Estabrook, E. and Falk., B. W. 1998. A small RNA identified as a component of California carrot motley dwarf resembles the beet western yellows luteovirus ST9-associated RNA. Phytopathology, 88:164-170.

White, J. G. 1988. Studies on the biology and control of cavity spot of carrots. Ann. Appl. Biol.. 113:259-268.

White, J. M. and Strandberg, J. O. 1979. Physical factors affecting carrot root growth: Water saturation of soil. J. Am. Soc. Hort. Sci., 104:414-416.

Whitney, N. J. 1954. Investigations of *Rhizoctonia crocorum* (Pers.) DC in relation to the violet root rot of carrots. Can. J. Bot., 32: 679-704.

Whitney, N. J. 1956. The control of violet root rot in Ontario. Can. J. Agric. Sci., 36:276-283.

Wilson, M. and Henderson, D. M. 1966. British Rust Fungi. University Press, Cambridge, Great Britain.

Celery Diseases and their Management

Richard N. Raid

*Everglades Research and Education Center, University of Florida,
3200 East Palm Beach Road, Belle Glade, FL 33430, USA*

Abstract: Celery ranks third among the world's salad vegetables in popularity. Grown primarily for its succulent leaf petioles, it is utilized in both fresh and processed forms. Celery seed is also popular for its tangy flavor. Celery has a relatively long production cycle, with seedlings being raised for 8-10 weeks as transplants and then for another 10-12 weeks in the field. The resultant long growing season exposes the crop to a myriad of pathogens over that time. Many are capable of inciting significant losses in both yield and quality under favorable conditions. In this chapter, the most widespread and significant diseases of celery are described, along with recommended control measures. Four important fungal diseases are discussed, including early blight, Fusarium yellows and wilt, late blight, and pink rot. Two bacterial diseases, bacterial leaf spot, and bacterial blight, are detailed, as are two viral diseases, celery mosaic virus and southern mosaic virus. Nematode pathogens discussed are divided into two major groups, root-knot nematodes and ectoparasitic nematodes, the latter including seven different types. Blackheart and cracked stem, two common physiological disorders of celery, are also described.

1. Introduction

Celery, *Apium graveolens* L. var. *dulce* (Miller) Pers., is a member of the family Umbelliferaceae. Although its origin is not clear, it is thought to have originated in the Mediterranean region (Ryder, 1979). Its first recorded cultivation took place in Egypt in about 300 B.C., where it was grown and used for medicinal purposes. However, it first cultivation as a vegetable may go back to as early as 850 B.C (Rubatzky *et al.,* 1999). Celery plants consist of a rosette of succulent, basal-clasping, long-petioled leaves in tight upright, stalk-like groupings. A well-developed celery 'stalk' has 7-15 clearly distinguished petioles surrounding a similar number of increasingly smaller petioles that lie interior. Referred to as the 'heart', the interior petioles are tightly grouped and are 'blanched' or shielded from light. These are the most tender tissues of the celery stalk.

Celery may develop a considerable root system, being somewhat shallow, with the exception of the taproot. Taproots are spindle-shaped, fleshy, and are nearly tuberous. Celery is a biennial, but subjected to vernalization, may complete its life cycle in one year. The inflorescence, known as an umbel, resembles that of carrot, but is smaller and somewhat more compact. Seed production on the umbrella-shaped reproductive structures is prolific, with the oval and corky-ribbed seeds being very small. There are about 2,500 seeds in one gram. To avoid self-dormancy, celery seeds should be stored at least one year prior to planting.

Celery is a relatively long-seasoned crop, most commonly produced from transplants. Celery seed germination and emergence is relatively slow, taking 7-12 days at optimal temperatures of 15-20 C. Above 30 C, celery seeds may exhibit thermodormancy. Since darkness may enhance prospects for thermodormancy, celery is typically sown very shallow, with seeds being kept moist and cool, frequently by shading.

Celery has high nutrient requirements, particularly for nitrogen. Nutrient inputs are typically 300 kg of nitrogen, 75 kg of phosphorus, and 250 kg of potassium per ha. Due to its high nitrogen requirement, celery is commonly produced on organic or 'muck' soils, high in plant-available nitrogen.

Celery transplants characteristically have a growth period of 45-75 days. It is common to clip or 'top' the foliage of celery seedlings to improve their hardiness and vigor prior to transplanting into the field. This operation has the potential for spreading disease pathogens, particularly bacteria, due to the wounding that takes place.

Field populations of celery ranges from 50,000 to 80,000 plants/has, with transplants usually being spaced 12-20 cm within rows planted on 50-75 cm spacing. Crops are harvested 80-100 days following transplanting, with the crop being sized and graded at harvest. Relative to head lettuce, which has a narrow harvest window of 1-2 days, celery has a rather wide harvest window, ranging from 7-10 days. After harvest, celery is typically hydo-cooled. Optimum storage and shipping conditions are 0-1 C with a relative humidity of greater than 95%. Celery can hold for more than 30 days under optimal conditions.

2. Celery diseases caused by Bacteria

There are three bacterial diseases of major importance on celery, two of which are covered in this chapter: bacterial leaf spot, and bacterial blight. The third disease, bacterial soft rot, is caused by *Erwinia caratovora* subsp. *caratovora* (Jones) Bergey *et al.,*. This disorder is covered in the chapter on lettuce disease management, (volume II) in the section on soft rot.

2.1 Bacterial blight and brown stem of celery

Caused by the bacterium *Pseudomonas cichorii* (Swingle) Stapp, bacterial blight has been a major disease of celery transplants, and occasionally field production, in warm celery growing regions such as Florida (Thayer and Wehlburg, 1965). The disease begins as small, water-soaked lesions on the leaf blade. Spots become necrotic, circular to angular, and may develop distinct halos. With age, lesions become dry and brown. A second symptom, known as brown stem may also develop (Pernezny *et al.,* 1994). Brown stem results in a brown discoloration of the petiole, usually more evident on the petiole interior, closest to the crown. Browning is confined to the parenchyma surrounding vascular bundles, which appear healthy and green within diseased cortical and pith tissues.

Bacterial blight is favored by warm temperatures and is capable of causing severe disease at temperatures of 25-30ºC (Thayer, 1965). Its relationship to warm temperatures, more common in southern areas of the U.S., is reflected in the common

moniker 'southern bacterial blight'. *P. cichorii* has a broad host range including a number of other vegetable crops and ornamentals. It is also thought to be capable of epiphytic survival on weeds.

Once established, bacterial blight is difficult to control in transplant beds and greenhouses. Care should be taken to disinfect transplant mowing or topping equipment between cuttings and movement between houses, since the mechanical wounding provides ample avenues for bacterial infection. Likewise, mowing should be performed when the foliage is dry to reduce opportunities for ingress. Streptomycin, once effective, has been rendered ineffective due to widespread selection for streptomycin-insensitive strains of the pathogen. Copper bactericides may give some control, but copper tolerant strains have also been reported (Pohronezny *et al.,* 1994). Varietal susceptibility to the disease is variable, holding promise for the development of more resistant celery lines.

2.2 Bacterial leaf spot

First described in New York in 1921 (Jagger 1921), bacterial leaf spot is frequently called northern bacterial blight to differentiate it from southern bacterial blight incited by *Pseudomonas cichorii* (Swingle) Stapp (Lacy *et al.,* 1996). Bacterial leaf spot remained a disease of minor importance in the eastern and Midwestern celery growing regions of the U.S. for decades. However, the disease has risen to prominence in recent years due to outbreaks in California since 1989. By 1991, the disease had spread to all celery-growing regions in California (Little *et al.,* 1997).

Symptoms begin as small, 2-5 mm angular, water-soaked lesions on leaves. Soon taking on a greasy appearance, lesions turn rusty brown, frequently coalesce, and may result in extensive tissue necrosis. With age, infected tissues become dry and papery. Fortunately, petioles are not affected and the disease rarely kills plants. However, leaf spot may significantly reduce the vigor and value of transplants.

Caused by *Pseudomonas syringe* pv. *apii* (Jagger) Young *et al.*, the disease is favored by cooler temperatures (20-25° C) than those that favor *P. cichorii*. For this reason, it is more prevalent in the cooler, temperate celery growing regions. The pathogen can survive in association with seed for 2-3 yrs. Thus contaminated seed is likely the primary inoculum source for most outbreaks (Little *et al.,* 1997). The bacterium then multiplies and survives epiphytically on transplants until environmental conditions become favorable for infection and spread. Such conditions are frequently related to wounding (mowing of the transplants) and overhead irrigation. Shoreflies may be involved in spreading the bacterium between transplant greenhouses. High nitrogen levels, which result in more succulent growth that is more easily damaged, also seems to increase disease severity.

Control should focus on the use of pathogen-free or hot-water treated seed (50° C for 25 min) for transplant production (Little *et al.,* 1997). Mowers used for topping should be disinfected between greenhouses and mowings. Should an outbreak occur, it may be necessary to restrict worker and tool movement between greenhouses and to take precautions regarding clothing and hand-disinfection. Excessive fertility, particularly N, should be avoided. Copper-based compounds provide only marginal control

and cannot be solely relied upon.

3. Celery diseases caused by fungi

Fungi make up the largest group of pathogens inciting disease on celery. Four of the most important and widely distributed fungal diseases are described in this chapter. Additional fungal disorders reported on celery include: Alternaria leaf spot (*Alternaria* sp.), anthracnose (*Colletotrichum* sp.), brown spot (*Acremonium apii*), crater rot and damping-off (*Rhizoctonia solani* Kühn), gray mold blight (*Botrytis cinerea* Pers.:Fr.), Phoma crown and root rot (*Phoma apiicola* Kleb.), powdery mildew (*Erysiphe heraclei* DC.), Pythium damping-off and root rot (*Pythium debaryanum* Auct.non R. Hesse), red root (*Fusarium solani* (Mart.) Sacc.), southern blight (*Sclerotium rolfsii* Sacc.) and *Stemphylium* leaf spot (*Stemphylium ramulosa* Sacc.) (Davis and Raid, 2002). Most of these diseases are of limited importance and/or range, although some may cause extensive damage in individual locations when conditions are favorable. Damping-off and southern blight, which are widely distributed, are covered in more detail in the chapter on lettuce diseases.

3.1 Early blight

Early blight, also known as *Cercospora* blight, is one of the most serious and potentially damaging diseases of celery worldwide. First described in Europe in 1863, it is present wherever celery is grown. Extensive necrosis caused by the fungal pathogen may result in plant stunting and necessitate significant levels of trimming at harvest. In Florida, early blight has resulted in entire fields being abandoned (Berger, 1973). Early blight management can be one of the most intense and expensive disease control programs on any vegetable crop.

Early blight is caused by the imperfect fungus, *Cercospora apii* Fresen. The pathogen has pale brown, septate hyphae that form hyphal knots in the substomatal cavities. Also referred to as pseudostromata, these knots give rise to brown, septate condiophores (4 x 55-100 μm) that emerge in clusters through the stomata. Conidia are colorless, long and slender (3.5-5.5 x 40-200 μm), straight to slightly curved, and tapered toward the apex. Several conidia are produced separately at the apex of each conidiophore, leaving visible scars when the spore detaches at the hilum.

Primary inoculum for early blight originates from either infected seeds or from plant residues in the soil. The fungus reportedly is capable of surviving as mycelium for more than 2 years on infected seeds. Levels of early blight in the field are correlated with blight levels that first develop in the transplant beds or houses. *Cercospora* blight generally appears before Septoria late blight, giving rise to its common name 'early blight'. Considered to be a warm-temperature disease, early blight is favored by temperatures of 15-30°C and by long periods of leaf wetness or high relative humidity. Sporulation is most prolific when leaf wetness exceeds 10 hr. Spores are readily wind disseminated and are released during late morning hours when relative humidity subsides. Conidia also are dispersed by field operations and rain splash. Spore germination and stomatal penetration may occur with as few as 5 hrs of leaf wetness. The pathogen

may complete its secondary cycle in 5-14 days, with colonization being most rapid at the warmer temperatures. The long celery field production season from transplanting is conducive to massive disease buildup, usually mandating some sort of fungicide control program.

Disease management of early blight should not rely on any single strategy. Efforts should be made to use pathogen-free seed and to limit disease development in the seedbeds. These efforts may include crop rotation and seedbed soil pasteurization or fumigation. Care should be taken to avoid overcrowding transplants as this prolongs leaf wetness. Optimal spacing of plants atop raised beds can also be a significant factor in reducing blight severity. In Florida, susceptible cultivars, such as 'Florida 683' and 'Florida 2-14', are planted only during the coolest months (Raid 2002). Only resistant or tolerant cultivars are planted during periods considered to be favorable for early blight (warm and moist). 'Florabelle', 'June-Belle', and 'Earlibelle' are several notable early blight tolerant cultivars. In high-risk growing regions, fungicidal spray programs may be required during both transplant and field production. A fungicide program based on disease forecasting, which was developed during the late 1960s for control of early blight in Florida, is still in use today, with some modification. Spray programs incorporating broad spectrum protectants, such as chlorothalonil, and locally systemic fungicides such as the sterol inhibitors or strobilurins have demonstrated the most efficacy (Miller and Hernandez 2000, Raid and Dyce 1999). Their use in tank-mixtures or alternation also aids in preventing selection for insensitive strains of the fungus.

3.2 *Fusarium* yellows and wilt

Incited by *Fusarium oxysporum* f. sp. *apii* (R. R. Nelson & Scherb) W. C. Snyder & H. N. Hans., *Fusarium* yellows and wilt is one of the most important soilborne diseases of celery. Formerly referred to as "stunting disease" or simply as "yellows", it has been reported from celery growing regions worldwide. Losses in quality and quantity may be significant. From the 1930s through the early 1970s, *Fusarium* wilt was successfully controlled using resistant varieties. However, reappearance of the disease in California in 1978 provided evidence for the existence of a new race, race 2, capable of attacking both self-blanched and the formerly resistant green varieties (Opgenorth and Endo 1979). By 1982, it was widespread and continues to be a problem, despite the availability of a few race 2 resistant lines.

The disease is characterized by yellowing of the foliage and plant stunting. When sectioned longitudinally, vascular tissues in the crown and taproot exhibit a reddish-brown discoloration. Fleshy roots may turn necrotic and older leaves may become brittle. Additionally, secondary growth from adventitious buds in the crown area may lead to split petioles.

F. oxysporum f. sp. *apii* produces single-celled microconidia as well as thick-walled, sickle-shaped macroconidia. These are multi-septate and have an attenuated apical cell and a foot-shaped basal cell. The fungus also produces abundant chlamydospores, which serve as survival structures.

The pathogen may persist indefinitely in the soil by surviving in plant debris, as

chlamydospores, or as a saprophyte on weeds and other crops. The fungus enters the root system and spread systemically throughout the plant. Research has demonstrated that thresholds of 37 propagules/gr of soil can cause significant growth reductions and above 42 propagules/gr, nearly all plants exhibit vascular discoloration and growth reductions. Symptom appearance is influenced by temperature, with latent periods shortening as soil temperature is raised.

Fusarium yellows is a monocyclic disease, meaning that there is no secondary spread of the disease within the same season. Infected plants serve to increase the amount of inoculum available for the next season. The disease is thought to spread to new fields and areas through the movement of infested soil or infested transplants.

Being long-lived in the soil, the *Fusarium* yellows pathogen is difficult to control once established. Therefore, in seedbeds established for transplant production, sanitation measures such as equipment disinfection and the use of pathogen-free soil or potting mix should be used to prevent introduction. If already present, inoculum levels may be reduced by soil fumigation or steam pasteurization.   However, such strategies are costly. Many growers have gone to the use of celery transplants grown in plastic or styrofoam planting trays using sterile potting mix. In production fields, soil amendments with rock salt, potassium or nitrate ions, and chitosan, have all shown promise but most are not feasible (Bell *et al.,* 1998, Schneider 1985). Short term crop rotations have for the most part been ineffective, but Lacy *et al.,* 1996 reported that onion/celery rotations have been demonstrated as efficacious in reducing inoculum levels short-term.

Resistance for many years provided effective control of *Fusarium* yellows. However, with the appearance of race 2, this control dissipated. Recent breeding efforts to reestablish control, while not yet entirely successful, display promise.

3.3 *Sclerotinia* **pink rot and foot rot**

Pink rot is caused by the fungal pathogens *Sclerotinia sclerotiorum* (Lib.) de Bary and foot rot is caused by *S. minor* Jagger. These fungi are worldwide problems in both production and storage. Both pathogens can attack celery at any stage of growth. The fungi cause damping-off in seedbeds, attacking plants at the soil surface. On more mature plants, the disease appears as a watery soft rot that develops on stalks at or near ground level. Infected tissues take on a pinkish hue, hence the name. Over time, these tissues become covered by a thick, white cottony mold. Hard, blackish-grey sclerotia frequently form within the crown and basal region, their shape and size dependent upon the fungal species. Sclerotia of *S. minor* are mostly spherical and uniform in diameter (approx. 0.5-2.0 mm), while those of *S. sclerotiorum* are more irregular in shape and are considerably larger, measuring 2-20 x 3-7 mm in diameter. Both pathogens may survive as sclerotia for up to 8-10 years in the soil, but they frequently differ in their mode of infection (Abawi and Grogan, 1979). Detailed disease cycles for the two *Sclerotinia* spp. are given in the section on 'drop' in the lettuce disease management chapter.

While sclerotia of the drop pathogens may be long-lived, crop rotation may

provide adequate control if non-host rotational crops are grown. Although *Sclerotinia* has a rather broad host-range, limiting crop selection, beet, onions, spinach, corn, and small grains are crops that are useful to include. Deep-plowing to bury sclerotia deep in the soil has also proven to be effective under low inoculum densities (Subbarao *et al*., 1996), as has flooding of celery fields during non-production periods where feasible (Moore, 1949). Irrigation methods which avoid wetting the top 5-8 cm of soil, such as subsurface drip or seepage irrigation, assist in controlling this disease. While fungicides may aid in the control of *Sclerotinia* when conditions are cool and moist for extended periods, chemical measures should not be solely relied upon.

3.4 *Septoria* late blight

Late blight, caused by the imperfect fungus *Septoria apiicola* Speg. (syn: *S. apii* and *S. apii-graveolentis*), is a major disease of celery worldwide. During cool, wet weather, losses can exceed 70%. Symptoms include irregularly shaped chlorotic spots on leaves and petioles. Oldest leaves are usually infected first. As lesions age, they become necrotci and may coalesce, turning large portions of tissue brown and blighted. The presence of small black specks, pycnidia, differentiate late blight from similar looking diseases such as early blight (*Cercospora apii*), bacterial spot (*Pseudomonas syringe* pv. *apii*) or bacterial blight (*P. cichorii*). Symptom development is typically 10 days on susceptible varieties and 16-21 days on more resistant varieties (Edwards *et al.,* 1997).

    S. apiicola pycnidia are black, flask-shaped and embedded in infected tissues. They typically measure 55-200 µm in diameter. Each pycnidium may produce 1,500-4,500 long, flexous, multicellular conidia, which are extruded in the presence of free-moisture. These conidia are then water-splashed by rain or overhead irrigation to foliage. No sexual stage has been reported. *S. apiicola* survives on infected seed and overwintering debris (Berger, 1970). Conidial viability within pycnidia on infested seed typically decreases over time (2-3 yrs) and seed held for periods longer than this may be virtually free of infective *Septoria*. Thus, storing seed for more than 3 yrs lends itself to very effective disease control (Maude, 1970). Seed may also be treated with a fungicide (24 hr at 30° C in a 2% thiram suspension) or hot-water dip (47°C for 30 min) to reduce seedborne inoculum. Crop rotations of two years or more are effective in reducing field-borne inoculum. Since late blight is disseminated by watersplash, overhead irrigation should be eliminated or minimized wherever possible.

    Should an outbreak of late blight occur, fungicides may provide effective control If used, the protectant fungicides chlorothalonil or mancozeb should be applied at 5-10 day intervals in advance of symptom development to be most effective (Mudita and Kushalappa, 1993). However, a program using protectant fungicides along with a sterol inhibiting and/or strobilurin fungicides may prove most effective (Raid, 2001a, Raid, 2001b). Fungicide programs using weather-based information for scheduling have also been developed (Lacy, 1994, Lacy *et al.,* 1996).

4. Celery diseases caused by Viruses

There are a number of important diseases of celery incited by plant viruses, and only

one attributed to a phytoplasma, aster yellows. Two viral diseases of widespread importance are covered in this chapter. These are celery mosaic and southern mosaic. Viral disorders not included but reported on celery include: Calico (*Alfalfa mosaic alfamovirus*), celery yellow net (*Celery yellow mosaic potyvirus*), curly top (*Beet curly top geminivirus*), strap leaf (*Strawberry latent ringspot nepovirus*), and tomato spotted wilt (*Tomato spotted wilt tospovirus*). Most of these are of limited distribution and importance. Descriptions of tomato spotted wilt and aster yellows are included in the chapter on lettuce diseases and their management.

4.1 Celery mosaic virus

Celery mosaic, caused by the *Celery mosaic potyvirus* (CeMV), formerly known as western celery mosaic virus, has been detected in most celery growing regions of the world. Whole fields have been lost to this disease. The virus systemically infects a number of other important umbelliferous crops, including carrot, coriander, dill, parsley, and parsnip. The moniker "western mosaic" was used to differentiate the disorder from "southern mosaic", now known to be caused by *Cucumber mosaic potyvirus* (CMV). Initial symptoms on celery include vein-clearing and mottling of interveinal areas on the youngest leaves (Purcifull and Shepard, 1967). Leaflets on mature leaves appear narrow, twisted and cupped, also having a shiny appearance. CeMV causes dark green mottled areas on petioles, in contrast to the brown sunken areas caused on petioles by CMV. Young plants infected with CeMV may be stunted, exhibiting horizontal growth of outer petioles.There are at least two distinct strains of CeMV, a common strain and a strain named celery crinkle-leaf mosaic for characteristic symptoms produced on the crop (Brunt *et al.,* 1996). The virus is transmitted in a semi-persistent manner by at least 19 species of aphids. It may also be mechanically transmitted but it is not seed transmitted. CeMV has a rather limited host range, with fewer than three plant families being reported as hosts.

Since CeMV is not seed transmitted, there are two primary sources of inoculum: cultivated umbelliferous crops such as carrots, dill, parsley, celery, etc., or umbelliferous weeds. CeMV is transmitted from infected hosts to celery by the many aphid vectors. These are capable of transmitting the virus after feeding for only 5-30 sec, but lose infectivity within 24 hrs. Symptoms may appear within 10 days of inoculation. CeMV management starts with eliminating or reducing the amount of primary inoculum. Programs using the establishment of a celery-free period of 1-3 months along with the elimination of weed hosts have been found effective in many areas (Orsenigo and Zitter, 1971).

Transplants should also be grown in virus-free areas or in highly monitored greenhouses. Due to its non-persistent transmission, attempts to manage the disease through vector control or by roguing infected plants are generally ineffective. Although CeMV tolerant varieties have been released, host-plant resistance has not offered total control in and of itself. However, when used in concert with other management strategies, the benefits of resistance may be enhanced.

4.2 Southern mosaic virus

Southern mosaic, now known to be caused by the *Cucumber mosaic potyvirus*, was described by Poole in New Jersey in 1922 (Poole, 1922). Worldwide in distribution, present-day losses seldom exceed 10%, although local outbreaks may be more serious.

CMV symptoms on celery are characterized by a prominent outward and downward curling of young petioles, giving plants an open or flattened appearance (Townsend, 1947). Infected plants tend to distinguish themselves, and the disease may be recognized from some distance away. Leaves of infected plants develop vein-clearing and mosaic. Interveinal areas may become dark green and thickened, producing a crinkled-leaf appearance. Cucumber mosaic symptoms may be transient. On plants infected when young, older leaves become chlorotic and develop veinal necrosis. The petioles on these plants may develop elongated, brown to translucent, sunken spots, significantly reducing their marketability.

There are at least two strains of CMV that have been identified on celery, the cucumber mosaic type strain and the celery calico virus strain. The calico strain produces milder symptoms and does not produce necrotic spots. CMV has one of the most extensive host ranges of any pathogen, including nearly 800 species of monocots and dicots (Brunt *et al.,* 1996). The virus is transmitted by more than 60 aphid species in a non-persistent, stylet-borne manner. The insect can acquire the virus within 20-60 seconds but remains infective for only a few hours. CMV has not been reported as being seed-borne on celery, although this has occurred with some other hosts.

The primary source of inoculum for CMV in celery is nearby infected weed, ornamental, or crop hosts. Aphids transmit the virus to celery after acquiring it by feeding on an infected plant. Major infection periods are typically well-correlated with major peaks in vector movement or activity. Mechanical transmission, although possible, is of minor consequence in the field. CMV outbreaks characteristically begin along field edges, and progress inward with secondary spread by the vectors. Small, scattered secondary foci then expand and coalesce until the entire field may ultimately be infected.

Management of CMV should target the primary inoculum source (Wellman, 1937). Strategies should include: use of disease-free transplants, eradication of weed hosts, isolating new celery plantings from old plantings or other host crops, and the prompt destruction of old crop debris. Such measures have made observation of CMV on celery a rarity in many regions. Efforts to control the vector have met with only limited success.

5. Celery diseases caused by Nematodes

Nematodes reported on celery include a number of ectoparasitic genera and a smaller number of endoparasitic species, primarily in the genus *Meloidogyne*. One migratory endoparasitic nematode, the stem nematode (*Ditylenchus dipsaci* Kühn) Filipjev) has been reported but is not covered in this chapter due to its limited distribution (Italy) (Davis and Raid, 2002).

5.1 Ectoparasitic nematodes on celery

A number of important ectoparasitic nematodes have been reported as causing injury to celery, as well as a number of related umbelliferous crops, such as carrot and parsley. In alphabetical order, these include: lesion nematodes, needle nematodes, pin nematodes, sting nematodes, stubby root nematodes, awl nematodes, and spiral nematodes. The lesion nematode species associated with celery is *Pratylenchus penetrans* (Cobb) Filip. & Stek. (Townsend and Wolynetz 1991), while *Longidorus africanus* Merny and *L. elongatus* (de Man) Thorne & Swanger are the needle nematode species most commonly associated with the crop (Ploeg 1998, Roberts and Mullens, 2002). *Paratylenchus hamatus* Thorne & Allen is the only species of pin nematode pathogenic on celery (Lownsbery *et al.,* 1952), while *Belonolaimus longicaudatus* Rau is the sting nematode species on celery (Christie *et al.,* 1952). *Paratrichodorus minor* (Colbran) Siddiqi (Synonym: *P. christiei* (Allen) Siddiqi), *Dolichodorus heterocephalus* Cobb, and *Rotylenchus* spp. are the stubby root, awl, and spiral nematodes, respectively, causing damage to celery (Ayala *et al.,* 1970, Roberts and Mullens 2002, Seinhorst and Kuniyasa, 1969, Tarjan *et al.,* 1952).

As ectoparasites, these nematodes remain outside of the host root as they feed from the root surface. Adult females deposit eggs into the root zone and the juveniles undergo several molts to become adults. Both juvenile and adult stages are vermiform or wormlike. Characteristic above-ground symptoms incited by these parasites are stunting, chlorosis, and deficiency-type symptoms. Regarding specific nematodes and symptoms, they are as follows: lesion nematodes produce reddish-brown lesions on roots, and root systems appear pruned; needle nematodes cause a reduction in the number of feeder roots and root distortion just behind the root tip; pin nematodes produce rust-colored cracks or root depressions; sting nematodes cause necrotic lesions and root forking; stubby nematodes also cause taproot stunting and forking; awl nematodes inhibit root elongation and produce tiny galls at feeding sites; and spiral nematodes turn roots yellow then brown. Due to similarities in resultant symptoms, the nematodes species listed above cannot be identified solely on the basis of symptom development. One must extract a representative sample from the soil and identify them microscopically using an appropriate taxonomic key.

Ectoparasitic nematodes generally can be controlled with preplant fumigants or contact nematicides. However, while such measures may be feasible in seedbeds and/or transplant potting mixes, they are seldom economically feasible for field production. In Florida, flooding of seedbeds or production fields for 1-3 months has proven effective in controlling these and other nematodes. Crop rotation may also prove to be of use, depending upon the nematode species and crop selection. Regarding the needle nematode, planting date manipulation offers some control. Planting may be delayed until soil temperatures drop below 22°C (Ploeg, 1988).

5.2 Root-knot nematodes

Meloidogyne hapla Chitwood is the root-knot nematode most commonly associated with celery (Taylor 1943). However, other *Meloidogyne* species reported on celery.

Although all cultivars can be affected, some are more prone to blackheart than others.

Blackheart is prevented by fostering steady rather than sporadic growth. Wide fluctuations in fertility or moisture should be avoided. Calcium applications in the form of calcium nitrate (10-20 kg/ha) or calcium chloride (5-10 kg/ha) drenches can help prevent or alleviate symptoms (Geraldson, 1954). Drenches are most effective when applied directly over the heart one or two times per week. Also, the planting of blackheart prone cultivars should be avoided during high risk periods.

6.2 Cracked stem or brown checking of celery

Cracked stem or brown checking is associated with boron deficiency ((Burdine, 1973). It is most frequently observed in old fields or in newly cultivated organic soils. Characteristic symptoms are crosswise cracks that occur across the prominent ribs of the petiole. The epidermis may curl back where cracks are few, giving the stem a shaggy appearance. Where cracks are plentiful, the epidermis remains intact. Disrupted tissue eventually turns brown. Below ground, roots may turn brown to orange, and root tips may die when boron deficiency is severe. Plants remain small and bushy, and petioles become brittle, developing a bitter taste. Leaves, and then petioles themselves, in the heart region may dry out, turn brown and die. This leaves a ring of outer petioles surrounding a brown cavity.

Cracked stem is incited by conditions limiting boron uptake in the celery plant. Although such conditions are most frequently associated with boron deficiency, low levels of calcium and high levels of potassium and ammonium nitrate may aggravate the disorder (Yamaguchi *et al.,* 1958). Management strategies include boron applications as either a supplemental spray or as a fertilizer amendment. Where boron is applied, accurate rates are critical to avoid boron toxicity to the current or future crops. Resistant or tolerant varieties have been identified and should be grown where possible.

7. References

Abawi, G. S. and Grogan, R. G. 1979. Epidemiology of diseases caused by *Sclerotinia* species. Phytopathology, 69:899-904.

Ayala, A., Allen, M. W., and Noffsinger, E. M. 1970. Host range, biology, and factors affecting survival and reproduction of the stubby root nematode. Journal of Agriculture, University of Puerto Rico, 54:341-369.

Bell, A. A., Hubbard, J. C., Liu, L., Davis, R. M., and Subbarao, K. V. 1998. Effects of chitin and chitosan on the incidence and severity of *Fusarium* yellows of celery. Plant Disease, 82:322-328.

Berger, R. D. 1970. Epiphytology of celery late blight. Proceeding of the Florida State Horticultural Society, 83:208-212.

Berger, R. D. 1973. Early blight of celery: Analysis of disease spread in Florida. Phytopathology, 63:1161-1165.

Brunt, A. A., Crabtree, K., Dallwitz, M J., Gibbs, A. J., and Watson, L. (eds.) 1996. Viruses of Plants. CAB International, Wallingford, UK 1484 p.

Burdine, H. W. 1973. The development of pencil stripe in celery. II. Internal induction. A manifestation of brown checking. Proceeding of the Soil and Crop Science Society of Florida,

32:77-83.

Christie, J. R., Brooks, A. N., and Perry, V. G. 1952. The sting nematode, *Belonolaimus gracilis*, a parasite of major importance on strawberries, celery, and sweet corn in Florida. Phytopathology, 42:173-176.

Davis, R. M. and Raid, R. N. (eds.) 2002. Compendium of Umbelliferous Crop Diseases. APS Press, St. Paul, MN. 75 p.

Edwards, S. J., Collin, H. A., and Isaac, S. 1997. The response of different celery genotypes to infection by *Septoria apiicola*. Plant Pathology, 46:264-270.

Foster, A. C. 1934. Blackheart disease of celery. Plant Disease Reporter, 18:177-185.

Geraldson, C. M. 1954. The control of blackheart of celery. Proceedings of the American Horticultural Society, 63:353-358.

Jagger, I. C. 1921. Bacterial leafspot disease of celery. Journal of Agricultural Research, 21:185-188.

Lacy, M. L. 1994. Influence of wetness periods on infection of celery by *Septoria apiicola* and use in timing sprays for control. Plant Disease, 78:975-979.

Lacy, M. L., Berger, R. D., Gilbertson, R. L., and Little, E. L. 1996. Currrent challenges in controlling diseases of celery. Plant Disease, 80:1084-1091.

Little, E. L., Koike, S. T., and Gilbertson, R. L. 1997. Bacterial leaf spot of celery in California: Etiology, Epidemiology, and Role of Contaminated Seed. Plant Disease, 81:892-896.

Lownsbery, B. F., Stoddard, E. M., and Lownsbery, J. W. 1952. *Paratylenchus hamatus* pathogenic to celery. Phytopathology, 42:651-653.

Maude, R. B. 1970. The control of Septoria on celery seed. Annuals of Applied Biology, 65:249-254.

Miller, M. E. and Hernandez, R. A. 2000. Evaluation of fungicides for early blight control on celery, 1999. American Phytopathological Society Fungicide and Nematicide Tests, 55:157.

Moore, W. D. 1949. Flooding as a means of destroying the sclerotia of *Sclerotinia sclerotiorum*. Phytopathology, 39:920-927.

Mudita, I. W. and Kushalappa, A. C. 1993. Ineffectiveness of the first fungicide application at different initial disease incidence levels to manage Septoria blight in celery. Plant Disease, 77:1081-1084.

Opgenorth, D. C. and Endo, R. M. 1979. Sources of resistance to *Fusarium* yellows of celery in California. Plant Disease Reporter 62:165-169.

Orsenigo, J.R. and Zitter, T. A. 1971. Vegetable virus problems in south Florida as related to weed science. Proceedings of the Florida State Horticultural Society, 84:168-171.

Ploeg, A. T. 1998. Horizontal and vertical distribution of *Longidorus africanus* in a filed in the Imperial Valley, California. Journal of Nematology, 30:592-598.

Pernezny, K., Datnoff, L., and Sommerfeld, M. L. 1994. Brown stem of celery caused by *Pseudomonas cichorii*. Plant Disease, 78:917-919.

Pohronezny, K., Sommerfeld, M. L., and Raid, R. N. 1994. Streptomycin resistance and copper tolerance among strains of *Pseudomonas cichorii* in celery seedbeds. Plant Disease, 78:150-153.

Poole, R. F. 1922. Celery mosaic. Phytopathology, 12:151-154.

Purcifull, D. E. and Shepard, J. F. 1967. Western celery mosaic virus in Florida celery. Plant Disease Reporter, 51:502-504.

Raid, R. N. 2001a. Evaluation of fungicides for control of late blight on celery. American Phytopathological Society Fungicide and Nematicide Tests, 57:V024.

Raid, R. N. 2001b. Evaluation of standard fungicides and SAR compounds for control of late blight on celery. American Phytopathological Society Fungicide and Nematicide Tests, 57:V025.

Raid, R. N. 2002. Early blight of celery. In: "Compendium of Umbelliferous Crop Diseases"

(eds. Davis, R. M. and Raid, R. N.) APS Press, St. Paul, MN pp. 20-21.

Raid, R. N. and Dyce, J. 1999. Evaluation of fungicides for control of early blight of celery. American Phytopathological Society Fungicide and Nematicide Tests, 54:135.

Roberts, P. A. and Mullens, T. R. 2002. Disease caused by nematodes. In: "Compendium of Umbelliferous Crop Diseases" (eds. Davis, R. M. and Raid, R. N.) APS Press, St. Paul, MN pp. 45-50.

Rubatzky, V. E., Quiros, C. F., and Simon, P. W. 1999. Carrots and Related Vegetable Umbelliferae. CABI Publishing, New York.

Ryder, E. J. 1979. Celery. In: "Leafy Salad Vegetables". AVI Publishing, Westport, CT. pp. 95-126.

Schneider, R. W. 1985. Suppression of *Fusarium* yellows of celery with potassium, chloride, and nitrate. Phytopathology, 75:40-48.

Subbarao, K. V., Koike, S. T., and Hubbard, J.C. 1996. Effects of deep plowing on the distribution and density of *Sclerotinia minor* sclerotia and lettuce drop incidence. Plant Disease, 80:28-33.

Seinhorst, J. W. and Kuniyasa, K. 1969. *Rotylenchus uniformis* (Thorne) on carrots. Netherlands Journal of Plant Pathology, 75:205-223.

Strandberg, J. O., and White, J. M. 1978. *Cercospora apii* damage on celery – effects of plant spacing and growth on raised beds. Phytopathology, 68:223-226.

Tarjan, A. C., Lownsberry, B. F., Jr., and Hawley, W. O. 1952. Pathogenicity of some plant-parasitic nematodes from Florida soils. I. The effect of *Dolichodorus heterocephalus* Cobb on celery. Phytopathology, 42:131-132.

Taylor, A. L. 1943. Nematode survey in Florida: Effect of rootknot and other nematodes on celery in the Sanford area. Plant Disease Reporter, 27:706-708.

Thayer, P. L. 1965. Temperature effects on growth and pathogenicity to celery of *Pseudomonas apii* and *P. cichorii.* Phytopathology, 55:1365.

Thayer, P. L. and Wehlburg, C. 1965. *Pseudomonas cichorii*, the cause of bacterial blight of celery in the Everglades. Phytopathology, 55:554-557.

Townsend, G. R. 1947. Celery mosaic in the everglades. Plant Disease Reporter, 31:118-119.

Townsend, J. L. and Wolynetz, M. S. 1991. Penetration of celery and alfalfa roots by *Pratylenchus penetrans* as affected by temperature. Journal of Nematology 23:194-197.

Wellman, F. L. 1937. Control of southern celery-mosaic virus in Florida by removing weeds that serve as sources of mosaic infection. United States Department of Agriculture, Technical Bulletin, 548:1-16.

Westgate, P. J., Blue, W. G., and Eno, C. F. 1954. Blackheart of celery and its relationship to soil fertility and plant composition. Proceedings of the Florida State Horticultural Society 67:159-163.

Yamaguchi, M., Howard, F. D., and Minges, P. A. 1958. Brown checking of celery. III. Effects of potassium, nitrogen, and boron in culture solutions on the physiological disorder and nutrient uptake. Journal of the American Society for Horticultural Science, 71:455-467.

Diseases of Cucurbits and their Management

Margaret Tuttle McGrath

Cornell University Department of Plant Pathology,
Long Island Horticultural Research and Extension Center,
3059 Sound Avenue, Riverhead, NY, USA

Abstract: Managing diseases is a very important component of production for melons, cucumbers, squashes, pumpkins, and other cucurbit crops. The already extensive list of more than 200 cucurbit diseases has expanded recently to include cucurbit yellow vine disease, Acremonium collapse, Rhizopycnis root rot, bacterial blight, cucumber root mat, *Cucurbit yellow stunting disorder virus, Cucurbit leaf crumple virus,* and Cucurbit leaf curl virus. Additionally, diseases that have recently increased in importance include the vine declines, bacterial wilt, powdery mildew on watermelon, Phytophthora blight, diseases caused by *Fusarium* species, and several diseases caused by viruses, including *Melon necrotic spot carmovirus* and several members of the crinivirus genus. Management practices effective for various diseases include rotation, deep plowing, fumigation, solarization, pathogen-free seed, treated seed, host plant resistance, fungicides, sanitation, manipulating the greenhouse environment, improving soil drainage, adjusting soil pH, drip irrigation, plastic mulch or other soil barrier, planting when soil is not too cold, controlling weeds and insects, avoiding moving pathogens on equipment or hands, roguing infected plants, minimizing injury during harvest, chlorine spray or hot water treatment after harvest, culling symptomatic fruit before storage, and providing proper storage conditions including refrigeration. Forecasting systems have been developed for diseases and insect vectors. Managing some diseases with fungicides has been challenged by development of resistance, which continues to be difficult to predict. Biocompatible materials such as bicarbonates, milk, oil, silicon, phosphate salts, plant extracts, and biological control agents are being developed as alternatives to conventional fungicides predominantly for powdery mildew. Some of these induce systemic resistance.

1. Introduction

Cucurbits are a large, diverse crop group that are susceptible to over 200 diseases (Zitter *et al.*, 1996). These crops as a group are an important part of a diverse and nutritious diet worldwide. Melons are eaten as fruits, whereas summer and winter squashes, cucumber, and pumpkin are eaten as vegetables. In addition, cucurbits are used for fiber, utensils, decoration, ceremonial and medicinal purposes. Diseases of cucurbits have been covered in recent publications that include several color photographs (Blancard *et al.*, 1994, Zitter *et al.*, 1996). Noninfectious disorders and insect pests are also covered in these publications. Good color photographs of cucurbit diseases are also in an article on watermelon disorders (Maynard and Hopkins, 1999). The goal of this chapter, therefore, is to focus on new developments pertaining to infectious

diseases and new diseases. Production is being challenged not only by the many new diseases that have been described recently, but also by previously described diseases that are increasing in severity and appearing in new areas. New cases of fungicide resistance developing recently indicate this problem continues to plaque cucurbit disease management. These new challenges to producing cucurbits have been partly balanced by new developments in disease management, including biocompatible alternatives to conventional fungicides, forecasting systems, induced systemic resistance, and marker-assisted selection and genetic engineering.

2. Major Diseases and their Management

Tables 1-4 contain a list of major diseases affecting cucurbit crops and their management. Table 2 contains fruit rots while the other tables are organized by pathogen type. Most of the information in these tables is from the 'Compendium of Cucurbit Diseases' (Zitter *et al.*, 1996). Additional information on disease management and specific diseases is presented in the following sections.

3. Important Developments in Disease Management

In addition to this section, other sections in this chapter on specific diseases also have information on important developments in disease management.

3.1 Alternatives to Conventional Fungicides for Managing Cucurbit Diseases

3.1.1 Biocompatible Materials

Several materials have been evaluated for cucurbit diseases that are less toxic and are considered to have lower potential for negative impact on non-target organisms than conventional chemical fungicides. Most have suppressed powdery mildew compared to nontreated plants. Materials found to be effective under glasshouse conditions include bicarbonates (Casulli *et al.*, 2000), milk (Bettiol, 1999), oil (Casulli *et al.*, 2000, McGrath and Shishkoff, 2000), silicon (Belanger *et al.*, 1995), phosphate salts (Reuveni *et al.*, 2000), plant extracts from *Reynoutria sachalinensis* (Konstantinidou-Doltsinis and Schmitt, 1998), and biological agents *Ampelomyces quisqualis* (Sztejnberg *et al.*, 1989), *Bacillus subtilis* (Bettiol *et al.*, 1997), *Pseudozyma flocculosa* (Fig. 1)(Belanger and Benyagoub, 1997), *Tilletiopsis minor* (Hijwegen, 1992), and *Verticillium lecanii* (Verhaar *et al.*, 1996). Some of these materials have also been effective when applied to field-grown Cucurbits. This group of materials includes the conventional fungicides copper and sulfur which have been approved for organic production. Biocompatible materials are especially useful for crops produced organically and crop requiring continuous harvesting. Most biocompatible materials tested have been sprayed on foliage. Silicon and phosphate can be applied through a hydroponics system as well as sprayed on foliage.

　　Biocompatible materials can be quite effective. Efficacy of *Reynoutria*

Table 1: Management practices for select diseases caused by fungi, bacteria, or phytoplasma that affect foliage and/or roots of cucurbits.

Disease	Pathogen	Management Practice [1]							
		Rotation	Deep Plowing	Fumigation	Solarization	Clean Seed	Resistance	Fungicides	Other
Disease Caused by Fungi									
Acremonium Collapse	*Acremonium cucurbitacearum*	ineffective	-	-	-	N/A	none	-	-
Alternaria Leaf Blight	*Alternaria cucumerina*	3 years	effective	-	-	N/A	low level	effective	-
Alternaria Leaf Spot	*Alternaria alternata* f. sp. *cucurbitae*	-	-	-	-	N/A	none	effective	good sanitation practices in greenhouse
Anthracnose	*Colletotrichum orbiculare*	2 years	effective	-	-	effective	race-specific	effective	-
Cercospora Leaf Spot	*Cercospora citrullina*	3 years	effective	-	-	N/A	none	effective	-
Charcoal Rot	*Macrophomina phaseolina*	ineffective	ineffective	ineffective	ineffective	N/A	available	ineffective	-
Damping-off	*Pythium* and *Phytophthora* spp.	-	-	-	-	-	-	moderately effective	control soil moisture
Downy Mildew	*Pseudoperonospora cubensis*	N/A	N/A	N/A	N/A	N/A	available	effective	-
Fusarium Wilt of Watermelon	*Fusarium oxysporum* f. sp. *niveum*	5-7 years	-	-	moderate effective	effective	race-specific	-	-
Fusarium Wilt of Melon	*Fusarium oxysporum* f. sp.	ineffective	-	effective	-	moderate effective	race-specific	-	-

table 1 contd......

Disease	Pathogen								
	melonis								
Fusarium Wilt of Cucumber	*Fusarium oxysporum* f. sp. *cucumerinum*	-	-	effective	-	effective	race-specific	-	lime soil to pH 6.5-7.0; apply nitrogen as NO$_3$ graft on resistant Cucurbita rootstock;induced resistance
Fusarium Crown and Foot Rot of Squash	*Fusarium solani* f. sp. *cucurbitae*	4 years	-	-	-	effective	none	-	-
Gummy Stem Blight	*Didymella bryoniae*	2 years	-	-	-	effective	potential	effective	minimize leaf wetness in greenhouse
Microdochium Blight	*Microdochium tabacinum*	-	-	-	-	N/A	none	effective	-
Monosporascus Root Rot and Vine Decline	*Monosporascus cannonballus*	ineffective	-	effective	ineffective	-	potential	-	graft on resistant Cucurbita rootstock.
Net Spot	*Leandria momordicae*	-	-	-	-	N/A	none	-	-
Powdery Mildew	*Podosphaera xanthii*	N/A	N/A	N/A	N/A	N/A	race-specific	effective	-
Phytophthora Blight	*Phytophthora capsici*	moderate effective	-	moderate effective	-	moderate effective	none	moderate effective	control soil moisture

table 1 contd.....

Disease	Pathogen								Remarks
Pink Root	*Phoma terrestris*	ineffective	-	-	-	N/A	none	-	none developed
Purple Stem	*Phomopsis cucurbitae*	-	-	-	-	-	-	ineffective	none developed
Rhizopycnis root rot	*Rhizopycnis vagum*	-	-	-	-	-	none	-	none developed
Scab	*Cladosporium cucumerinum*	3 years	-	-	-	effective	available	effective	well-drained soil
Septoria Leaf Spot	*Septoria cucurbitacearum*	2 years	-	-	-	N/A	none	effective	-
Target Leaf Spot	*Corynespora cassiicola*	-	-	-	-	N/A	available	effective	good sanitation practices in greenhouse
Ulocladium Leaf Spot	*Ulocladium cucurbitae*	2 years	-	-	-	N/A	available	effective	-
Verticillium wilt	*Verticillium dahliae*	effective	-	-	-	-	available	-	delay planting when cool
Disease Caused by Bacteria									
Angular Leaf Spot	*Pseudomonas syringae* pv. *lachrymans*	3 years	-	-	-	effective	available	effective	2, cultivate soil when dry to reduce bacterial survival
Bacterial Fruit Blotch	*Acidovorax avenae* subsp. *citrulli*	3 years	-	-	-	effective	none	effective	2, Good sanitation practices in greenhous.
Bacterial Leaf Spot	*Xanthomonas campestris*	-	-	-	-	effective	none	effective	2, cultivate soil when dry to reduce bacterial

table 1 contd....

Disease	Pathogen					Resistance[1]	Comments
.							survival
Bacterial Rind Necrosis	*Erwinia* sp. and others	-	-	-	-	low level	-
Bacterial Wilt	*Erwinia tracheiphilia*	N/A	N/A	N/A	N/A	available	control cucumber beetle vectors
Cucurbit yellow vine disease	*Serratia marcescens*	-	-	ineffective	-	none	delay planting
Disease Caused by Phytoplasma							
Aster Yellows	-	N/A	N/A	N/A	N/A	N/A	eradicate nearby overwintering hosts, insecticide for leaf-hopper vectors often ineffective

[1] N/A means not applicable. Blank space indicates no information found. Potential is entered under resistance where tolerance or resistance has been detected but no commercial cultivars are available.

[2] Avoid overhead irrigation. Avoid working in field when foliage is wet. Copper fungicides are only moderately effective and provide most benefit when applications are started before symptoms are seen and conditions are not highly favorable for disease development.

Table 2: Management practices for select preharvest and postharvest fruit rots caused by fungi or bacteria.

Fruit Rots	Pathogen	Management Practice[1]							Other
		Fumigation	Deep Plowing	Soil Barrier	Control soil moisture	Proper handling	Culling	Refrigeration	
Disease Caused by Fungi									
Alternaria Rot	*Alternaria alternata*	-	-	-	-	effective	effective	effective	avoid sunscald; don't hold fruit beyond shelf life
Belly Rot	*Rhizoctonia solani*	effective	effective	effective	-	-	-	-	-
Black Rot	*Didymella bryoniae*	-	-	-	-	effective	-	effective	control foliar phase; cure winter squash and pumpkin at 20-25 °C for 1-2 weeks
Blue Mold	*Penicillium* spp.	-	-	-	-	effective	effective	effective	don't hold beyond shelf life
Choanephora Fruit Rot	*Choanephora cucurbitarum*	-	-	-	-	-	-	-	none developed
Crater Rot	*Myrothecium roridum*	-	-	-	-	-	-	-	none developed
Fusarium Rot	*Fusarium* spp.	-	-	-	-	effective	-	effective	fungicides plus hot-water treatment (1 min at 57°C)
Lasiodiplodia Fruit Rot	*Lasiodiplodia theobromae*	-	-	-	-	effective	effective	effective	leave some peduncle on

table 2 contd...

Disease	Pathogen							Notes
Phomopsis Black Rot	*Phomopsis cucurbitae*	-	-	-	-	-	-	none developed; limited post-harvest control with hot water plus fungicide
Phytophthora Fruit Rot	*Phytophthora capsici*	-	-	effective	-	effective	-	apply fungicide in field
Pythium Cottony Leak	*Pythium* spp.	-	effective	effective	-	-	-	apply fungicide in field
Red Rot	*Epicoccum nigrum*	-	-	-	effective	effective	effective	-
Rhizopus Soft Rot	*Rhizopus stolonifer*	-	effective	-	effective	effective	effective	-
Sclerotinia Rot	*Sclerotinia sclerotiorum*	moderately effective	effective	-	effective	-	-	
Southern Blight	*Sclerotium rolfsii*	moderately effective	-	-	-	-	-	rotation; lime soil to raise pH to about 7
Disease Caused by Bacteria								
Bacterial Brown Spot	*Erwinia ananas*	-	-	-	-	-	-	none developed
Bacterial Fruit Blotch	*Acidovorax avenae* subsp. *citrulli*	-	-	effective	effective	effective	effective	control foliar phase
Bacterial Soft Rot	*Erwinia carotovora* subsp. *carotovora*	-	effective	effective	effective	effective	effective	chlorine spray harvest

[1] Plastic mulch and other soil barriers minimize opportunity for soil-borne pathogen to reach fruit. Proper handling to avoid injury. Refrigerate to proper temperature for commodity during transit and storage. Blank space indicates no information found.

Table 3: Management practices for select diseases caused by viruses in cucurbits.

Virus	Resistance	Clean seed	Insecticide	Host-free period	Control weed hosts	Remove infected debris	Other
				Management Practice [1]			
Cucumber Mosaic	available	N/A	moderately effective	-	infeasible	-	mineral oil and reflective mulch to control vector
Lettuce Infectious Yellows	none	N/A	effective	effective	effective	-	soft insecticides (soaps, etc.) plus biological control
Papaya Ringspottype W	available	N/A	moderately effective				
Squash Leaf Curl	tolerance reported	N/A	ineffective	effective	effective	effective	soft insecticides (soaps, detergents, and various plant derived products)
Squash Mosaic	potential	effective	effective	-	-	-	-
Tobacco Ringspot	potential	-	-	-	effective	-	intense cultivation and eradication of weeds to control nematode vector
Tomato Ringspot	potential	-	-	-	effective	-	intense cultivation and eradication of weeds; fumigate to control nematode vector
Watermelon Mosaic	available	N/A	moderately effective	-	-	-	mineral oil to control aphid vector
Zucchini Yellow Mosaic	available	-	moderately effective				mineral oil and reflective mulch are moderately effective for for aphid vector

[1] N/A = not applicable. - = no information found. For some viruses tolerance \ resistance has been detected but no commercial cvs are available.

sachalinensis extract formulated as Milsana can reach 90% under high disease pressure (Konstantinidou-Doltsinis and Schmitt, 1998). More than 80% control can be achieved with milk (Santomauro *et al.*, 2001). Control obtained with potassium bicarbonate (formulated as Kaligreen) or *Bacillus subtilis* (Serenade) can be at least 88% (Matheron and Porchas, 2000), but the degree of control can be considerably less under different environmental conditions (McGrath, 2002). High nitrogen fertilization, which increases susceptibility to powdery mildew, may partly account for discrepancy in results with some biocompatible materials (Reuveni *et al.*, 1994). Sulfur can be highly effective, providing control similar to some systemic fungicides and superior to other biocompatible materials (Matheron and Porchas, 2001).

Biocompatible materials often, however, are not as effective as conventional fungicides. Although quite effective, Milsana is less effective than the combination of sulfur and myclobutanil (Konstantinidou-Doltsinis and Schmitt, 1998). Pyrazophos is more effective than *Ampelomyces quisqualis* (Sztejnberg *et al.*, 1989). Biocontrol agents generally are less effective when disease pressure is high (Belanger and Benyagoub, 1997). Additionally, dependence on humid conditions for biocontrol agents to infect probably makes it more challenging to use them successfully on outdoor crops (Sundheim

Table 4: Management practices for select nematodes affecting cucurbits.

Nematode	Management Practice		
	Fumigation	Rotation	Resistance
Root-Knot Nematodes	effective	infeasible	potential
Reniform Nematode	effective	possible	none

and Amundsen, 1982). There have been exceptions: high concentrations of milk (up to 50%) are at least as effective as benomyl or fenarimol under greenhouse conditions (Bettiol, 1999). Phosphate is more effective than pyrifenox for about one week following application to cucumber leaves with established powdery mildew colonies (Reuveni *et al.*, 1995). However, phosphate is less effective than pyrifenox at two weeks after treatment, which suggests more frequent application may be needed with biocompatible materials.

Better control can be achieved by using an integrated management program, such as applying biocompatible materials to less susceptible cultivars (Konstantinidou-Doltsinis and Schmitt, 1998, Verhaar *et al.*, 1996). Bicarbonates and oil are synergistic (Casulli *et al.*, 2000). Another recommended approach is to alternate applications with conventional chemical fungicides (Sztejnberg *et al.*, 1989). Control achieved with NPK fertilizers can be improved by either alternating with a systemic fungicide or by applying to resistant varieties (Reuveni and Reuveni, 1998). Including a spreader/sticker improved effectiveness, most likely due to increased retention of phosphate ions (Reuveni *et al.*, 1994).

Additional research is needed to assess other biocompatible materials under field conditions. For example, almost all work with silicon has involved adding the

product metasilicate (potassium silicon) to nutrient solutions for hydroponically-grown crops. Limited work with foliar applications has shown these can be effective, but require 10-fold higher rates than applications to roots; however, silicon sprayed on foliage was effective for at least 7 days after treatment, which was considerably longer than silicon added to nutrient solutions (Menzies *et al.*, 1992).

Direct and indirect modes of action have been identified for biocompatible materials. Some materials have multiple modes of action. Bicarbonates inhibit formation and germination of conidia (Homma *et al.*, 1981). Mycelia are collapsed and conidia are of irregular shape on leaves treated with phosphates, indicating a direct effect (Reuveni *et al.*, 1994). *Ampelomyces quisqualis* attacks the powdery mildew fungus directly rather than inducing plant defenses (Abo-Foul *et al.*, 1996). *Pseudozyma flocculosa* acts rapidly by means of antibiosis (Fig. 1) (Belanger and Benyagoub, 1997). Metasilicate
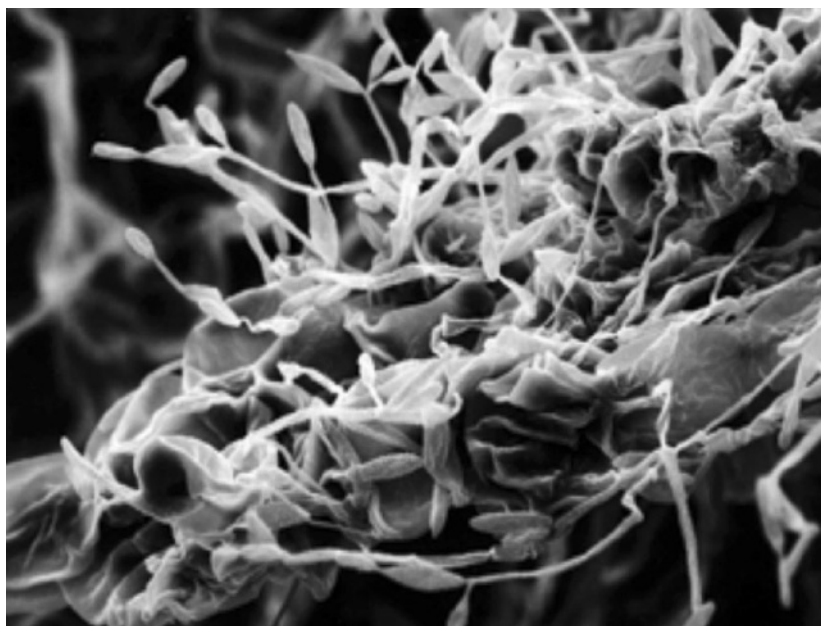
Figure 1: Micrograph of the antagonist *Pseudozyma flocculosa* attacking powdery mildew on cucumber. (courtesy of R. R. Belanger, Universite Laval, Quebec, Canada)

crystals may also act as a physical barrier when the pathogen tries to penetrate its host (Menzies *et al.*, 1992). Silicon, at least in the form of metasilicate, does not have a direct negative effect on the pathogen. Some materials have curative activity. Phosphate applied to large, heavily-infected plants resulted in 99% of colonies disappearing within 2 days of application (Reuveni *et al.*, 1995). Some biocompatible materials, including Milsana, silicon and phosphate, trigger the plant's defense system. This response, while often systemic, can sometimes be local with protection only occurring on treated plant tissue. For example, Milsana is effective on adaxial leaf surfaces but it only some-

times provides control on the abaxial surface and then at a lower level (Konstantinidou-Doltsinis and Schmitt, 1998). Induced systemic resistance is covered in the next sub-section. Plant extracts have been shown to delay senescence, which could account for the weight and number of fruit from Milsana-treated plants exceeding that expected based on powdery mildew control (Konstantinidou-Doltsinis and Schmitt, 1998).

One challenge is controlling powdery mildew (Fig. 2) on abaxial leaf surfaces (Casulli *et al.*, 2000, McGrath and Shishkoff, 1999). Few biocompatible materials have been found with systemic or translaminar activity, which is the most important charac-teristic of the conventional fungicides relied on for controlling powdery mildew. Phos-phate, however, is extremely mobile in plants, and it is absorbed rapidly into plant tissue (Reuveni *et al.*, 1995). Sulfur has been shown to suppress powdery mildew on abaxial leaf surfaces, perhaps because of its volatility. It can be as effective as some systemic

Figure 2: Colonies of powdery mildew are larger with denser sporulation on abaxial than adaxial leaf surfaces.

fungicides in muskmelon (Matheron and Porchas, 2001), but not in pumpkin (McGrath, 2002), perhaps due to differences between these crops in leaf size and canopy structure that affect spray deposition. Sulfur has performed well compared to other biocompatible materials (Matheron and Porchas, 2000, Matheron and Porchas, 2001, McGrath, 2002). Another challenge is controlling other diseases when using biocompatible materials for controlling powdery mildew (McGrath and Shishkoff, 1999). Gummy stem blight and Alternaria leaf blight are suppressed by oil, but not as effectively as powdery mildew (Ziv and Zitter, 1992). Neither Alternaria leaf blight on muskmelon or anthracnose on watermelon was controlled with sodium bicarbonate applied with Sunspray ultrafine oil (Damicone *et al.*, 1993, MacNab, 1993). Conventional fungicides such as chlorothalonil have activity against several diseases while most biocompatible materials are limited to

powdery mildew. One exception is silicon, which has not only been effective for other diseases, including Pythium root rot and stem lesions caused by *Botrytis* and *Didymella*, but apparently also alleviates abiotic stress (Belanger *et al.*, 1995). Extracts from *R. sachalinensis* are effective for diseases caused by a diversity of pathogens, including viruses and non-obligate pathogens (Konstantinidou-Doltsinis and Schmitt, 1998).

Biocompatible materials are being used in commercial cucurbit production. Silicon is being used by about 60% of cucumber growers in Europe (Belanger *et al.*, 1995). Organic growers are using milk in Brazil (Bettiol, 1999). Products are being marketed in several countries for use in greenhouse and/or field that contain oil, monopotassium phosphate, potassium bicarbonate, sulfur, or a biocontrol agent (*Ampelomyces quisqualis, Bacillus subtilis*, or *Pseudozyma flocculosa*). Additionally, some Australian melon growers are producing their own compost tea (McGrath, personal observation). Similar to conventional contact fungicides, biocompatible products are often recommended for use in a fungicide program with conventional systemic fungicides including strobilurin and DMI (demethylation inhibiting) fungicides.

3.1.2 Induced Systemic Resistance

Some biocompatible materials enhance the natural defense response in treated plants, which is also known as induced systemic resistance (ISR) or systemic acquired resistance (SAR). For example, silicon hastens accumulation of phenolic materials in infected host epidermal cells and increases the number of cells that respond (Belanger *et al.*, 1995). Milsana induces production of phenolic compounds in both susceptible and tolerant cultivars (Daayf *et al.*, 2000). Haustoria in Milsana-treated plants rapidly collapse and become encased by electron-dense substances, most likely through the involvement of phenolics (Wurms *et al.*, 1999). ISR can be induced with a nutrient solution for hydroponics containing 20 ppm phosphorus (Reuveni *et al.*, 2000). Also, phosphate or potassium salts applied once before inoculation induce up to 94% systemic protection (Reuveni *et al.*, 1994) also: (Reuveni and Reuveni, 1998). A similar level of systemic resistance can be induced by one spray of certain micronutrients, including B, Mn and Cu (Reuveni *et al.*, 1997).

Plant growth-promoting rhizobacteria (PGPR) induce resistance to angular leaf spot, anthracnose, and bacterial wilt (Wei *et al.*, 1996, Zehnder *et al.*, 2001). ISR to bacterial wilt is two pronged (Zehnder *et al.*, 2001). Beetle feeding and thus pathogen transmission is reduced because of decreased concentrations of cucurbitacin, a secondary plant metabolite and powerful beetle-feeding stimulant. Plant defense mechanisms against the pathogen are also involved as less wilt occurs in PGPR-treated cucumber than in non-treated plants when injected with the pathogen. PGPR-ISR can be more effective than weekly applications of the insecticide esfenvalerate with field-grown plants. PGPR-ISR also suppresses angular leaf spot and anthracnose under field conditions, as well as promotes early-season plant growth and enhances yield (Wei *et al.*, 1996). In addition to ISR, PGPR may help compensate for reduced plant growth that often occurs when fields are not fumigated with methyl bromide (Raupach and Kloepper, 2000).

3.1.3 Planting Method

No-till production of pumpkin into a cover crop can reduce disease compared to conventional production on bareground (Everts, 2001). A suitable cover crop is hairy vetch or hairy vetch and rye seeded after the previous crop and killed prior to seeding pumpkin. Incidence or severity of powdery mildew, black rot, and Microdochium blight, the main diseases that occurred in Everts' study, were reduced in at least one year. The number of fungicide applications needed for effective control can be further reduced by planting powdery mildew resistant cultivars.

3.2 Fungicide Resistance

Application of fungicides is the principal practice for managing many cucurbit diseases. Fungicides that are systemic or have translaminar activity are especially valuable. Unfortunately, these fungicides generally have a high risk of developing resistance because they have specific modes of action. Dealing with resistance continues to be challenging, as it is not always possible to predict resistance risk of a fungicide or the potential for a pathogen to develop resistance. Strobilurins, an important new group of fungicides, were predicted to have low resistance risk. However, resistance developed quite quickly in some areas. As expected based on past history with other fungicides, the cucurbit powdery mildew fungus, *Podosphaera xanthii,* was one of the first pathogens to develop resistance (McGrath, 2001a). Resistant strains were found in 1999 after just two years of commercial use in four countries. Surprisingly, resistance was not detected in the United States, despite the size of the *P. xanthii* population, until 2002, the fifth year of commercial use (McGrath and Shishkoff, 2003). Just as unexpectedly, resistance developed in the United States first in the gummy stem blight fungus, *Didymella bryoniae* (Olaya and Holm, 2001). In sharp contrast, control failure associated with resistance to benomyl was observed in *P. xanthii* just 1 year after its registration for use on Cucurbits in the United States (McGrath, 2001a) but it was not observed for *D. bryoniae* until 23 years after its registration (Keinath and Zitter, 1998). Other cucurbit pathogens have also developed resistance. Resistance to metalaxyl and its active enantiomer, mefenoxam, has developed in *Pseudoperonospora cubensis*, which causes downy mildew (Reuveni *et al.*, 1980), and in *Phytophthora capsici*, which causes Phytophthora blight (Lamour and Hausbeck, 2001a). Control failure associated with resistance in *P. cubensis* occurred in commercial greenhouses following just three years of use. Frequency of mefenoxam insensitivity in *P. capsici* did not decrease over a period of two years when it was not used; thus it will be difficult to resume use of this fungicide following resistance development. It appears to be an incompletely dominant trait that is not linked to compatibility type (Lamour and Hausbeck, 2000).

3.3 Forecasting

Three types of forecasting systems have been developed for cucurbit diseases. One uses data on environmental conditions within a crop to schedule fungicide applications for when conditions are favorable for disease development. This approach is

typical of most disease forecasters. Another uses reports of disease occurrence and models pathogen movement from these areas to other locations. This system differs from the previous one in that it uses macro-scale weather patterns, rather than site-specific weather, and it predicts pathogen dispersal. A third system using aphid flights to forecast occurrence of virus diseases is in development.

Melcast is a spray advisory system utilizing daily measurements of leaf wetness periods and temperature to schedule fungicide applications when environmental conditions are conducive to disease development in melons (http://www.agcom. purdue.edu/AgCom/Pubs/BP/BP-64-W.pdf; Daniel S. Egel, Southwest Purdue Ag Center, Vincennes, IN). The length of time that leaves are wet and air temperature during these wet periods are key inputs for this and other disease-warning systems because many fungal and bacterial pathogens are active only when free water is present and temperatures are conducive to their activity. Melcast was originally developed for Alternaria leaf blight of muskmelon. The model was developed through research conducted under controlled environmental conditions, then validated under field conditions. Subsequently it was adapted to forecast anthracnose and gummy stem blight in watermelon. Growers depend on repeated applications of fungicides to manage these diseases because there are no other effective practices: there is little genetic resistance available in commercially preferred cultivars and crop rotation alone is not adequate. Under Melcast, an initial fungicide application is made between the time when plants begin to vine and when the vines of adjacent plants in a row meet. With this 'cover spray', applied two weeks after transplanting, gummy stem blight is more effectively controlled with Melcast in watermelon (Keinath, 2000). Environmental data for a field are obtained from sensors located in or near the field or from a weather data service. Environmental favorability index (EFI) values are accumulated daily to determine when subsequent applications are needed. EFI values range from 0 when conditions are not favorable to 10 when conditions are ideal. An application is recommended when EFI values reach 20 for muskmelons and 35 for watermelons or when 14 days have lapsed since the previous application and plants are growing rapidly. Melcast implemented without this 14-day 'safety net' has not performed as well as a 7 to 10 day calendar-based schedule for controlling gummy stem blight (Langston *et al.*, 2000). However, insufficient control with Melcast in this study may have been due to the high disease pressure in this area (D. B. Langston, University of Georgia, personal communication). Using a lower EFI threshold may be necessary where disease pressure is very high. In drier areas, on the other hand, it is anticipated that this 'safety net' might not be necessary (J. P. Damicone, Oklahoma State University, personal communication).

Growers using Melcast in Indiana have reduced fungicide use to muskmelon by 10-20%, while maintaining control of Alternaria leaf blight, compared to the conventional schedule of applying fungicides every 1 to 2 weeks often without considering weather conditions. Alternaria leaf blight and gummy stem blight were controlled in watermelon as effectively, and Anthracnose almost as effectively, with four fungicide applications scheduled with Melcast as with eight applications on a weekly schedule in experiments conducted in the eastern United States (Everts and Shields, 2000, Everts *et al.*, 2001). Anthracnose was controlled in muskmelon as effectively with two fungicide applications scheduled with Melcast as with five applications on a weekly schedule in

an experiment conducted in the mid-western United States (Gleason *et al.*, 2001). Remote-estimated data purchased from a commercial source can be sufficiently similar to on-site weather data that its use in Melcast results in a similar degree of disease control as using on-site data (Gleason *et al.*, 2000). However, in another experiment the two types of weather data were sufficiently different that using remote-estimated data in Melcast resulted in scheduling of applications such that Anthracnose was not controlled (Gleason *et al.*, 2001). An algorithm has been developed to adjust estimates of leaf wetness (Mark Gleason, Iowa State University, Ames, personal communication). Research is underway to evaluate Melcast in commercial fields in southeastern United States (D. S. Egel, personal communication). Insurance policies are being developed through a project of the American Farmland Trust to promote adoption of Melcast and other IPM practices by offering a guaranteed payment for confirmed crop losses (Anthony P. Keinath, Clemson University, South Carolina, personal communication). The policies are less expensive for growers than the pesticide savings obtained through adoption.

Regional occurrence of downy mildew in the eastern United States is being forecast based on disease observations and modeling long-distance movement of the pathogen with large-scale weather systems (Holmes *et al.*, 1998)(http://www.apsnet.org/online/feature/forecast/top.htm). The pathogen, *Pseudoperonospora cubensis*, is believed to survive winters south of the 30th latitude and from there is reintroduced to the north each year. Disease reports are provided by a network of approximately 40 plant pathologists and horticulturists representing Mexico and various states. Weather forecasting models and pathogen epidemiology are then used to determine the likelihood of disease outbreaks along weather trajectories. The model utilizes factors important to sporulation at the source, survivability during transport, and deposition from rainout and washout. The HY-SPLIT trajectory model from NOAA's Air Resources Laboratory is used to calculate spore transport in the atmosphere. Twice weekly forecasts are made of the likelihood of inoculum spread and disease risk 48 hours into the future. Risk is described as high, moderate, or low. Disease reports and forecasts are placed on the world wide web (http://www.ces.ncsu.edu/depts/pp/cucurbit/; Gerald J. Holmes, North Carolina State University, Raleigh, NC). This system is related to the Tobacco Blue Mold forecaster developed previously for a biologically similar pathogen.

A forecasting system similar to Melcast has been developed for downy mildew in Ukraine (Chaban *et al.*, 2000). In addition to temperature and leaf wetness duration, this system also uses relative humidity and concentration of sporangia in the air to schedule fungicide applications. This system is recommended for use as a component of a management program that also includes resistant cultivars and highly effective systemic and contact fungicides. With this program, the amount of fungicide can be halved for managing downy mildew, which has been one of the most widespread and economically important diseases of cucumber in Ukraine since 1985.

A system is being developed to forecast mosaic virus outbreaks in squash based on flights of aphid vectors (www.aphidwatch.com/squash/index.htm; John D. Fletcher, New Zealand Institute for Crop & Food Research, Canterbury). High level suction traps are being used to monitor the aerial aphid population. Aphid flights are regularly updated at the web site during the growing season. This information is being

related to aphid infestation and virus occurrence in crops, as well as historical data on virus incidence, for developing a system to predict the risk of virus infection and the need for insecticide applications.

3.4 Advances in Developing Resistant Cultivars

Developing resistant cultivars using conventional breeding methods can be tedious and time-consuming, and sometimes impossible. Marker-assisted selection and genetic engineering are valuable new tools. These advances are particularly important for diseases such as Fusarium wilt that cannot be controlled by any other method. Robust markers tightly linked to *Fom-2,* which confers resistance to race 1, have already been developed and markers linked to *Fom-1,* which confers resistance to races 0 and 2, are being developed (Karsies *et al.*, 2000).

4. Recently Described and Emerging Diseases

4.1 Cucurbit vine declines

The general term 'vine decline' is used for symptoms of decline caused by soilborne pathogens that develop as fruit approach maturity (Bruton *et al.*, 1998b). Typical symptoms include yellowing and death of crown leaves with gradual vine decline as the plant approaches maturity (Fig. 3), then rapid collapse just before harvest which exposes fruit to damaging solar radiation and causes fruit to ripen prematurely resulting in fruit also being unmarketable due to small size and low sugars (Miller *et al.*, 1995, Martyn and Miller, 1996). Muskmelon and watermelon are affected more than other Cucurbits (Bruton *et al.*, 1998b). Other common descriptors for declines are crown rot, root rot, sudden death, sudden wilt, wilt, and vine collapse. Specific diseases include Acremonium collapse, Botryodiplodia decline, charcoal rot, gummy stem blight, Monosporascus root rot and vine decline, cucurbit yellow vine disease, purple stem, and Rhizopycnis root rot. Vine declines have become a limiting factor to melon production in several areas. They are the primary reason that production of muskmelon in Spain, one of that country's most important horticultural crops, has decreased more than 40% (García Jiménez *et al.*, 2000).

These diseases have increased in importance and also in number over the past 20 years. This is thought to be at least partly due to changes in cultural practices that have also occurred, including use of hybrid cultivars, transplants, higher plant density, plastic mulch, drip irrigation, and inadequate rotation (Bruton *et al.*, 1998b). Plants grown with plastic mulch and drip irrigation often receive constant water and nutrients, consequently they produce large vines with small root systems, thereby making them more sensitive to root damage (Martyn and Miller, 1996). On the other hand, symptoms of Monosporascus root rot/vine decline develop later for plants grown with mulch and drip than plants on bare ground (Miller and Bruton, 2000).

This is likely due to the fact this pathogen damages primary roots, rather than fine roots as occurs with other pathogens causing decline, thereby shifting the root system to a higher proportion of roots in the top soil layer where soil moisture varies

little under plastic (Biernacki and Bruton, 2001).

Plants are predisposed to vine decline by heat, drought, fruit load, insect feeding, and other stressful conditions (Martyn and Miller, 1996). For example, early-maturing cultivars develop symptoms of Monosporascus root rot/vine decline sooner than late-maturing cultivars relative to seeding date, but not relative to maturity date. Also, removing fruit can delay symptoms as much as 14 days. Genotypes with a concentrated fruit set appear to be more susceptible.

Accurately diagnosing vine declines is challenging (Martyn and Miller, 1996). There are few distinguishing symptoms. The 'Compendium of Cucurbit Diseases' contains a table of the principal diagnostic characteristics of these and other soilborne diseases affecting melon (Zitter *et al.*, 1996). However, once symptoms are expressed

Figure 3: Yellowing of vines in the middle of the row, which is a typical initial symptom of most vine declines, due to Acremonium collapse. (courtesy of B. D. Bruton, USDA, ARS, Lane, OK, USA)

the disease is well underway, thus it is too late to implement control measures. Detecting and identifying soilborne plant pathogens is very difficult. A PCR-mediated detection protocol has been developed that can accurately detect *Monosporascus* in plant tissue and soil (Martyn and Miller, 1996).

Controlling vine declines is also challenging. Resistance is the most desirable management practice. It has played an important role in managing other soilborne pathogens affecting Cucurbits, including Fusarium and Verticillium wilts (Bruton, 1998). New opportunities to identify general resistance to vine declines now exist with recent advances in molecular biology that have provided tools being used to develop molecu-

lar markers and to construct genetic maps of Cucurbits. Biological control, another desirable management practice, has shown good potential in preliminary experiments conducted under controlled conditions in a glasshouse (Zhang *et al.*, 1999). Additional information on management follows for specific vine declines.

4.1.1 Acremonium collapse

Acremonium sp. have been recognized as causing a hypocotyl rot of muskmelon and

Figure 4: Micrograph of *Acremonium cucurbitacearum.* (courtesy of B. D. Bruton, USDA, ARS, Lane, OK, USA)

watermelon seedlings (Zitter *et al.*, 1996). A new vine decline caused by *Acremonium cucurbitacearum* appeared in Spain in the early 1980s (Armengol *et al.*, 1998)(Fig. 4). It has spread throughout Spain, becoming the predominant fungal pathogen associated with vine decline and resulting in many fields being taken out of melon production (García Jiménez *et al.*, 2000). This pathogen was also found frequently in melon fields throughout California when surveyed from 1995 through 1997 (Aegerter *et al.*, 2000). Although under glasshouse conditions all Cucurbits tested were susceptible to *A.*

cucurbitacearum isolates from Spain and Texas, with watermelon having a similar disease reaction as muskmelon, fortunately only muskmelon production has been affected under field production indicating that watermelon has field resistance (Armengol *et al.*, 1998, Bruton *et al.*, 2000b). *Benincasa hispida*, three *Cucumis sativus* L. cultigens, and all species within *Cucurbita, Lagenaria*, and *Luffa*, were rated as highly resistant to *A. cucurbitacearum*. In another study, *A. cucurbitacearum* failed to cause symptoms on summer squash (*Cucurbita pepo*) cv. Super Set (Aegerter *et al.*, 2000).

The collapse and sudden death of plants as fruit ripening begins is similar to other vine declines (Fig. 3 and 5); however, symptoms on roots begin to develop on younger plants than with other declines (García-Jiménez *et al.*, 1994). The seedling hypocotyl of affected direct-seeded plants develops a light yellow-brown discoloration soon after planting. Both transplanted and direct-seeded plants develop dry, corky,

Figure 5: Acremonium collapse of Piel de Sapo melon in Spain caused by *Acremonium cucurbitacearum.* (courtesy of B. D. Bruton, USDA, ARS, Lane, OK, USA)

brown lesions on the upper root. Secondary roots die (Fig. 6) and superficial roots are produced above the dead ones. Affected plants when young typically do not show aboveground symptoms such as stunting, slow growth, or decline. *A. cucurbitacearum* causes little damage to melon in Texas most likely because temperatures there exceed the optimum for growth of this pathogen when collapse typically occurs, in contrast with California and Spain (Bruton *et al.*, 2000b), and Spanish isolates are more virulent than those from Texas (Bruton *et al.*, 2000a).

Managing Acremonium collapse with rotation is difficult because this pathogen produces chlamydospores, is pathogenic to all members of Cucurbitaceae, and can colonize, albeit to a limited extent, roots of plants in other families, including Asteraceae, Fabaceae, Malvaceae, Poaceae and Solanaceae (Armengol *et al.*, 1998). However, weeds

are unlikely involved in the disease cycle as the pathogen was rarely isolated from weeds in melon fields. An 8-year rotational period was inadequate (Bruton, 1998). No muskmelon cultivar has been found exhibiting sufficient tolerance for production in highly infested fields.

4.1.2 Cucurbit yellow vine disease

Yellow vine was first observed in 1988 affecting squash and pumpkin in south central

Figure 6: Root rot of muskmelon caused by *Acremonium cucurbitacearum* showing extensive rot of secondary roots. (courtesy of B. D. Bruton, USDA, ARS, Lane, OK, USA)

United States (Central Texas and Oklahoma)(Bextine *et al.*, 2001, Pair and Bruton, 1998). It has since been confirmed in Tennessee and Massachusetts, and is suspected of occurring in another eastern state, New York (Pair *et al.*, 2000). Two unique symptoms distinguish yellow vine from other vine declines (Bextine *et al.*, 2001). Affected plants often suddenly wilt and die within a single day following fruit set and the beginning of fruit enlargement, without preliminary symptoms such as leaf yellowing. This is earlier

in the growing season than with most vine declines. Plants also can gradually decline with prominent leaf yellowing about one to two weeks before harvest. Phloem is discolored honey-brown in the primary root and crown, often extending throughout the terminal portion of the vine section (Fig. 7). While root rot is not a symptom, roots may be attacked by secondary invaders. Symptoms have been observed in summer and winter squash, pumpkin, watermelon, and cantaloupe, but not in cucumber or gourd. Squash and pumpkin are most susceptible and cantaloupe is least (Pair *et al.*, 2000). This disease is caused by the phloem-inhabiting bacterium *Serratia marcescens* which is transmitted by squash bug (*Anasa tritis*) adults (Bruton *et al.*, 2001). Transfer of photosimilates to roots is disrupted in infected plants. Although yellow vine has been seen sporadic, substantial economic losses can result. Complete crop loss has occurred (Bruton *et al.*, 1998a) (Fig. 8).
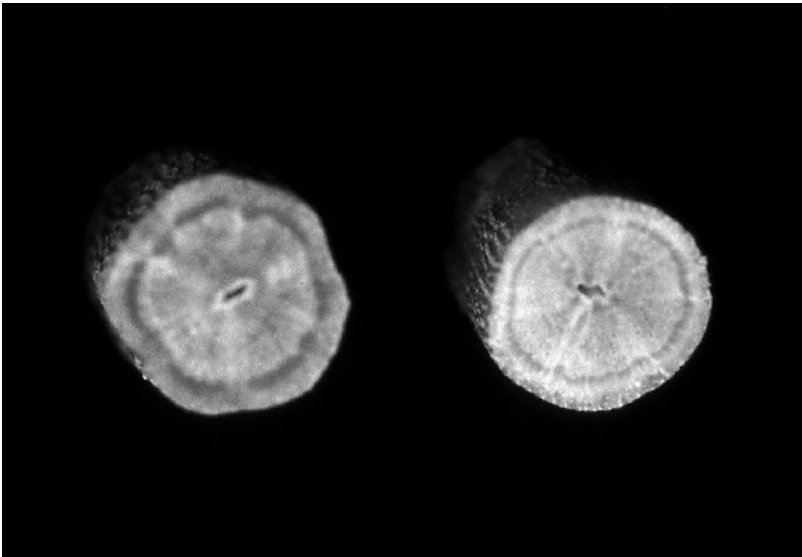
Figure 7: Honey-brown discoloration of the phloem in diseased plants, which is characteristic and diagnostic for cucurbit yellow vine disease, contrasts with the healthy cross-section on the left. (courtesy of B. D. Bruton, USDA, ARS, Lane, OK, USA)

Yellow vine cannot be controlled adequately (Pair *et al.*, 2000, Pair *et al.*, 1998). Applying insecticides to control the vector can provide some control, but results have been erratic and this practice is considered uneconomical. Crops planted after June 1 are less severely affected than earlier plantings, but prices are usually lower for late-harvested melons. Triploid seedless watermelons are less susceptible than diploid open-pollinated and hybrid cultivars, but triploid cultivars exhibit a range of reaction. Early crops grown on plastic mulch are more severely affected than those on bare ground most likely because crops on plastic are more attractive to the insect vector due to the resulting increased plant vigor and higher temperatures. Fumigating soil does not have an effect.

4.1.3 Monosporascus root rot and vine decline

Vine decline caused by *Monosporascus cannonballus* is generally associated with hot arid to semi-arid environments. It has been found in southern United States (Texas, Arizona, California), Mexico, Honduras, Guatemala, southern Spain, Israel, Iran, Tunisia, Libya, Saudi Arabia, India, Pakistan, Japan, and Taiwan (Karlatti *et al.*, 1997, Martyn and Miller, 1996, Miller and Bruton, 2000). It was first discovered in 1970 in Arizona, but likely was present for many years prior either at very low levels or it was attributed to other pathogens (Martyn and Miller, 1996). The first report of this disease that included work on determining pathogenicity is from Japan in 1978. Economically significant losses were first attributed to this disease in Texas in 1986. It continues to be a major constraint to melon production in some important production areas, with complete crop

Figure 8: Total crop loss in watermelon due to cucurbit yellow vine disease. (courtesy of B. D. Bruton, USDA, ARS, Lane, OK, USA)

loss occurring in some fields and yearly losses fluctuating between about 10 to 25% of the crop. This disease has only been reported on muskmelon and watermelon in the field; however, cucumber, summer squash, pumpkin, and several winter squashes are susceptible under controlled conditions. Lesions develop on roots, particularly at their junctions. Secondary and tertiary roots die. The tap root may rot under wet conditions. Diagnostic small black perithecia develop in roots (Fig. 9 and 10). Each ascus has one ascospore (Fig. 11). Typical symptoms of vine decline develop aboveground. The name *Monosporascus eutypoides* was used in some countries; it is now considered synonymous with *M. cannonballus* (Bruton, 1998). Geographical differences in virulence have been detected with Texas isolates are more virulent than those from Spain (Bruton *et al.*,

2000a).

Monosporascus root rot and vine decline is a challenging disease to manage. Short-term crop rotation is of little utility because all cucurbit crops are susceptible, several noncucurbits may serve as hosts, and ascospores are presumably long-lived (Martyn and Miller, 1996). Hosts include corn, wheat, and grain sorghum (Miller and Bruton, 2000). Traditional soil solarization has not been successful because this fungus is thermophilic (Cohen *et al.*, 2000a). However, it is possible to obtain a high enough temperature using a modified method developed for production in soilless culture.

The only control practice presently is pre-plant fumigation of beds. Methyl bromide is effective; however, its use will be banned in the near future. Fumigation with methyl bromide has been the most common management practice in some areas (Cohen *et al.*, 2000a), while growers in other areas have been reluctant to use fumigation be-

Figure 9: Root rot of muskmelon by *Monosporascus cannonballus* illustrating diagnostic small black perithecia embedded in the small secondary roots. (courtesy of B. D. Bruton, USDA, ARS, Lane, OK, USA)

cause they do not feel it is economical (Miller and Bruton, 2000). Control also has been achieved with 1,3 dichloropropene and chloropicrin, used alone or combined, and with metham sodium (Miller and Bruton, 2000). Newer fumigants that can be injected through drip irrigation lines may overcome some objections to older materials. Costs can be further reduced because treating a 0.3-m-wide area was as effective as treating an area three times as wide and fruit yield was also not improved by covering the beds with plastic tarps for 10 days following fumigation (Miller *et al.*, 2000). Control can be obtained by using fumigant at reduced dosage combined with solarization (Cohen *et al.*, 2000a).

All commercially-available melon cultivars tested to date are susceptible (Cohen *et al.*, 2000a). Breeding work is underway to introduce resistance into melon from other genotypes. Selecting cultivars based on root structure also may be advantageous as tolerant cultivars have longer total root length and larger root number than susceptible ones (Crosby *et al.*, 2000). Plants irrigated less frequently or provided less water than the conventional daily regime have deeper root systems, begin to wilt later, and produce more marketable fruit. Optimizing irrigation regime needs to be integrated with other management practices to achieve an acceptable level of disease control.

Another management practice entails grafting melon on to *Cucurbita* rootstock, which is now being done in Israel (Cohen *et al.*, 2000a). The rootstock produces large root systems. While disease development often is slowed, results can be variable and there is the potential for inoculum build-up as the rootstocks are infected. On the other

Figure 10: Perithecia of *Monosporascus cannonballus* on muskmelon root. (root is about 2 mm in diameter). (courtesy of B. D. Bruton, USDA, ARS, Lane, OK, USA)

hand, there is the potential for additional yield improvement due to greater water and nutrient uptake. *Monosporascus*-resistant melons may be valuable as rootstocks .

An integrated management program has been developed in California (Stanghellini *et al.*, 2001) consisting of (i). pre-plant soil fumigation with either methyl iodide or chloropicrin, (ii). several applications of the fungicide fludioxonil through drip irrigation, and (iii). cultivation or fumigation with metam sodium immediately after harvest (M. E. Stanghellini, University of California, personal communication). The first fludioxonil application needs to be made before the first root infection, which can occur as early as 9 days after planting in a fall crop in California but usually does not occur

until about 35 days after planting in the spring unless soil temperature is warmer than typical. Fludioxonil is not registered yet for this use; registration is being pursued in the United States (David Thompson, USDA IR-4 program, Rutgers, personal communication).

4.1.4 Rhizopycnis root rot

A new fungus associated with vine decline was first reported on melon in Texas in 1991 and later identified as *Rhizopycnis vagum* (Farr *et al.*, 1998). This disease has also been detected in Guatemala, Honduras, and Spain (Armengol *et al.*, 2000b). Symptoms include browning of roots, lesions on the primary root and at the junction of primary to
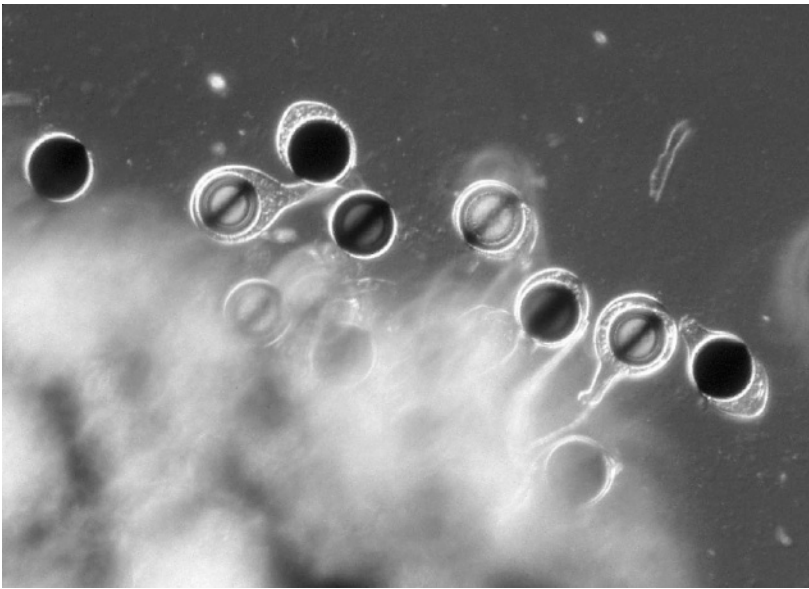
Figure 11: Ascospores of *Monosporascus cannonballus* illustrating one ascospore per ascus. (courtesy of B. D. Bruton, USDA, ARS, Lane, OK, USA)

secondary root, stubbed-off and necrotic root hairs, reduced root systems, pinkish-brown to red discoloration of root (Fig. 12 and 13) and hypocotyl, and sometimes microsclerotia in secondary roots (Fig. 14 and 15)(Aegerter *et al.*, 2000, Armengol *et al.*, 2000b).

Phoma terrestris causes pink root on Cucurbits which can easily be confused with *Rhizopycnis vagum* (Fig. 16)(B. D. Bruton, USDA, ARS, Lane, OK, USA, personal communication).

4.2 Bacterial fruit blotch

Acidovorax avenae subsp. *citrulli* (formerly *Pseudomonas pseudoalcaligenes* subsp. *citrulli*) affecting watermelon was first observed in 1967 in Australia (O'Brien and Martin, 1999). The pathogen was misidentified, but the symptoms photographed were conclusive. It was then observed in 1987 in the Mariana Islands and in 1989 in the United States (Walcott *et al.*, 2000). Since then bacterial fruit blotch (BFB) has been observed in commercial plantings of melons, pumpkin and cucumber (Fig. 17 - 20). . All Cucurbits, tomato and eggplant are susceptible when artificially inoculated (Shirakawa *et al.*, 2000). While squash, tomato and eggplant are susceptible to BFB, outbreaks in commercial plantings have not been reported thus far. This disease has also been
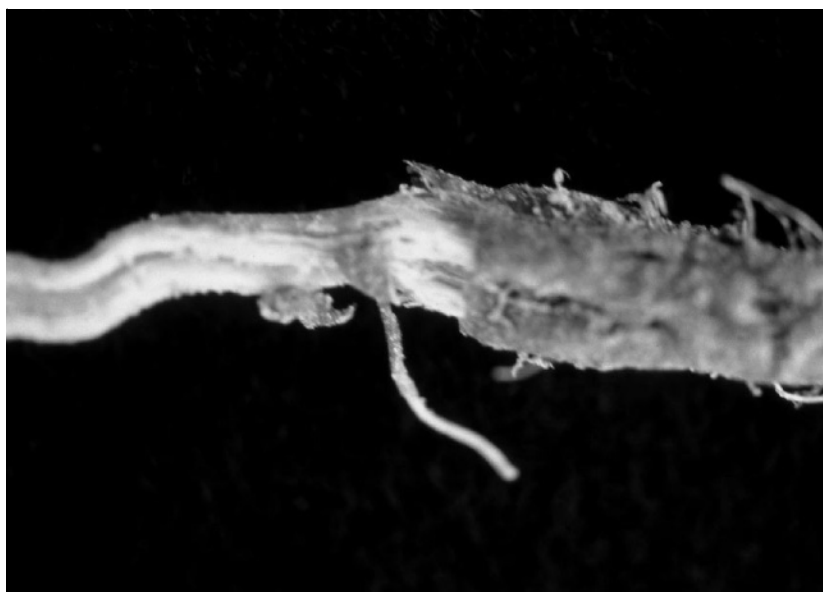
Figure 12: *Rhizopycnis vagum* causing red discoloration and root rot on cantaloupe. (courtesy of B. D. Bruton, USDA, ARS, Lane, OK, USA)

observed in other countries, including Brazil, Japan, Taiwan, Turkey and Venezuela (Assis *et al.*, 1999, Cheng *et al.*, 2000, Demir, 1996, O'Brien and Martin, 1999, Shirakawa *et al.*, 2000) (R. R. Walcott, University of Georgia, personal communication). BFB continues to be found in new areas, including most recently in 2001 in Illinois, United States (Babadoost and Pataky, 2002) and in 2002 in the Northern Territory, Australia (Heidi Martin, Queensland Department of Primary Industries, Australia, personal communication).

BFB outbreaks in the United States have been increasing over the past 4 years, despite improvements in management (R. R. Walcott, personal communication). Con-

siderable effort is being made to ensure seeds are pathogen-free. This is the most important control measure. Seeds are being produced in arid regions and seed production fields are inspected before harvest. Field inspection is especially important because symptomless watermelons from fields with BFB can contain infested seed. The pathogen has been isolated from pollinating insects, which suggests they may play a role in seed infestation. The most widely used seed test is the seedling grow-out assay which is routinely conducted on 10,000 to 50,000 seeds per seedlot. Seed testing has significantly reduced, but not eliminated, disease outbreaks. Several polymerase chain

Figure 13: Secondary roots exhibiting pink to red discoloration caused by *Rhizopycnis vagum*. (courtesy of B. D. Bruton, USDA, ARS, Lane, OK, USA)

reaction (PCR)-based seed tests are now available and work is underway to further improve their efficiency (Walcott and Gitaitis, 2000). Treating seed in hot water (10 to 30 min at 54 to 56 C) reduces incidence but does not eradicate the pathogen (Nomura and Shirakawa, 2001). A procedure is being developed for treating seeds with peroxyacetic acid to eliminate the pathogen (Hopkins *et al.*, 2001).

Practices recommended for managing BFB include greenhouse sanitation, ebb and flow irrigation to prevent splash dispersal in greenhouses, rotation, and routine

application of copper-based bactericides, in addition to using tested, uncontaminated seed (Zitter *et al.*, 1996). The pathogen can be moved from infected to healthy seedlings when plants are handled, such as during grafting, as well as by overhead irrigation (Shirakawa *et al.*, 2001). Growers have been able to reduce losses from BFB with copper-based bactericides when applications are started before bloom and conditions are not hot and humid with frequent rainfall, which is highly favorable for BFB development. Post-harvest management begins with looking for symptoms during harvest, so that affected fruit are left in the field. Inspections also continue during packing to eliminate symptomatic fruit missed in harvest (Rushing *et al.*, 1999). Since warm temperatures are favorable for the pathogen, fruit should be pre-cooled soon after harvest and kept cool during shipment and storage. In storage, fortunately, symptoms that develop on fruit that are asymptomatic at harvest do not become sufficiently severe to
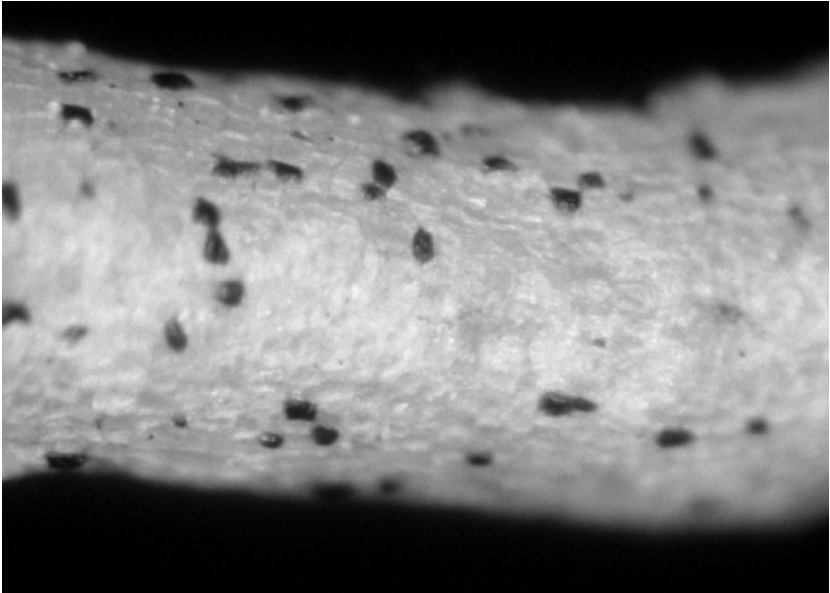
Figure 14: Secondary root of cantaloupe with microsclerotia of *Rhizopycnis vagum*. (courtesy of B. D. Bruton, USDA, ARS, Lane, OK, USA)

affect fruit marketability. Additionally, BFB severity does not increase dramatically on fruit with minor symptoms at harvest (less than 10-cm-diameter area affected) and healthy fruit in contact with diseased fruit do not develop symptoms for at least 3 weeks. Finally, surface abrasions on either diseased or healthy fruit do not promote pathogen transmission. Following a BFB outbreak, fields should be plowed soon after harvest and planted to non-cucurbit crops for at least three years (Zitter *et al.*, 1996). Volunteer cucurbit plants and wild cucurbit species should be controlled during the rotation; however, there is no conclusive evidence that they represent a significant source of inoculum.

Developing cultivars resistant to fruit blotch is an important goal. Resistance is especially sought after for bacterial diseases due to the difficulty of achieving effective control with chemicals. Inconsistent results obtained previously with watermelon were most likely due to genetic variation among the pathogen strains used in the different screens. Two subgroups have been identified (Walcott *et al.*, 2000) of which group I includes the American Type Culture Collection (ATCC) type strain from watermelon and strains from cantaloupe and pumpkin. Group II consists mainly of bacterial strains from watermelon. Thus there appears to be some degree of host specificity. Identifying these subgroups also clarifies taxonomic confusion described in the 'Compendium of Cucurbit Diseases'. The 'seedling bacterium' described in this book is the ATCC type strain while the 'fruit blotch bacterium' belongs to Group II with other isolates obtained from symptomatic watermelon.
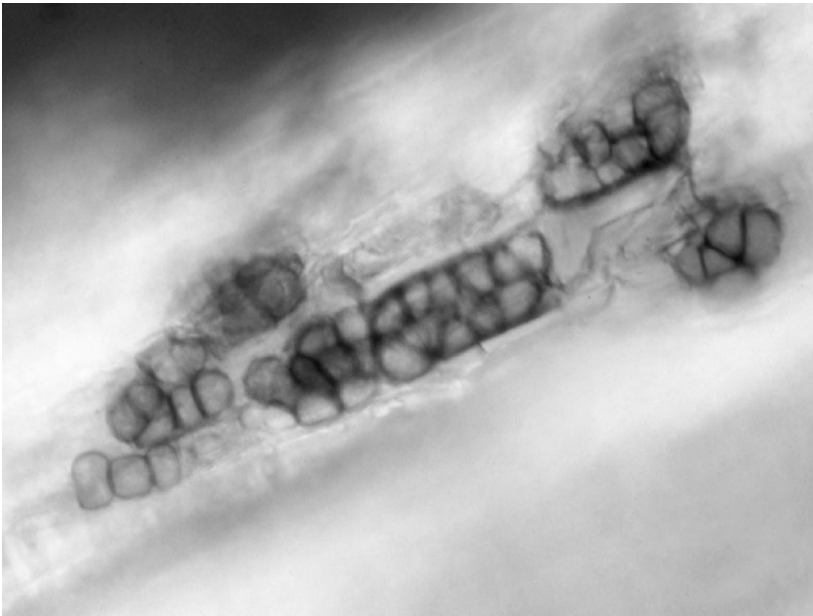
Figure 15: Micrograph of cantaloupe root showing microsclerotia of *Rhizopycnis vagum*. (courtesy of B. D. Bruton, USDA, ARS, Lane, OK, USA)

4.3 Bacterial blight

Pseudomonas syringae pv. *aptata* has been causing substantial losses in cantaloupe in France since 1993 (Morris *et al.*, 2000). Total loss of crop has occurred in some fields. Symptoms include necrotic spots on leaves that may have a surrounding water-soaked halo and cankers on stems and petioles. Fruit can drop when infected early or develop sunken lesions sometimes with large, dry-rotted cavities extending into the flesh (Fig. 21). Previously *P. syringae* has been reported occasionally on Cucurbits as a minor

pathogen. In southwestern France where severe blight epidemics first occurred, the pathogen has also been isolated from nearby sugar beet seed production fields, and rainy weather provided particularly favorable conditions for disease development during the cantaloupe production seasons from 1993 to 1997.

4.4 Bacterial wilt

Importance of bacterial wilt has been increasing in areas where it was endemic; and recently the disease has occurred in new areas, severely affecting crops previously thought to be moderately to highly resistant (especially pumpkin, summer and winter squash, and some gourds). Historically, pumpkin has been considered substantially less susceptible to bacterial wilt than cantaloupe and cucumber. However, the trend for
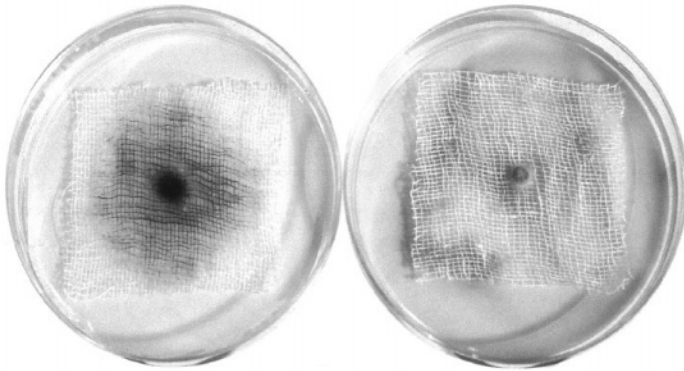
Figure 16: *Rhizopycnis vagum* and *Phoma terrestris* growing on cotton cloth showing characteristic red pigmentation of *Phoma terrestris*, which is the primary way to distinguish these two pathogens which look similar in culture and cause similar diseases. (courtesy of B. D. Bruton, USDA, ARS, Lane, OK, USA)

increased disease incidence in pumpkin has been noted in many areas of the United States. Complete crop loss has occurred. Most alarmingly, commercially available powdery mildew resistant pumpkin cultivars were found to be more susceptible to wilt than other pumpkin cultivars (McGrath, 2001b). Equally important, *Erwinia tracheiphila* has been associated with plants exhibiting some unique symptoms. These were first noted in 1998 in New York. Affected pumpkin plants had shortened internodes, interveinal yellow and chlorosis of the leaves, and tufted and reduced apical growth with marginal browning and eventual wilting and death. Wilt was virtually absent in south central United States until 1998-99 when cantaloupe, pumpkin, and squash were severely affected.

Presently, growers rely on insecticide applications. Pesticide management of

beetles and wilt is complicated because the presence of beetles alone is not indicative of an impending wilt epidemic. In the absence of the pathogen, a much higher beetle density can be tolerated by the crop. But if growers wait until disease symptoms occur to treat the beetle vectors, subsequent control of wilt is erratic.

4.5 Wilt and stem rot caused by *Pseudomonas cichorii*

This new disease was found affecting seedlings of muskmelon and watermelon growing under plastic in 1989 and 1998 in Serbia (Obradovic and Arsenijevic, 2002).

Figure 17: Watermelon seedlings with symptoms of bacterial fruit blotch caused by *Acidovorax avenae* subsp. *citrulli.* (courtesy of D. L. Hopkins, University of Florida, Apopka, FL, USA)

4.6 Cucumber root mat

Rhizogenic *Agrobacterium* biovar 1 (*A. radiobacter*) is the primary causal agent of this new disease (O'Neill *et al.*, 2001, Weller *et al.*, 2000). Only strains harboring an Ri-plasmid are virulent. Root mat was first described in 1973. It affected many soil and straw bed crops during the 1970s in the United Kingdom, then disappeared until 1993 when observed in crops grown hydroponically on a rockwool medium. Since then root mat has increased in importance, causing severe economic loss for some growers, and it has been observed in France, Spain and Holland (personal communication). Loss

occurs because affected plants produce more leaves and fewer fruit than healthy plants.

Roots on affected plants usually are thickened, grow upwards, and proliferate (Fig. 22). Root proliferation is the most noticeable symptom and results in rockwool cubes and slabs becoming swollen and distorted such that irrigation water runs off the cube. Roots become thickened and corky later in disease development. Occasionally affected plants, including roots, grow slowly and they produce many small, bent fruit. Tomatoes are also susceptible. Some isolates can cross-infect the two hosts. While predominantly in the root and crown, the bacterium has been isolated from within upper stems.

Management of root mat centers around avoiding pathogen introduction and sanitation (O'Neill *et al.*, 2001). Growers have found this pathogen to be difficult to eradicate. Infected plants or contaminated trays are the likely initial source of inoculum for a commercial operation. Rhizogenic *Agrobacterium* can survive in soil for more than
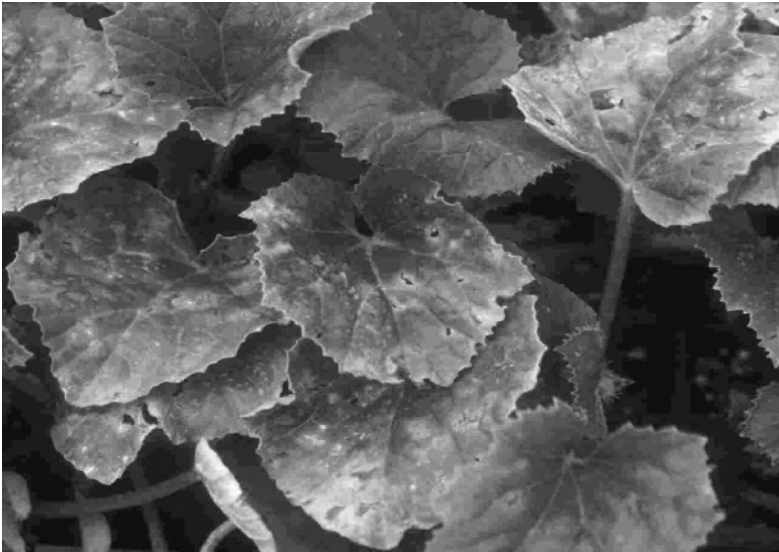
Figure 18: Leaf spots due to bacterial fruit blotch. (courtesy of H. Martin, Queensland Department of Primary Industries, Gatton Research Station, Australia)

a year and it has been found in soil both in and around commercial glasshouses as well as in growing media and run-off solution and on knives, concrete paths, and other equipment and glasshouse surfaces. Therefore boots should be worn in glasshouses or shoes should be disinfected prior to entering. Equipment and glasshouse surfaces also need to be disinfected. The pathogen could also move in irrigation systems. Using a power hose to clean irrigation lines and drip pegs followed by spraying a disinfectant has been effective. Re-planting during a growing season is an option for reducing yield loss because there is a long latent period (4 to 8 weeks). Root mat is less of a problem when plants are grown off the floor and when coir is used instead of rockwool or peat.

Thorough clean-up after a crop is important. Rockwool slabs from an affected crop should not be re-used. Although *Agrobacterium* can be easily killed by heat, steam-sterilizing rockwool slabs between crops is not recommended because the Ri plasmid survives this treatment and can be acquired by non-rhizogenic *Agrobacterium* re-populating the steamed slabs. Biocontrol agents have shown potential in reducing incidence of the disease.

Detection of the Ri-plasmid is critical with this pathogen as isolates of *Agrobacterium* biovar 1 without it have been detected in glasshouses. Pathogen detection will be facilitated by the recent development of a fluorogenic Taqman PCR assay that is less expensive, faster, and less labor-intensive than the previous procedure which entailed plating on semiselective media, profiling fatty acids of resulting colonies, and conventional PCR to detect the Ri-plasmid (Weller and Stead, 2001).

Figure 19: Cracks on watermelon fruit due to bacterial fruit blotch. (courtesy of D. L. Hopkins, University of Florida, Apopka, FL, USA)

4.7 Phytoplasma diseases

Phytoplasmas have been detected in pumpkin and melon plants in Queensland, Australia, by PCR assay using Phytoplasma-specific primers (Davis *et al.*, 1997). Infected plants are stunted with shortened internodes giving plants a compact appearance. Leaves are reduced in size and have marginal and interveinal chlorosis. Phyllody sometimes occurs on plants. The incidence of the disease has been low and is not currently of economic importance. The relationships of the phytoplasmas involved to those causing serious diseases in papaya, tomato, grapevine, strawberry and *Stylosanthes* in Australia is being investigated (Gibb *et al.*, 1996, Padovan *et al.*, 2000)(Denis Persley,

Queensland Department of Primary Industries, Australia, personal communication).

4.8 Phytophthora blight

Importance of Phytophthora blight, also known as Phytophthora fruit and crown rot, has been increasing to the point that it is now considered a major limitation to cucurbit production, most notably in several areas in the United States (Fig. 23 - 25). It is also an important disease of pepper. In addition to becoming more severe and widespread in areas where it occurred previously, Phytophthora blight of Cucurbits has recently been detected in new states (Georgia and Wisconsin). It was also first reported in Ontario in 1994 (Anderson and Garton, 2000). Crown and root rot were observed in 1999 for the first time on glasshouse-grown cucumber and melon in southeastern Spain (Herrero *et*
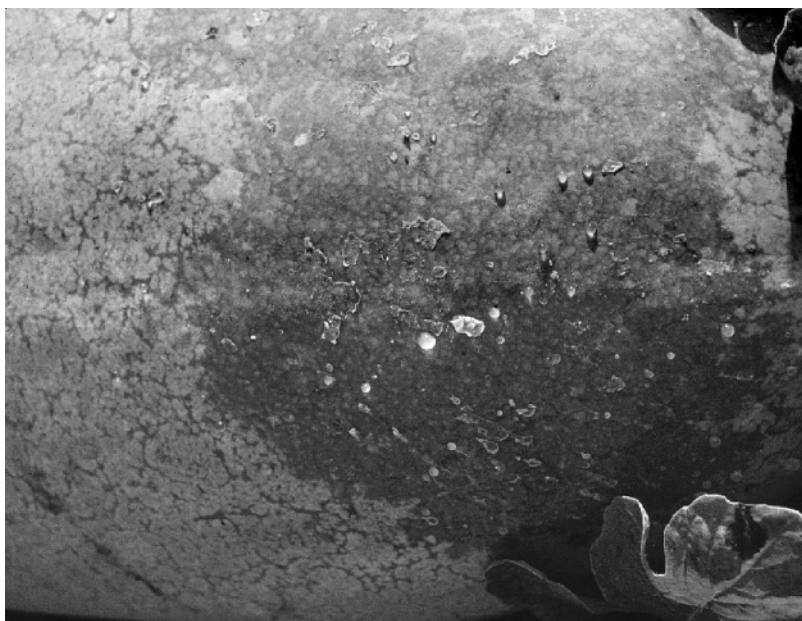
Figure 20: Water-soaked lesion on watermelon fruit due to bacterial fruit blotch. (courtesy of D. L. Hopkins, University of Florida, Apopka, FL, USA)

al., 2002). *Phytophthora capsici* was recently detected on non-cucurbit hosts in western and eastern Australia (Weinert *et al.*, 1999).

First occurrence of Phytophthora blight of Cucurbits in an area may follow recent introduction of the pathogen or extremely favorable conditions. *Phytophthora capsici* was first observed in the United States on pepper in 1918. First reports on Cucurbits in a state range from the 1930s (California and Colorado) to 1994 (Georgia and Wisconsin). In Florida and the northeastern states, *P. capsici* was noted on pepper several years before Cucurbits. Detection on Cucurbits often followed major rainstorms.

Phytophthora blight is a more important problem in areas where other susceptible crops (especially pepper) are also grown intensively and where water stands after excessive rain or irrigation. Many reports of Phytophthora blight mention the occurrence was associated with high rainfall or flooding from rain or irrigation (*e.g.* (Anderson and Garton, 2000, Babadoost, 2000, Wasilwa *et al.*, 1995). Once blight becomes important on Cucurbits in an area, it tends to occur annually.

Substantial losses have occurred because neither fungicides nor cultural practices provide adequate control when conditions are very favorable. Disease impact is related to pathogen distribution (not all fields are infested), soil moisture, and intensity of production of susceptible crops. It is difficult to manage fruit rot with contact fungicides because the disease typically starts on the fruit side contacting soil (Fig. 25). Soil solarization with cabbage leaf amendment is not sufficiently effective under conditions

Figure 21: Sunken lesions due to bacterial blight in cantaloupe. (courtesy of C. E. Morris, INRA, Station de Pathologie Vegetale, Montfavet, France)

in northern Florida to be a viable replacement for methyl bromide (Coelho *et al.*, 1999). Resistance may be an option in the future as cucumber cultivars have been identified with a high level of resistance to seedling root rot (Henz and Lima, 1998). To be commercially viable, resistance will need to include the fruit rot phase as well. Mature fruit of pumpkin cultivars producing hard, gourd-like rinds are substantially less susceptible to fruit rot than cultivars with conventional rinds (McGrath, 1998). Hard rinds are suitable for small ornamental pumpkins to be painted or left whole, but not for pumpkins to be carved. Managing Phytophthora blight of pepper as well as Cucurbits currently focuses on soil moisture management (Ristaino and Johnston, 1999). For example, decreasing frequency of furrow irrigation delays disease onset, slows disease progress,

and reduces final severity (Cafe-Filho *et al.*, 1995).

Fungicides may have a more important role in managing Phytophthora blight in the future if recently developed fungicides prove more effective than previous ones. Ethaboxam, a derivative of aminothiazole carboxamide, performed well for Phytophthora blight affecting pepper under field conditions compared to fluazinam, fosetyl, mancozeb, metalaxyl and copper oxychloride (Kim *et al.*, 1999). A novel systemic cyanoimidazole fungicide, IKF-916, is very effective against a broad spectrum of oomycetes including Phytophthora (Mitani *et al.*, 1998).

Information from research conducted recently has increased our understanding of *P. capsici*, which will be useful for improving control. Oospores are now known to be important for survival of this pathogen, at least in the area of the United States where investigated, based on both compatibility types occurring in a 1:1 ratio in most popula-

Figure 22: Root proliferation due to cucumber root mat occurring in hydroponically-grown crop on a rockwool medium. (courtesy of T. M. O'Neill, ADAS Arthur Rickwood, Mepal, Ely, Cambs, UK)

tions (Lamour and Hausbeck, 2000) and the high level of genotypic diversity detected between years in a field (Lamour and Hausbeck, 2001a). Identical genotypes were not recovered between years even though clonal reproduction does occur within years. Thick-walled oospores enable the pathogen to survive longer between susceptible crops than other structures, thus dictating the need for long rotational periods. They can survive over at least five years in temperate climates (Lamour and Hausbeck, 2001b). Additionally, when sexual reproduction plays a significant role in a pathogen's life cycle, the potential exists for generating a genotypically diverse array of isolates with an important trait, such as fungicide resistance, thus increasing the likelihood that this trait will be combined with other important traits such as fitness. As pointed out in

Section 3.2, resistance to mefenoxam has developed and this trait is stable in a population when this fungicide is not used (Lamour and Hausbeck, 2000). Half the Michigan isolates have unique multilocus genotypes and are fully insensitive to mefenoxam. Structure of the *P. capsici* populations examined is being shaped by sexual recombination as evidenced by the high frequency of isolates found with unique multilocus AFLP genotypes over consecutive years (Lamour and Hausbeck, 2001a). Some progeny from crossing mefenoxam-sensitive and insensitive isolates are fully insensitive, indicating the potential for selfing as well as outcrossing once the sexual stage of this heterothallic species has been stimulated (Lamour and Hausbeck, 2000).

Movement of inoculum among fields can be rare in the absence of equipment movement and flowing water. At one farm, for example, a field where blight occurred the previous year was unlikely the source of inoculum for another field, only about 1 mile
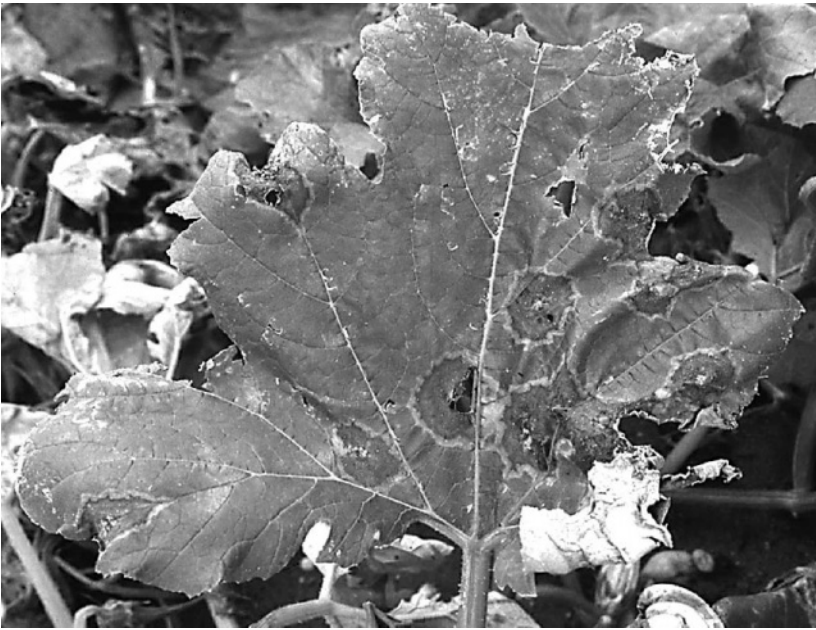
Figure 23: Leaf spots caused by *Phytophthora capsici.*

away, that had been planted to soybean and corn since blight occurred five years before (Lamour and Hausbeck, 2001b). This conclusion was based on the fact the isolates examined from these fields clearly separated into two discrete populations based on patterns of diversity at the DNA level and frequencies of mefenoxam sensitivity. Significant local spread of *P. capsici* can occur via water. Dispersal over 70 m was documented with furrow irrigation (Cafe and Duniway, 1995).

Cucurbit crop types differ in degree of susceptibility, specific host tissue affected and symptomology (Holmes *et al.*, 2002). Summer squash, pumpkin and zucchini are most susceptible and develop symptoms on fruit, crowns, and occasionally leaves.

Cucumber, muskmelon and watermelon are highly susceptible to fruit rot while symptoms on leaves range from uncommon in muskmelon to not observed in watermelon.

4.9 Powdery mildew on watermelon

Until recently, watermelon generally had been considered an unimportant host for powdery mildew in many areas. This disease had been described as a serious problem in Australia, Egypt, India, Japan, USSR, and the Philippines prior to 1970 (Robinson and Provvidenti, 1975). Watermelon more commonly remained asymptomatic while nearby plantings of other Cucurbits were affected. A single recessive gene conditions susceptibility (Robinson *et al.*, 1975). Thus, in contrast with the typical situation of resistance arising as a mutation in a susceptible host, resistance in watermelon to *Podosphaera*

Figure 24: *Phytophthora* blight usually starts in summer squash with die back of the growing tip. Symptoms can also begin at the plant crown.

xanthii (published as *Sphaerotheca fuliginea*) is thought to represent the ancestral condition (Robinson and Provvidenti, 1975). The cucurbit powdery mildew fungus has recently been re-named (Shishkoff, 2000). Sudden occurrence of powdery mildew on all cultivars and breeding lines of watermelon in a new area, as occurred in Sudan and the United States, suggests introduction or evolution of a new strain or race (Omara and Taha, 1995). Powdery mildew on watermelon is now considered an emerging disease (Davis *et al.*, 2001) and a limiting factor to production (Cohen *et al.*, 2000b).

Several different types of symptoms are recognized (Davis *et al.*, 2001). Symptoms on watermelon can be difficult to recognize as sporulation often is very sparse, in

sharp contrast with other Cucurbits (D. B. Langston, University of Georgia, Tifton, personal communication). Signs of the fungus can be invisible to the unaided eye, thus the disease may go undetected until chlorotic spots develop and leaves begin to senesce prematurely. Additionally, powdery mildew can begin in discrete foci in watermelon fields, but then the pathogen can spread quickly throughout the field like other powdery mildew fungi. Elsewhere, typical powdery fungal growth characteristic of powdery mildews develops on petioles and stems while leaves remain devoid of symptoms (Christine Horlock, Queensland Department of Primary Industries, Australia, personal communication). This typical powdery fungal growth has been observed on both leaf surfaces in other areas (Davis *et al.*, 2001)(McGrath, unpublished). Young watermelon fruit can be infected (Cohen *et al.*, 2000b).

Identify of the pathogen has been investigated. The causal agent is suggested

Figure 25: Fruit rot caused by *Phytophthora capsici* often begins on the part of a fruit in contact with the ground.

to be *Podosphaera xanthii* f. sp. *citrullus* based on results from cross-infectivity studies conducted in Israel in which conidia from watermelon were able to infect some other Cucurbits but conidia from other Cucurbits were unable to infect watermelon (Cohen *et al.*, 2000b). All isolates from watermelon in the United States are race 2 *Podosphaera xanthii* (Davis *et al.*, 2001). However they appear to be a different strain as they are more aggressive than other race 2 isolates (Davis *et al.*, 2001). Some race 1 isolates from muskmelon are able to infect watermelon under controlled conditions whereas no isolates from pumpkin or squash are virulent on watermelon (Shishkoff and McGrath, 2001).

4.10 Diseases caused by *Fusarium* species

Several potentially destructive diseases caused by *Fusarium* species have appeared in new areas recently. These include crown and root rot caused by *Fusarium solani* f. sp. *cucurbitae* race 1 in Spain affecting squash and watermelon (Armengol *et al.*, 2000a). Potential impact could be great as Spain is the main European producer of watermelons and no resistant rootstocks have been found. Root and stem rot of cucumber caused by

Figure 26: Melon necrotic spot virus (MNSV) affecting cucumber. (courtesy of H. Lecoq, INRA, Station de Pathologie Vegetale, Montfavet cedex, France)

Fusarium oxysporum f. sp. *radicis-cucumerinum* is spreading among greenhouses in Greece where it is causing a lot of damage (Vakalounakis and Fragkiadakis, 2000).

Changes have been detected in race composition that could compromise management using resistant cultivars. Races 0, 1, and 1,2 of *Fusarium oxysporum* f. sp. *melonis* have been found since 1985 in several areas in North America (Zitter, 1999), where resistance to race 2 is much more available than to race 1 in cultivars of eastern-type melons (Zuniga *et al.*, 1997). Race 2 of *F. oxysporum* f. sp. *niveum*, for which there is not adequate resistance, has been detected in south central United States where

cultivars resistant to race 1 are a principal control practice (Bruton and Damicone, 1999). Fortunately some important advancements in control have been made recently. The moderate level of Fusarium wilt control achieved in watermelon with soil solarization was improved substantially by first amending the soil with monoammonium phosphate or ammonium sulphate (Ioannou *et al.*, 2000). This synergy is likely due to production of gaseous ammonia. Duration of solarization, a common impediment to its adoption, can be halved to 2 to 4 weeks by using impermeable plastics (Antoniou and Tjamos, 2000). Grafting melon onto resistant rootstocks is more effective than prolonged crop rotation or soil disinfestation, and allows melon to be grown in monoculture without an excessive rise in the level of the *F. oxysporum* f. sp. *melonis* population (Languasco *et al.*, 2000). Inoculum increases more rapidly in sandy soils than in clay, silty or loam soils, and can reach high levels after just two successive crops. Additionally, molecular

Figure 27: *Moroccan watermelon mosaic virus* (MWMV) affecting zucchini squash. (courtesy of H. Lecoq, INRA, Station de Pathologie Vegetale, Montfavet cedex, France)

techniques that greatly facilitate identifying pathogens have been used for *Fusarium* species (*see:* section 3.5).

4.11 Viruses

Diseases caused by viruses are increasing in importance with recent reports of new viruses detected on Cucurbits and reports of previously detected viruses occurring in new areas. This adds to the already long list of different viruses affecting Cucurbits (Zitter *et al.*, 1996). They can cause substantial economic losses and limit production.

For example, in Lebanon, virus diseases are considered the major cause of economic losses in Cucurbits, which are among the most important vegetable crops grown there (Abou Jawdah *et al.*, 2000).

4.11.1 *Melon necrotic spot carmovirus*

Melon necrotic spot carmovirus (MNSV), which was first reported in 1966 in Japan, has

Figure 28: Widespread yellowing of melon foliage due to *Cucurbit yellow stunting disorder virus* (CYSDV) in a commercial glasshouse. (courtesy of B. W. Falk, University of California, Davis, CA, USA)

since been observed in protected crops in several European countries and the United States, and most recently in Italy where it has been occurring since 1995 on field-grown melon (Tomassoli and Barba, 2000). Symptoms include necrotic spotting on leaves and necrotic streaks near the base of the stem and along main branches (Fig.26). This disease resembles vine declines in that affected plants suddenly decline during fruit ripening. It has resulted in total loss of commercial product in some fields. MNSV is

spread in seed and by the fungus *Olpidium bornovanus*. Suggested management practices are using virus-free seed, selecting cultivars in the 'cantalupensis' group that are resistant, and crop rotation.

4.11.2 *Moroccan watermelon mosaic virus*

Moroccan watermelon mosaic virus (MWMV) has also been spreading worldwide since its first report in 1972 (Fig. 27)(Lecoq *et al.*, 2001). It is now considered a new threat to cucurbit production in the Mediterranean basin. This virus belongs to the genus Potyvirus, which includes some of the most frequently occurring and economically important viruses affecting Cucurbits on a worldwide basis: *Zucchini yellow mosaic virus* (ZYMV), *Papaya ringspot virus* type watermelon (PRSV-W), and *Water-*

Figure 29: Symptoms of *Cucurbit yellow stunting disorder virus* (CYSDV) on glasshouse-grown melon. (courtesy of B. W. Falk, University of California, Davis, CA, USA)

melon mosaic virus (WMV, formerly WMV-2)

The crinivirus genus of bipartite closteroviruses is a fairly new and rapidly growing group that includes several cucurbit pathogens (Nuez *et al.*, 1999). Yellowing diseases caused by closteroviruses have been reported occurring in Cucurbits growing in both field and greenhouse throughout the world since the late 1970s (Celix *et al.*, 1996). These viruses are transmitted by the greenhouse whitefly. Cucurbit pathogens in this group include *Beet pseudo-yellows virus* (BPYV), *Lettuce infectious yellows virus* (LIYV), *Cucumber yellows virus*, *Cucumber infectious chlorosis virus*, *Cucumber chlorotic spot virus*, and *Melon yellows virus* (Celix *et al.*, 1996, Nuez *et al.*, 1999).

4.11.3 *Cucurbit yellow stunting disorder virus*

Cucurbit yellow stunting disorder virus (CYSDV) is one of the newest closteroviruses affecting Cucurbits. It was first observed in North America in 1999 in both field- and greenhouse-grown melon plants (Kao *et al.*, 2000). This virus has emerged as a serious pathogen on cucumber and melon in southern Europe and the Middle East (Livieratos *et al.*, 1999). It is becoming even more widespread with recent introduction into Lebanon (Abou Jawdah *et al.*, 2000), Portugal (Louro *et al.*, 2000), and Morocco (Desbiez *et al.*, 2000). CYSDV also occurs in Israel and Jordan (Berdiales *et al.*, 1999). Symptoms of CYSDV begin as interveinal mottle on older leaves and progress to severe yellowing (Fig. 28 and 29)(Abou Jawdah *et al.*, 2000). Incidence in some greenhouses has reached 100%. This virus is transmitted by both the 'B' and 'Q' biotypes of the tobacco white-

Figure 30: *Cucurbit leaf crumple virus* (CuLCrV) affecting watermelon. (courtesy of R. L. Gilbertson, University of California, Davis, CA, USA)

fly, *Bemisia tabaci*, whereas the American resident biotype 'A' is an inefficient vector (Berdiales *et al.*, 1999). In Spain, CYSDV has displaced BPYV, a closely related virus which had been present there since the late 1970s (Berdiales *et al.*, 1999). This shift coincided with CYSDV's vector displacing BPYV's vector, the greenhouse whitefly (*Trialeurodes vaporariorum*). CYSDV is now among the most important cucurbit pathogens in the Mediterranean Region causing millions of dollars in losses annually (Livieratos *et al.*, 1999). A polyclonal antiserum against the CYSDV coat protein is now available for diagnosis.

4.11.4 *Cucurbit leaf crumple virus*

Cucurbit leaf crumple virus (CuLCrV) is a new begomovirus first observed in 1998 on volunteer watermelons with leaf curl, crumpling and yellowing symptoms that were growing in a commercial honeydew melon field in the Imperial Valley of California, United States (Fig. 30 and 31)(Guzman *et al.*, 2000).

A begomovirus was associated with a severe disease of bitter melon that occurred in 2001 in India (Khan *et al.*, 2002). Symptoms are stunted and deformed fruit and leaves that are curled upward, shortened or distorted. Impact of this disease is potential high as the crop is grown widely in tropical countries because of its nutritive and medicinal values.

Leaf curl diseases of melon and pumpkin and/or zucchini squash were observed simultaneously in Arizona, Texas, and Mexico during 1998-99. Symptoms were found to

Figure 31: *Cucurbit leaf crumple virus* (CuLCrV) affecting cantaloupe. (courtesy of R. L.
       Gilbertson, University of California, Davis, CA, USA)

be caused by a newly described begomovirus, provisionally *Cucurbit leaf curl virus* (CuLCV) (Brown *et al.*, 2000). The same virus was later determined to cause both leaf crumple disease of watermelon in California and leaf curl diseases of Cucurbits in Arizona, Texas, and Mexico. Koch's Postulates have now been completed for this emergent virus and retention of the CuLCV nomenclature has been proposed owing to its more accurate description of symptoms observed for most infected cucurbit species (Brown *et al.*, 2002). More recently, a second emergent begomovirus of melons was identified in Guatemala and assigned the provisional name, *Melon chlorotic leaf curl virus* (MCLCV) (Brown *et al.*, 2001). In contrast to *Squash leaf curl virus* strain E (SLCV-E), which causes only mild symptoms in melon (Fig. 32) and does not typically

cause yield loss, this virus causes severe leaf curl and chlorosis (Fig. 33) and fruit cracking, particularly when plants become infected at early growth stages (Brown *et al.*, 2001).

Virus diseases are in general more challenging to control than other disease groups. Most management programs focus on the vector for the virus because there are no pesticides that target viruses. Insecticides applied for insect vectors, however, are often ineffective when the virus can be transmitted quickly (Berdiales *et al.*, 1999). Floating row covers have been used to prevent insects from getting to the crop. This practice has been found more effective than others (El Zammar *et al.*, 2001). Significant yield increase over nontreated plants was also obtained with cross protection using a mild strain of *Zucchini yellow mosaic virus.* Grey mulch was less effective. Recommended management practices for MNSV include use of virus-free seed, resistant cul-

Figure 32: Leaf curl and chlorosis caused by *Squash leaf curl virus* strain E (SLCV-E) (courtesy of J. K. Brown, University of Arizona, Tucson, AZ, USA)

tivars where melons in the 'cantalupensis' group are grown, and crop rotation (Tomassoli and Barba, 2000). A good level of protection against virus diseases is being obtained in Lebanon with a combination of insect-proof nets, sticky yellow traps and application of insecticides including imidacloprid (Abou Jawdah *et al.*, 2000).

Considerable effort is being made to develop resistant cultivars. Two mechanisms of resistance have been found for *Melon yellows virus* (MYV): an antixenotic reaction against the vector (*Trialeurodes vaporariorum*), which prevents it from selecting resistant plants for feeding and oviposition, and resistance to the virus (Nuez *et al.*, 1999). Antixenosis is considered generally a successful strategy to combat white-

fly-borne virus diseases. MYV is a major cause of crop losses in protected melons in southeastern Spain. Monogenic, dominant resistance against CYSDV has been described in melon germplasm (Berdiales *et al.*, 1999). Genetic engineering is proving to be a valuable new tool for developing virus-resistant Cucurbits. There are now transgenic Cucurbits available for commercial production.

5. References

Abo-Foul, S., Raskin, V. I., Sztejnberg, A. and Marder, J. B. 1996. Disruption of chlorophyll organization and function in powdery mildew-diseased cucumber leaves and its control by the hyperparasite *Ampelomyces quisqualis*. Phytopathology, 86: 195-199.
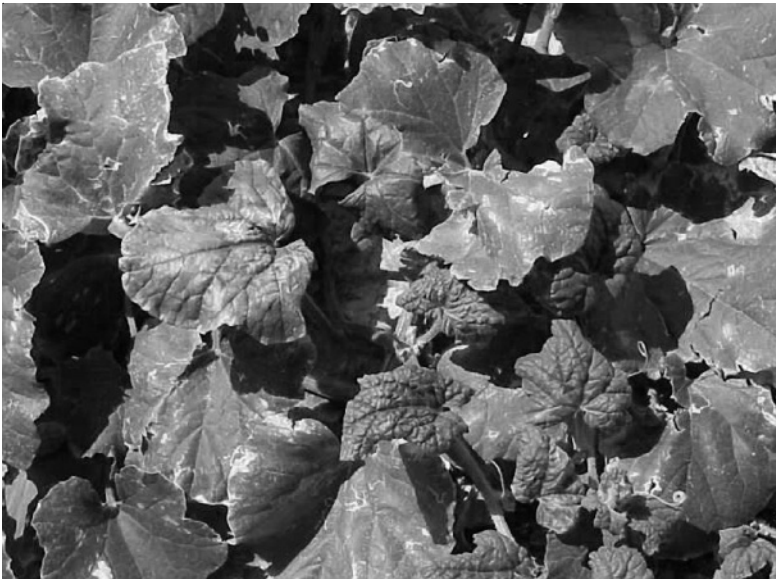
Figure 33: Severe leaf curl and chlorosis caused by *Melon chlorotic leaf curl virus* (MCLCV) (courtesy of J. K. Brown, University of Arizona, Tucson, AZ, USA)

Abou Jawdah, Y., Sobh, H., Fayad, A., Lecoq, H., Delecolle, B. and Trad Ferre, J. 2000. Cucurbit yellow stunting disorder virus: A new threat to Cucurbits in Lebanon. Journal of Plant Pathology, 82: 55-60.

Aegerter, B. J., Gordon, T. R. and Davis, R. M. 2000. Occurrence and pathogenicity of fungi associated with melon root rot and vine decline in California. Plant Disease, 84: 224-230.

Anderson, T. R. and Garton, R. 2000. First report of blight of field peppers caused by *Phytophthora capsici* in Ontario. Plant Disease, 84: 705.

Antoniou, P. P. and Tjamos, E. C. 2000. Control of *Fusarium oxysporum* f. sp. *cucumerinum* of cucumbers by soil solarization with impereable plastics and/or reduced doses of methyl bromide. Bulletin OEPP 30: 165-167.

Armengol, J., Jose, C. M., Moya, M. J., Sales, R., Vicent, A. and Garcia-Jimenez, J. 2000a.

Fusarium solani f. sp. *cucurbitae* race 1, a potential pathogen of grafted watermelon production in Spain. Bulletin OEPP. 30: 179-183.

Armengol, J., Pellicer, I., Vicent, A., Sales, R., Bruton, B. D. and García-Jiménez, J. 2000b. *Rhizopycnis vagum* D. F. Farr, un nuevo coelomycete asociado a raíces de plantas de melón con síntomas do colapso en España. Bol. San. Veg. Plagas 26: 103-112.

Armengol, J., Sanz, E., Martínez-Ferrer, G., Sales, R., Bruton, B. D. and García-Jiménez, J. 1998. Host range of *Acremonium cucurbitacearum*, cause of Acremonium collapse of muskmelon. Plant Pathology 47: 29-35.

Assis, S. M. P., Mariano, R. L. R., Silva Hanlin, D. M. W. and Duarte, V. 1999. Bacterial fruit blotch caused by *Acidovorax avenae* subsp. *citrulli* in melon in the state of Rio Grande do Norte, Brazil. Fitopatologia Brasileira 24: 191.

Babadoost, M. 2000. Outbreak of Phytophthora foliar blight and fruit rot in processing pumpkin fields in Illinois. Plant Disease, 84: 1345.

Babadoost, M. and Pataky, N. 2002. First report of bacterial fruit blotch of watermelon caused by *Acidovorax avenae* subsp. *citrulli* in Illinois. Plant Disease, 86: 443.

Belanger, R. R., Bowen, P. A., Ehret, D. L. and Menzies, J. G. 1995. Soluble silicon: Its role in crop and disease management of greenhouse crops. Plant Disease, 79: 329-336.

Belanger, R. R. and Benyagoub, M. 1997. Challenges and prospects for integrated control of powdery mildews in the greenhouse. Canadian Journal of Plant Pathology 19: 310-314.

Berdiales, B., Bernal, J. J., Saez, E., Woudt, B., Beitia, F. and Rodriguez Cerezo, E. 1999. Occurrence of cucurbit yellow stunting disorder virus (CYSDV) and beet pseudo-yellows virus in cucurbit crops in Spain and transmission of CYSDV by two biotypes of Bemisia tabaci. European Journal of Plant Pathology, 105: 211-215.

Bettiol, W. 1999. Effectiveness of cow's milk against zucchini squash powdery mildew (*Sphaerotheca fuliginea*) in greenhouse conditions. Crop Protection, 18: 489-492.

Bettiol, W., Garibaldi, A. and Migheli, Q. 1997. *Bacillus subtilis* for the control of powdery mildew on cucumber and zucchini squash. Bragantia, 56: 281-287.

Bextine, B., Wayadande, A., Bruton, B. D., Pair, S. D., Mitchell, F. and Fletcher, J. 2001. Effect of insect exclusion on the incidence of yellow vine disease and of the associated bacterium in squash. Plant Disease, 85: 875-878.

Biernacki, M. and Bruton, B. D. 2001. Quantitative response of *Cucumis melo* inoculated with root rot pathogens. Plant Disease, 85: 65-70.

Blancard, D., Lecoq, H. and Pitrat, M. 1994. A colour atlas of cucurbit diseases: observation, identification and control. INRA Vegetable Pathology Unit, Villenave-d'Ornon, France.

Brown, J. K., Idris, A. M., Olsen, M. W., Miller, M. E., Isakeit, T. and Anciso, J. 2000. Cucurbit leaf curl virus, a new whitefly transmitted geminivirus in Arizona, Texas, and Mexico. Plant Disease, 84: 809.

Brown, J. K., Idris, A. M., Rogan, D., Hussein, M. H. and Palmieri, M. 2001. Melon chlorotic leaf curl virus, a new begomovirus associated with *Bemisia tabaci* infestations in Guatemala. Plant Disease, 85: 1027.

Brown, J. K., Idris, A. M., Alteri, C. and Stenger, D. C. 2002. Emergence of a new cucurbit-infecting begomovirus species capable of forming viable reassortants with related viruses in the squash leaf curl virus cluster. Phytopathology, 92: 734-742.

Bruton, B., Brady, J., Mitchell, F., Bextine, B., Wayadande, A., Pair, S., Fletcher, J. and Melcher, U. 2001. Yellow vine of cucurbits: Pathogenicity of *Serratia marcescens* and transmission by *Anasa tristis*. Phytopathology, 91: S11-S12.

Bruton, B. D. 1998. Soilborne diseases in cucurbitaceae: pathogen virulence and host resistance. In: "Cucurbitaceae '98: Evaluation and Enhancement of Cucurbit Germplasm"(ed. McCreight, J. D.) ASHS Press, Alexandria, VA. pp. 143-166.

Bruton, B. D., Fletcher, J., Pair, S. D., Shaw, M. and Sittertz Bhatkar, H. 1998a. Association of

a phloem-limited bacterium with yellow vine disease in cucurbits. Plant Disease,. 82: 512-520.

Bruton, B. D., Russo, V. M., Garcia-Jimenez, J. and Miller, M. E. 1998b. Carbohydrate partitioning, cultural practices, and vine decline diseases of cucurbits. In: "Cucurbitaceae '98: Evaluation and Enhancement of Cucurbit Germplasm"(ed. McCreight, J. D.) ASHS Press, Alexandria, VA, Pacific Grove, CA, pp. 189-200.

Bruton, B. D. and Damicone, J. P. 1999. Fusarium wilt of watermelon: Impact of race 2 of *Fusarium oxysporum* f. sp. *niveum* on watermelon production in Texas and Oklahoma. Subtropical Plant Science, 51: 4-9.

Bruton, B. D., Garcia Jimenez, J., Armengol, J. and Popham, T. W. 2000a. Assessment of virulence of *Acremonium cucurbitacearum* and *Monosporascus cannonballus* on *Cucumis melo*. Plant Disease, 84: 907-913.

Bruton, B. D., Popham, T. W., Garcia Jimenez, J., Armengol, J. and Miller, M. E. 2000b. Disease reaction among selected Cucurbitaceae to an *Acremonium cucurbitacearum* isolate from Texas. Hortscience, 35: 677-680.

Cafe-Filho, A. C. and Duniway, J. M. 1995. Dispersal of *Phytophthora capsici* and *P. parasitica* in furrow-irrigated rows of bell pepper, tomato and squash. Plant Pathology, 44: 1025-1032.

Cafe-Filho, A. C., Duniway, J. M. and Davis, R. M. 1995. Effects of the frequency of furrow irrigation on root and fruit rots of squash caused by *Phytophthora capsici*. Plant Disease, 79: 44-48.

Casulli, F., Santomauro, A. and Faretra, F. 2000. Natural compounds in the control of powdery mildew on Cucurbitaceae. Bulletin OEPP. 30: 209-212.

Celix, A., Lopez Sese, A., Almarza, N., Gomez Guillamon, M. L. and Rodriguez Cerezo, E. 1996. Characterization of cucurbit yellow stunting disorder virus, a *Bemisia tabaci*-transmitted Closterovirus. Phytopathology, 86: 1370-1376.

Chaban, V. S., Okhrimchuk, V. N. and Sergienko, V. G. 2000. Optimization of chemical control of *Pseudoperonospora cubensis* on cucumber in Ukraine. Bulletin OEPP 30: 213-215.

Cheng, A. H., Hsu, Y. L., Huang, T. C. and Wang, H. L. 2000. Susceptibility of cucurbits to *Acidovorax avenae* subsp. *citrulli* and control of fruit blotch on melon. Plant Pathology Bulletin, 9: 151-156.

Coelho, L., Chellemi, D. O. and Mitchell, D. J. 1999. Efficacy of solarization and cabbage amendment for the control of *Phytophthora* spp. in North Florida. Plant Disease, 83: 293-299.

Cohen, R., Pivonia, S., Burger, Y., Edelstein, M., Gamliel, A. and Katan, J. 2000a. Toward integrated management of Monosporascus wilt of melons in Israel. Plant Disease, 84: 496-505.

Cohen, Y., Baider, A., Petrov, L., Sheck, L. and Voloisky, V. 2000b. Cross-infectivity of *Sphaerotheca fuliginea* to watermelon, melon, and cucumber. Acta Horticulturae: 85-88.

Crosby, K., Wolff, D. and Miller, M. 2000. Comparisons of root morphology in susceptible and tolerant melon cultivars before and after infection by *Monosporascus cannonballus*. Hortscience, 35: 681-683.

Daayf, F., Ongena, M., Boulanger, R., El Hadrami, I. and Belanger Richard, R. 2000. Induction of phenolic compounds in two cultivars of cucumber by treatment of healthy and powdery mildew-infected plants with extracts of *Reynoutria sachalinensis*. Journal of Chemical Ecology,. 26: 1579-1593.

Damicone, J. P., Jacobs, J. L. and Walker, R. M. 1993. Efficacy of fungicides for control of foliar diseases of watermelon, 1992. Fungicide and Nematicide Tests, 48: 204.

Davis, A. R., Bruton, B. D., Pair, S. D. and Thomas, C. E. 2001. Powdery mildew: an emerging disease of watermelon in the United States. Cucurbit Genetics Cooperative Report, 24: 42-48.

Davis, R. I., Schneider, B. and Gibb, K. S. 1997. Detection and differentiation of phytoplasmas in Australia. Australian J. of Agricultural Research, 48: 535-544.

Demir, G. 1996. A new bacterial disease of watermelon in Turkey: bacterial fruit blotch of watermelon (*Acidovorax avena*e subsp. *citrulli* (Schaad *et al.*) Willems *et al.*). Journal of Turkish Phytopathology, 25: 43-49.

Desbiez, C., Lecoq, H., Aboulama, S. and Peterschmitt, M. 2000. First report of cucurbit yellow stunting disorder virus in Morocco. Plant Disease, 84: 596.

El Zammar, S., Abou Jawdah, Y. and Sobh, H. 2001. Management of virus diseases of squash in Lebanon. Journal of Plant Pathology, 83: 21-25.

Everts, K. L. and Shields, P. L. 2000. Evaluation of fungicides for control of gummy stem blight and leaf blight on watermelon, 1999. Fungicide and Nematicide Tests, 55: 287.

Everts, K. L. 2001. Reduced fungicide applications for disease management of pumpkin with no-till production. Phytopathology, 91: S27.

Everts, K. L., Shields, P. L. and Armentrout, D. K. 2001. Evaluation of fungicides for control of gummy stem blight and Anthracnose on watermelon, 2000. Fungicide and Nematicide Tests, 2001: V114.

Farr, D. F., Miller, M. E. and Bruton, B. D. 1998. *Rhizopycnis vagum* gen. et sp. nov., a new coelomycetous fungus from roots of melons and sugarcane. Mycologia, 90: 290-296.

García-Jiménez, J., Velázquez, M. T., Jordá, C. and Alfaro García, A. 1994. *Acremonium* species as the causal agent of muskmelon collapse in Spain. Plant Disease, 78: 416-419.

García Jiménez, J., Armengol, J., Sales, R., Jordá, C. and Bruton, B. D. 2000. Fungal pathogens associated with melon collapse in Spain. Bulletin OEPP 30: 169-173.

Gibb, K. S., Persley, D. M., Schneider, B. and Thomas, J. E. 1996. Phytoplasmas associated with papaya diseases in Australia. Plant Disease, 80: 174-178.

Gleason, M. L., Petit, E., Wegulo, S. N. and Taber, H. G. 2000. Evaluation of SkyBit data input to the Melcast disease-warning system for control of watermelon anthracnose, 1999. Fungicide and Nematicide Tests, 55: 289.

Gleason, M. L., Wegulo, S. N. and Escassut, H. 2001. Performance of SkyBit data input to a disease warning model for muskmelon anthracnose, 2000. Fungicide and Nematicide Tests, 2001: V23.

Guzman, P., Sudarshana, M. R., Seo, Y. S., Rojas, M. R., Natwick, E., Turini, T., Mayberry, K. and Gilbertson, R. L. 2000. A new bipartite geminivirus (Begomovirus) causing leaf curl and crumpling in cucurbits in the Imperial Valley of California. Plant Disease, 84: 488.

Henz, G. P. and Lima, M. F. 1998. Plantlet resistance of cucurbit cultivars to root rot caused by Phytophthora capsici. Pesquisa Agropecuaria Brasileira, 33: 853-859.

Herrero, M. L., Blanco, R., Santos, M. and Tello, J. C. 2002. First report of *Phytophthora capsici* on cucumber and melon in Southeastern Spain. Plant Disease, 86: 558.

Hijwegen, T. 1992. Biological control of cucumber powdery mildew with *Tilletiopsis minor* under greenhouse conditions. Netherlands Journal of Plant Pathology, 98: 221-225.

Holmes, G. J., Main, C. E. and Keever, T. 1998. Forecasting long-distance movement of cucurbit downy mildew. In: "Cucurbitaceae '98: Evaluation and Enhancement of Cucurbits Germplasm"(ed. McCreight, J. D.) ASHS Press, Alexandria, VA, Pacific Grove, CA, pp. 186-188.

Holmes, G. J., Lancaster, M. E., Rodriguez, R. J. and Redman, R. S. 2002. Relative susceptibility of cucurbit and solanaceous crops to Phytophthora blight, 2001. Biological and Cultural Tests, 17: V10.

Homma, Y., Arimoto, Y. and Misato, T. 1981. Effect of sodium bicarbonate on each growth stage of cucumber powdery mildew fungus (*Sphaerotheca fuliginea*) in its life cycle. Journal of Pesticide Science, 6: 201-209.

Hopkins, D., Thompson, C., J., H. and Lovic, B. 2001. Wet seed treatment with peroxyacetic

acid for the control of bacterial fruit blotch of watermelon. Phytopathology, 91: S40.

Ioannou, N., Poullis, C. A. and Heale, J. B. 2000. Fusarium wilt of watermelon in Cyprus and its management with soil solarization combined with fumigation or ammonium fertilizers. Bulletin OEPP 30: 223-230.

Kao, J., Jia, L., Tian, T., Rubio, L. and Falk, B. W. 2000. First report of cucurbit yellow stunting disorder virus (genus Crinivirus) in North America. Plant Disease, 84: 101.

Karlatti, R. S., Abdeen, F. M. and Al Fehaid, M. S. 1997. First report of *Monosporascus cannonballus* on melons in Saudi Arabia. Plant Disease, 81: 1215.

Karsies, T., Dean, R. and Thomas, C. 2000. Toward the development of molecular markers linked to race 2 Fusarium wilt resistance in melon (*Cucumis melo* L.). Acta Horticulturae,: 415-419.

Keinath, A. P. 2000. Effect of protectant fungicide application schedules on gummy stem blight epidemics and marketable yield of watermelon. Plant Disease, 84: 254-260.

Keinath, A. P. and Zitter, T. A. 1998. Resistance to benomyl and thiophanate-methyl in *Didymella bryoniae* from South Carolina and New York. Plant Disease, 81: 479-484.

Khan, J. A., Siddiqui, M. K. and Singh, B. P. 2002. The association of begomovirus with bitter melon in India. Plant Diseas,e 86: 328.

Kim, D., Park, H., Chun, S., Yu, S., Choi, K., Oh, J., Shin, K., Koh, Y., Kim, B., Hahm, Y. and Chung, B. 1999. Field performance of a new fungicide ethaboxam against cucumber downy mildew, potato late blight and pepper Phytophthora blight in Korea. Plant Pathology Journal, 15: 48-52.

Konstantinidou-Doltsinis, S. and Schmitt, A. 1998. Impact of treatment with plant extracts from *Reynoutria sachalinensis* (F. Schmidt) Nakai on intensity of powdery mildew severity and yield in cucumber under high disease pressure. Crop Protection, 17: 649-656.

Lamour, K. H. and Hausbeck, M. K. 2000. Mefenoxam insensitivity and the sexual stage of *Phytophthora capsici* in Michigan cucurbit fields. Phytopathology, 90: 396-400.

Lamour, K. H. and Hausbeck, M. K. 2001a. The dynamics of mefenoxam insensitivity in a recombining population of *Phytophthora capsici* characterized with amplified fragment length polymorphism markers. Phytopathology, 91: 553-557.

Lamour, K. H. and Hausbeck, M. K. 2001b. Investigating the spatiotemporal genetic structure of *Phytophthora capsici* in Michigan. Phytopathology, 91: 973-980.

Langston, D. B., Lewis, K. L. and Jennings, W. T. 2000. Evaluation of fungicides and spray programs for control of gummy stem blight of watermelon, 1999. Fungicide and Nematicide Tests, 55: 291.

Languasco, L., Giosue, S., Rossi, V. and Gualazzi, M. 2000. Influence of soil and cultural variables on Fusarium wilt of melon. Bulletin OEPP.*, 30: 185-190.

Lecoq, H., Dafalla, G., Desbiez, C., Wipf-Scheibel, C., Delecolle, B., Lanina, T., Ullah, Z. and Grumet, R. 2001. Biological and molecular characterization of Moroccan watermelon mosaic virus and a potyvirus isolate from Eastern Sudan. Plant Disease, 85: 547-552.

Livieratos, I. C., Avgelis, A. D. and Coutts, R. H. A. 1999. Molecular characterization of the cucurbit yellow stunting disorder virus coat protein gene. Phytopathology, 89: 1050-1055.

Louro, D., Vicente, M., Vaira, A. M., Accotto, G. P. and Nolasco, G. 2000. Cucurbit yellow stunting disorder virus (genus Crinivirus) associated with the yellowing disease of cucurbit crops in Portugal. Plant Disease,. 84: 1156.

MacNab, A. A. 1993. Muskmelon Alternaria leaf blight control by fungicide applications timed according to various programs. Fungicide and Nematicide Tests, 48: 135.

Martyn, R. D. and Miller, M. E. 1996. Monosporascus root rot and vine decline: an emerging disease of melons worldwide. Plant Disease, 80: 716-725.

Matheron, M. E. and Porchas, M. 2000. Comparative efficacy of fungicides for control of powdery mildew on muskmelon, 1999. Fungicide and Nematicide Tests, 55: 174-175.

Matheron, M. E. and Porchas, M. 2001. Comparative fungicide performance for control of

powdery mildew on muskmelon, 2000. Fungicide and Nematicide Tests, 56: V24.

Maynard, D. N. and Hopkins, D. L. 1999. Watermelon fruit disorders. HortTechnology, 9: 155-161.

McGrath, M. T. 1998. Susceptibility of pumpkin experimentals to Phytophthora fruit rot in pumpkin, 1997. Biological and Cultural Tests, 13: 175.

McGrath, M. T. 2001a. Fungicide resistance in cucurbit powdery mildew: Experiences and challenges. Plant Disease, 85: 236-245.

McGrath, M. T. 2001b. Variation among cucurbit crop types and cultivars in susceptibility to bacterial wilt and attractiveness to cucumber beetles. Phytopathology, 91: S60.

McGrath, M. T. 2002. Evaluation of fungicide programs for managing powdery mildew of pumpkin, 2001. Fungicide and Nematicide Tests, 2002: V93.

McGrath, M. T. and Shishkoff, N. 1999. Evaluation of biocompatible products for managing cucurbit powdery mildew. Crop Protection, 18: 471-478.

McGrath, M. T. and Shishkoff, N. 2000. Control of cucurbit powdery mildew with JMS Stylet-Oil. Plant Disease, 84: 989-993.

McGrath, M. T., and Shishkoff, N. 2003. Evaluation of fungicide programs for managing powdery mildew of pumpkin, 2002. Fungicide and Nematicide Tests, 58: (in press).

Menzies, J., Bowen, P., Ehret, D. and Glass Anthony, D. M. 1992. Foliar applications of potassium silicate reduce severity of powdery mildew on cucumber, muskmelon, and zucchini squash. Journal of the American Society for Horticultural Science,. 117: 902-905.

Miller, M. E. and Bruton, B. D. 2000. Management of cucurbit vine declines in Texas. In: "Proc. 19th Ann. Hort. Ind. Conf".(ed. Motes, J.) Oklahoma State University, Stillwater,, pp. 137-140.

Mitani, S., Araki, S., Matsuo, N. and Camblin, P. 1998. IKF-916 - a novel systemic fungicide for the control of oomycete plant diseases. Brighton Crop Protection Conference: Pests & Diseases, 2: 16-19.

Miller, M. E., Martyn, R. D. and Bruton, B. D. 2000. Muskmelon growth and yield in response to fumigation. In: "Cucurbitaceae 2000"(eds. Katzir, N. and Paris, H. S.) ASHS Press, Alexandria, VA,, pp. 179-185.

Miller, M. E., Martyn, R. D., Lovi, B. R. and Bruton, B. D. 1995. An overview of vine decline diseases of melons. In: "Cucurbitaceae '94"(eds. Lester, G. E. and Dunlap, J. R.) Gateway Printing,, pp. 31-35.

Morris, C. E., Glaux, C., Latour, X., Gardan, L., Samson, R. and Pitrat, M. 2000. The relationship of host range, physiology, and genotype to virulence on cantaloupe in Pseudomonas syringae from cantaloupe blight epidemics in France. Phytopathology, 90: 636-646.

Nomura, T. and Shirakawa, T. 2001. Efficacy of hot water and bactericide treatments of watermelon seeds infested by *Acidovorax avenae* subsp. *citrulli*. Proc. Kansai Pl. Prot., 43: 1-6.

Nuez, F., Pico, B., Iglesias, A., Esteva, J. and Juarez, M. 1999. Genetics of melon yellows virus resistance derived from *Cucumis melo* ssp. *agrestis*. European Journal of Plant Pathology, 105: 453-464.

O'Brien, R. G. and Martin, H. L. 1999. Bacterial blotch of melons caused by strains of *Acidovorax avenae* subsp. *citrulli*. Australian Journal of Experimental Agriculture, 39: 479-485.

O'Neill, T. M., Weller, S. A., Stead, D. E., Jackson, A. and McPherson, G. M. 2001. Cucumber and tomato root mat. HDC News, 70: 11-13.

Obradovic, A. and Arsenijevic, M. 2002. First report of a wilt and stem rot of muskmelon and watermelon transplants incited by *Pseudomonas cichorii* in Serbia. Plant Disease, 86: 443.

Olaya, G. and Holm, A. 2001. Sensitivity of *Didymella bryoniae* isolates to azoxystrobin. Phytopathology, 91: S67.

Omara, S. K. and Taha, M. 1995. Powdery mildew attacks commercial watermelon cultivars in Sudan. Report Cucurbit Genetics Cooperative, 18: 55.

Padovan, A. C., Gibb, K. S. and Persley, D. M. 2000. Association of *Candidatus* Phytoplasma Australiense with green petal and lethal yellows diseases of strawberry. Plant Pathology, 49: 362-369.

Pair, S. D. and Bruton, B. D. 1998. Relationship of watermelon genotype and ploidy to incidence of yellow vine disease. In: "Cucurbitaceae '98"(ed. McCreight, J.) ASHS Press, Alexandria, Virginia.

Pair, S. D., Bruton, B. D., Mitchell, F. and Fletcher, J. 1998. Advances in yellow vine disease research. In: "17th Ann. Hort. Ind. Conf".(ed. McCraw, B. D.) Oklahoma State University, Stillwater, pp. 287-290.

Pair, S. D., Bruton, B. D., Mitchell, F. and Fletcher, J. 2000. Yellow vine management. In: "19th Ann. Hort. Ind. Conf".(ed. Motes, J.) Oklahoma State University, Stillwater, pp. 145-148.

Raupach, G. S. and Kloepper, J. W. 2000. Biocontrol of cucumber diseases in the field by plant growth-promoting rhizobacteria with and without methyl bromide fumigation. Plant Disease, 84: 1073-1075.

Reuveni, M., Eyal, H. and Cohen, Y. 1980. Development of resistance to metalaxyl in *Pseudoperonospora cubensis*. Plant Disease, 64: 1108-1109.

Reuveni, M., Agapov, V. and Reuveni, R. 1994. Induced systemic protection to powdery mildew in cucumber by phosphate and potassium fertilizers: effects of inoculum concentration and post-inoculation treatment. Canadian Journal of Plant Pathology, 17: 247-251.

Reuveni, M., Agapov, V. and Reuveni, R. 1995. Suppression of cucumber powdery mildew (*Sphaerotheca fuliginea*) by foliar sprays of phosphate and potassium salts. Plant Pathology, 44: 31-39.

Reuveni, M., Agapov, V. and Reuveni, R. 1997. A foliar spray of micronutrient solutions induces local and systemic protection against powdery mildew (*Sphaerotheca fuliginia*) in cucumber plants. European Journal of Plant Pathology, 103: 581-588.

Reuveni, R., Dor, G., Raviv, M., Reuveni, M. and Tuzun, S. 2000. Systemic resistance against *Sphaerotheca fuliginea* in cucumber plants exposed to phosphate in hydroponics system, and its control by foliar spray of mono-potassium phosphate. Crop Protection, 19: 355-361.

Reuveni, R. and Reuveni, M. 1998. Foliar-fertilizer therapy - a concept in integrated pest management. Crop Protection, 17: 111-118.

Ristaino, J. B. and Johnston, S. A. 1999. Ecologically based approaches to management of Phytophthora blight on bell pepper. Plant Disease, 83: 1080-1089.

Robinson, R. W. and Provvidenti, R. 1975. Susceptibility to powdery mildew in *Citrullus lanatus* (Thunb.) Matsum. & Nakai. Journal of the American Society for Horticultural Science, 100: 328-330.

Robinson, R. W., Provvidenti, R. and Shail, J. W. 1975. Inheritance of susceptibility to powdery mildew in the watermelon. Journal of Heredity, 66: 310-311.

Rushing, J. W., Keinath, A. P. and Cook, W. P. 1999. Postharvest development and transmission of watermelon fruit blotch. HortTechnology, 9: 217-219.

Santomauro, A., F., C., Gatto, M. A. and Faretra, F. 2001. Evaluation of the natural compounds and microbial antagonists effectiveness towards agents of powdery mildew. Notiziario sulla protezione delle piante (News on plant protection), 13: 149-151.

Shirakawa, T., Kikuchi, S., Kato, T., Abiko, K. and Kawai, A. 2000. Occurrence of watermelon bacterial fruit blotch in Japan. Japanese Journal of Phytopathololology, 66: 223-231.

Shirakawa, T., Komiya, Y. and Abiko, K. 2001. Second transmission of *Acidovorax avenae* subsp. *citrulli* at watermelon nursery plants production. Japanese Journal of Phytopathololology, 67: 208.

Shishkoff, N. 2000. The name of the cucurbit powdery mildew: *Podosphaera* (sect. *Sphaerotheca*) *xanthii* (Castag.) U. Braun & N. Shish. comb. nov. (Abstr.). Phytopathology, 90: S133.

Shishkoff, N. and McGrath, M. T. 2001. Distribution of cucurbit powdery mildew races 1 and

2 on watermelon and muskmelon. Phytopathology, 91: S197.

Stanghellini, M. E., Kim, D. H., Waugh, M. M., Radewald, K. C., Sims, J. J., Ohr, H. D., Mayberry, K. S., Turini, T. and McCaslin, M. A. 2001. Vine-decline of melons caused by *Monosporascus cannonballus*: I. Preplant disease management strategies. Phytopathology, 91: S84.

Sundheim, L. and Amundsen, T. 1982. Fungicide tolerance in the hyperparasite *Ampelomyces quisqualis* and integrated control of cucumber powdery mildew. Acta. Agric. Scand., 32: 349-355.

Sztejnberg, A., Galper, S., Mazar, S. and Lisker, N. 1989. *Ampelomyces quisqualis* for biological and integrated control of powdery mildews in Israel. Journal of Phytopathology, 124: 285-295.

Tomassoli, L. and Barba, M. 2000. Occurrence of melon necrotic spot carmovirus in Italy. Bulletin OEPP., 30: 279-280.

Vakalounakis, D. J. and Fragkiadakis, G. A. 2000. Genetic variation among *Fusarium oxysporum* isolates from cucumber. Bulletin OEPP, 30: 175-177.

Verhaar, M. A., Hijwegen, T. and Zadoks, J. C. 1996. Glasshouse experiments on biocontrol of cucumber powdery mildew (*Sphaerotheca fuliginea*) by the mycoparasites *Verticillium lecanii* and *Sporothrix rugulosa*. Biological Control, 6: 353-360.

Walcott, R. R. and Gitaitis, R. D. 2000. Detection of *Acidovorax avenae* subsp. *citrulli* in watermelon seeds using immunomagnetic separation and the polymerase chain reaction. Plant Disease, 84: 470-474.

Walcott, R. R., Langston , D. B., Sanders, F. H. and Gitaitis, R. D. 2000. Investigating intraspecific variation of *Acidovorax avenae* subsp. *citrulli* using DNA fingerprinting and whole cell fatty acid analysis. Phytopathology, 90: 191-196.

Wasilwa, L. A., Correll, J. C. and Morelock, T. E. 1995. Phytophthora blight of squash caused by *Phytophthora capsici* in Arkansas. Plant Disease, 79: 1188.

Wei, G., Kloepper, J. W. and Tuzun, S. 1996. Induced systemic resistance to cucumber diseases and increased plant growth by plant growth-promoting rhizobacteria under field conditions. Phytopathology, 86: 221-224.

Weinert, M. P., Smith, B. N., Wagels, G., Hutton, D. and Drenth, A. 1999. First record of *Phytophthora capsici* from Queensland. Australasian Plant Pathology, 28: 93.

Weller, S. A., Stead, D. E., O'Neill, T. M., Hargreaves, D. and McPherson, G. M. 2000. Rhizogenic Agrobacterium biovar 1 and cucumber root mat in the UK. Plant Pathology, 49: 43-50.

Weller, S. A. and Stead, D. E. 2001. Detection of root mat associated *Agrobacterium* strains from plant material and other sample types by post-enrichment TaqMan PCR. Journal of Applied Microbiology, 91: 1-9.

Wurms, K., Labbe, C., Benhamou, N. and Belanger, R. R. 1999. Effects of Milsana and benzothiadiazole on the ultrastructure of powdery mildew haustoria on cucumber. Phytopathology, 89: 728-736.

Zehnder, G. W., Murphy, J. F., Sikora, E. J. and Kloepper, J. W. 2001. Application of rhizobacteria for induced resistance. European Journal of Plant Pathology, 107: 39-50.

Zhang, J. X., Bruton, B. D., Howell, C. R. and Miller, M. E. 1999. Potential of *Trichoderma virens* for biocontrol of root rot and vine decline in *Cucumis melo* L. caused by *Monosporascus cannonballus*. Subtropical Plant Science, 51: 29-37.

Zitter, T. A. 1999. Fusarium wilt of melon, a worldwide problem in temperate and tropical regions. Acta Horticulturae, 492: 157-161.

Zitter, T. A., Hopkins, D. L. and Thomas, C. E. 1996. Compendium of Cucurbit Diseases, APS Press, St. Paul.

Ziv, O. and Zitter, T. A. 1992. Effects of bicarbonates and film-forming polymers on cucurbit diseases. Plant Disease, 76: 513-517.

Zuniga, T. L., Zitter, T. A., Gordon, T. R., Schroeder, D. T. and Okamoto, D. 1997. Characterization of pathogenic races of *Fusarium oxysporum* f.sp. *melonis* causing Fusarium wilt of melon in New York. Plant Disease, 81: 592-596.

Diseases and Disorders of Mango and their Management

Om Prakash

Department of Crop Protection,
Central Institute for Subtropical Horticulture, Rehmankhera,
P.O.- Kakori, Lucknow-227 107, India.

Abstract: The mango (*Mangifera indica* Linn.) is an important fruit crop in India and other tropical and subtropical countries of the world. It is grown in at least 87 countries but no where it is so greatly valued as in India where 40 per cent of total fruits grown in our country is only mango. Although India is the largest producer of mango but in terms of productivity, it ranks sixth. The low productivity is mainly due to the associated disease problem. Mango is affected by a number of diseases at all stages of its development, right from the plants in the nursery to the fruits in storage or transit. Hardly any plant organ is immune and almost every part viz. stem, branch, twig, root, leaf, petiole, flower and fruit are affected by various pathogens, yet there are few diseases which are of great economic importance. These diseases manifest themselves as several kinds of rot, die back, mildew, necrosis, scab, blotch, stem bleeding, wilt, spots, canker, sooty mould and malformation. Leaf spot diseases cause great loss and hamper the efforts made to increase the yield of mango tree. They impoverish the leaves, diminish the phyto-synthetic efficiency and upset normal physiological activity of the host. Some of these diseases take heavy toll of trees, and have become limiting factor in mango cultivation in some regions. Bloom blight or Blossom blight in some years causes a complete failure of the crop. Other diseases like bacterial canker, black tip, powdery mildew, sooty mould and die back in India are the sources of great loss to the orchardists. The chemical based strategies have been so far dominating for management of mango diseases but it has caused serious imbalance in the agro-ecosystem. Strangely, about 70 percent of the amount of sprayed chemicals, does not stick to the plants. Enormous quantities of chemicals that fall on to earth get mixed up with soil adversely affect microbial life. Some problems like nontarget effects of chemicals as well as chemical induced diseases are being experienced. A shift towards nonchemical strategies is likely to correct the imbalance in our approach. The most logical approach is known as IDM, which is being used for few important diseases of mango and discussed in this chapter.

1. Introduction

The mango is a very important cultural and religious symbol of India. It has developed its own importance all over the world but its cultivation is nearly as old as Indian civilization. The fruit has been in cultivation in Indian subcontinent for well over 4000 years and favourite of the kings and common people because of its nutritive value, taste, attractive fragrance and health promoting qualities. One medium size mango (about 200 gm.) provides more than daily requirement of vitamin 'A' of an adult and three fourth requirement of vitamin 'C'. Thus it is recongnized as one of the best fruit

in the world market. Hinduism, Buddhism and Islam were introduced into South East Asia from India. Although, at present it is being cultivated commercially in eighty seven countries of the world, no where it has achieve the same premier position as in the Indian subcontinent where it thrives throughout the length and breadth of the country and is considered as the king of all fruits.

Mango is adopted to wide range of soils, climate and altitude and is relatively easy to cultivate. It plays a vital role in supplementing the diet of millions of people throughout the tropics in Africa, America and Asia were local consumption is tremendous.

India is the largest producer of mango in the world accounting for 52.63 per cent of total mango production. The mango accounts for 22.06 per cent of total area under fruit and 23.93 per cent of total fruit production in the country. The country produced 10.99 million tones of mangoes from an area of 1.23 million ha. The area under mango is highest in Uttar Pradesh *i.e.* 0.30 million ha followed by 0.26 and 0.15 million ha in Andhra Pradesh and Bihar, respectively. However, Andhra Pradesh is the largest producer of mangoes with production of 3.07 million tones as against 2.39 and 1.79 million tones produced by Uttar Pradesh and Bihar, respectively. Wide fluctuations are recorded in the productivity in different states of the country. The productivity of mango is highest *i.e.* 12 tones per ha in Andhra Pradesh and Bihar. It is lowest in the states like Arunachal Pradesh, Himachal Pradesh and Jammu & Kashmir, where it ranged between 0.04 and 1.63 tones per ha. Goa, Gujarat and Karnataka reported a productivity of 10.26, 10.00 and 0.50 tones per ha, respectively. In Uttar Pradesh, it has increased from 6.35 tones per ha during 1987 to 9.42 per ha during 1994-95. Maharashtra, which exports Alphonso mango, reported productivity of only 5.50 tones per ha during 1994-95. It will not be out of place to mention that there is wide divergence in the area, production and productivity.

The low productivity is due to the wide range of climatic conditions, environment situation and the diversity of the associated disease and disorder problems. Over 140 pathogens are known to cause damage to the crop. The first step in overcoming the threats from diseases is to accurately identify the problems.

Diseases are caused by fungi, bacteria, algae, unknown etiology, phanerogamic parasites and epiphytes, nutritional deficiencies and smokes. Disorders are ailment not caused by infecting organism, they are mostly the results of some form of physical damage or an upset to mango physiology. Many such factors produce blemishes on the fruit surface and hence downgrade quality and lower the prices. Faulty post harvest handling procedures or lack of adequate post harvest treatments are the cause of many disorders. Often they can be avoided if careful attention is paid to correct handling and management at harvest and during marketing. Several disorders do not produce symptoms until fruit ripens. It is utmost important that growers receive prompt, accurate and adequate feedback from the market about any problems that occur with their fruit during marketing.

The most critical times for disease control are at flowering, at fruit set and after harvest. Diseases at flowering and fruit set can badly affect the quality and subsequent yield. Some post harvest diseases damage fruit at very crucial time, when they are ripening and ready to display before buyers. Others show symptoms at harvest, en-

abling affected fruit to be culled out during grading and packaging. Many, however, become established on fruit in the field and control of these diseases at field level is an important step in reducing post harvest losses. Certain diseases appear during long-term storage which are not encountered in fruit stored for shorter periods. These diseases are a problem for export and must be adequately controlled.

Mango is affected by a number of diseases at all stages of its development, right from the seedling in the nursery to the fruits in storage or transit. Field diseases result in the crop loss while post harvest diseases are directly linked with the losses in export and domestic market. Hardly any plant organ is immune and almost every part viz., stem, branch, twig, root, leaf, petiole, flower and fruit are affected by various diseases. These diseases manifest themselves as several kinds of rots, die back, mildew, necrosis, scab, blotch, stem bleeding, wilt, spots, canker, sooty mould, malformation, unknown etiology and disorders. Some of these diseases have become limiting factor in mango cultivation. Bloom blight or blossom blight in some year causes a complete failure of the crop.

Bacterial canker, anthracnose, black tip, dieback, mildew, malformation, sooty mould and phoma blight are of major concern to the growers in India. Bacterial canker earlier restricted to few South Indian varieties has recently been observed even on the choicest variety *i.e.* Dashehari. Overall, fungal diseases of foliage and fruits are prevalent more in the Indian conditions.

Prevention is the only effective means of reducing losses from most mango diseases. The use of chemical to control diseases is justified only if significant economic losses are anticipated and there is no practical alternative. In mango, cost of chemical to be used against control of diseases in the tree canopy has major impact because of large quantity of spray material is needed. Therefore, chemicals having long residual and protectant action or can inhibit inoculum production for a long period are practical for controlling diseases.

More intensive horticulture through increased cropping intensity may eventually result in more problems of diseases. The commercial control of plant diseases has become very complex and the extent of losses inflicted has increased. In addition to the losses, the yield fluctuation between years also causes great concern. Such a situation leads to over usage of fungicides which is neither ecologically safe nor economically viable. The issue facing horticultural and in particular disease control is, therefore, managing the plant disease severity below the economic threshold following ecologically safe, economically viable and easily operational procedures.

The integrated disease management (IDM) strategy is targeted to achieve this objective. The most common method for controlling management of mango diseases in the use of fungicide. Other methods used are sanitation practices, tillage operations, fertility management, manipulation in dates and duration of spraying, destruction of alternate hosts, destruction and rouging of diseased twigs/seedlings, pruning and defoliation and water management etc. The IDM is structured to use an assortment of procedures rather than relying only on fungicides to control the disease. Some commonly used specific practices as management strategy are disease surveillance and forewarning for integrated disease management, variety, horticultural practices and biological control.

2. Diseases caused by Fungi

2.1 Powdery Mildew (*Oidium mangiferae*) Berthet

Berthet first recorded powdery mildew of mango in Brazil in 1914 and he named it *Oidium mangiferae* Berthet. Wagle (1928) recorded the disease in India. Earlier, it was considered a disease of minor importance but now it is becoming increasingly important in most of the commercial mango growing countries of the world affecting almost all cultivars, either in severe or mild form (Prakash and Srivastava, 1987a, Prakash and Misra, 1993a,b, Prakash and Raoof, 1994, Prakash *et al.*, 1996, Ploetz and Prakash, 1997). Although infection occurs both on foliage and blossom, the losses mainly occur as a result of blossom infection. Hence, spraying of fungicides during flowering stage is essential for the control of disease. In India, incidence of mildew has assumed such devastating proportions that the disease has become the single limiting factor in the expansion of mango cultivation.

2.1.1 Geographical distribution

The disease is known to cause extensive damage mainly up to latitude 40ºNS of the Equator. It is reported from India, Myanmar, Bangladesh, Nepal, Pakistan, Sri Lanka, Israel, Lebanon (Asia), New South Wales (Zaire), Queensland and New Caledonia (Australia), Congo, Egypt, Ethiopia, Kenya, Malawi, Mozambique, Mauritius, Reunion, Tanzania, Zambia, Zimbabwe, South Africa (Africa), USA (California and Florida), Mexico, Jamaica, Costa Rica, Guatemala (Central America), Brazil Venezuela, Colombia, Peru (South America) (Prakash *et al.*, 1996). It has also been reported from Canary Island (Hernandez *et al.*, 1955) and Cuba (Padron, 1983). In India, the disease is present in almost all the states of country (Prakash and Raoof, 1994).

2.1.2 Economic importance

Dropping of unfertilized infected flowers and immature fruits causes serious losses. In Australia, Peterson *et al.,* (1991) reported the incidence of mildew up to 23% on unsprayed trees, whereas it was 11.5% in Mancozeb sprayed trees. Anonymous (1968) reported 20% decreasing yield in Venezuela. The disease can result in yield reduction upto 90% mainly due to its effect on fruit set and development (Schoeman *et al.*, 1995). In India, due to powdery mildew, the yield of mango decreases about 20% in some years (Anonymous, 1930). In one estimate, the loss varied from 22.35 to 90.41% (Prakash and Srivastava, 1987b, Prakash and Misra, 1993a, Prakash and Raoof, 1994).

2.1.3 Symptoms

The symptoms can be noticed on the inflorescence, stalk of the inflorescence, leaves and young fruits (Fig.1). Inflorescences are susceptible during the period beginning when the main axis changes colour and ending at fruit set. The characteristic symptom of the disease is the white superficial powdery growth of the fungus on these parts

having millions of conidia borne in chains on conidiophores. Mildew pathogen attacks flowers resulting in its shedding. The sepals are relatively more susceptible than the petals. The main branches of inflorescence are affected in part only on the less susceptible cultivars. The affected flowers fail to open and may fall prematurely.

Young fruits are covered entirely by the mildew (Fig. 2) epidermis of the infected fruit cracks and corky tissues are formed. Purplish brown blotchy areas appear on the skin of older fruits. Recently, the disease has been noticed on young mango fruits in
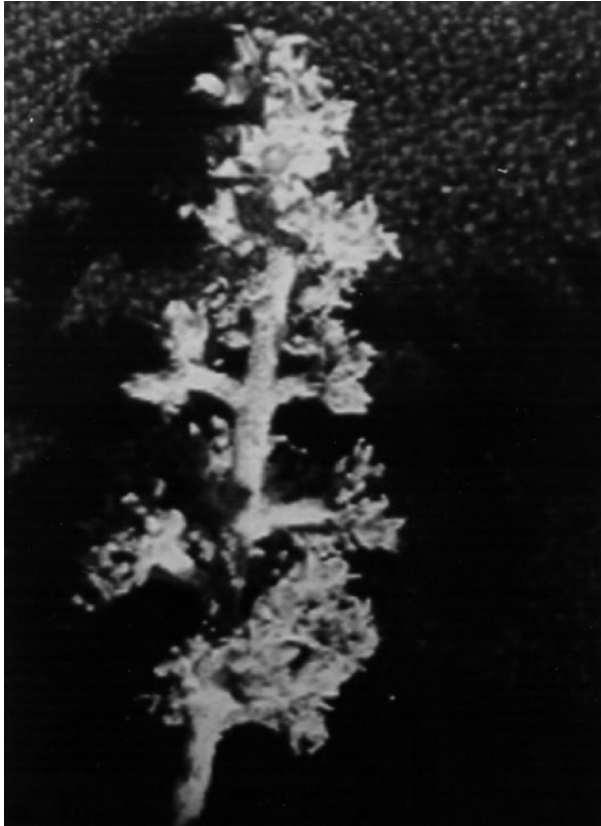
Figure 1: Powdery mildew on the inflorescence

leading varieties viz. Dashehari, Kishan Bhog. Such fruits may remain on the tree until they reach up to pea size and then drop prematurely. In normal infection conditions, 20-40% flowers and fruits are destroyed but during epidemic, it may result in complete failure of the mango crop (Prakash *et al.*, 1996).

Infection is frequently noticed on young leaves also, when their colour changes from brown to light green. Young leaves are attacked on both the sides as small irregular greyish patches, but on the underside the symptoms are generally more conspicuous.

Often, these patches coalesce and occupy larger areas turning into purplish brown in colour. At a later stage, patches become darker in colour. Under favourable environmental conditions, the invaded areas are covered with a luxuriant whitish growth, consisting of mycelial mat and conidia. The pathogen is frequently restricted to the area of the central and lateral veins of infected leaves and such leaves often twist, curl and get distorted. Recently, Misra and Prakash (1995) have observed that while distortion of leaves is more common in plains, in the foot hill areas, it showed ashy brown patches
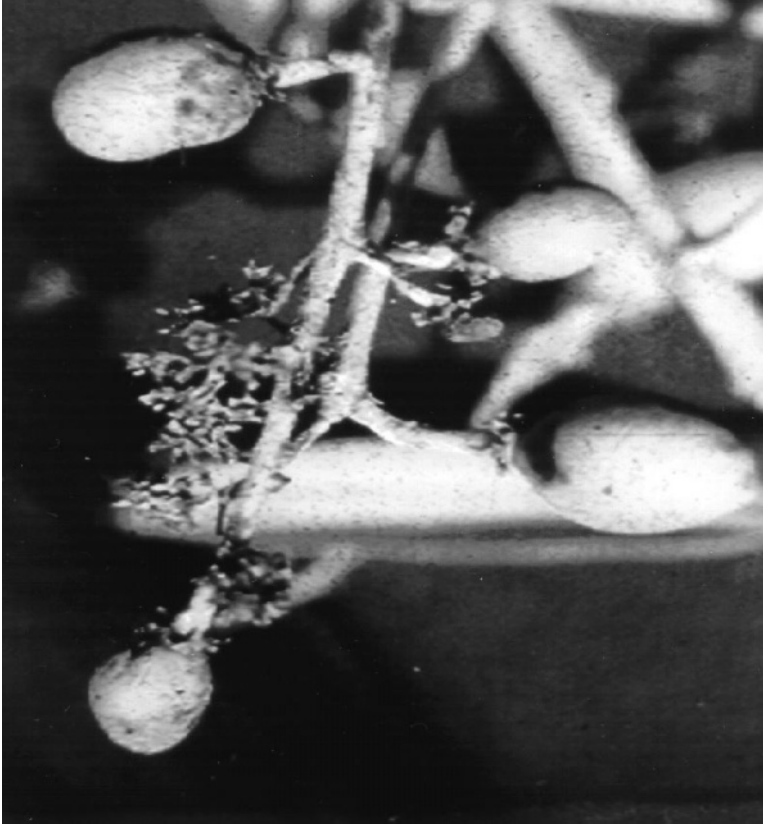
Figure 2: Young fruits are covered by powdery mildew

with white powdery growth on leaf surface.

2.1.4 Causal organism

Powdery mildew of mango was attributed to *Erysiphe cichoracearum* (Wagle, 1928). On the basis of histological studies (globular haustoria) and the type of conidial germination, the pathogen was kept under *E. polygoni* group (Uppal, 1937). It was later

observed that the pathogen produces saccate or lobed haustoria which is not characteristic of *E. cichoracearum* (Uppal *et al.*, 1941). Since the description of the perfect stage of the pathogen has not been given by Uppal (1937), the name of the conidial stage *Oidium mangiferae* Berthet is preferred.

The morphology of *O. mangiferae* has been described in detail (Uppal *et al.*, 1941). The fungus causing mildew produces septate mycelium, which ramifies over the surface of the host, forming a white dense coating of branched hyphae, measuring 4.1-8.2 μm. The hyphae in contact with the host form saccate haustoria on the under surface, which arise as slender tubes and pierce the cuticle and cell walls, these tubes then swell up inside the epidermal cells and form sac like structures. Appressoria also develop on the underside of the hyphae and fix it firmly to the epidermal wall. Sometimes, two appressoria may arise at the same point on the opposite side of a hypha. From the superficial mycelium, numerous branches arise as conidiophores (pseudoidium type) with two to more basal cells straight ranging from 64-163 μm in length and at tips bear unicellular, hyaline, elliptical conidia having truncated ends. The conidia are produced in a basipetal fashion and are sometimes seen singly or in pairs of two. These are also produced in chains of 20-40 on detached leaves kept in close containers but the conidia easily fall off when mature. Conidia measure 25 to 48.9 μm in length and 16 to 23.9 μm in width, mostly 33 to 42.9 x 18 to 21.9 μm and germinate by a germ tube. The length of germ tube vary depending upon relative humidity, and they terminate in hook like appressoria (Ploetz and Prakash, 1997).

2.1.5 Perpetuation of pathogen

The pathogen does not infect hosts other than mango, *Mangifera indica* (Prakash and Srivastava, 1987, Gupta, 1989a). During off season, the pathogen remains present in intact green malformed panicles, mostly hidden under dense foliage. Datar (1985), Munshi *et al.,* (1988) and Gupta (1989b) studied the perpetuation of *O. mangiferae* on leaves and malformed inflorescence. The conidia could not be located after August in malformed bunches. Germination was highest in June, decreased in July and no germination occurred in August. Studies conducted at Lucknow (India) revealed that mildew pathogen persists on infected leaves of the previous year's flush which are retained on the plant in the succeeding year. During flowering (Jan.-March), the conducive environmental conditions activate the dormant mycelium already persisting in necrotic tissue of previous year's infected leaves. Abundant conidia are produced and blown over to the new flushes of growth or young flowers which provide sufficient spore load for initiating the disease. Fresh infection of mildew on young leaves in the first week of December in Rajasthan, (when flowers are not present), further confirms that the fungus perpetuates in asexual form on leaves of mango (Prakash and Raoof, 1985a, Prakash and Raoof, 1994, Prakash *et al.*, 1996). In recent studies, Misra and Prakash (1995) found severe foliar powdery mildew in foot hill areas of Pinjore, Dehradun, Udaipur, Ajmer and Sangareddy during off season which further confirms mildew pathogen, survives during off season. Out break of disease is initiated either from inoculum harboured on the tree or by air borne conidia from other infection sites. Secondary infection with in the tree is mainly due to air borne conidia.

The pathogen colonized on oak (*Quercus robur* L.) leaves in the laboratory, report suggested that the characteristics of this fungus were identical to those of the anamorph of *Microsphaera alphitoides* recorded from oak, and that oak mildew may have been introduced into Europe on mango plants brought to Portugal. No cleistocorps of *Microsphaera alphitoides* f. sp. *mangiferae* were found on heavily mildewed parts of mango tree. Perennating mycelium in the leaves produced conidia ready to infect the susceptible flowers from an affected area easily stick to the hairy unopened flowers near the tip of the inflorescence (Munshi *et al.*, 1988). In India, *Croton sparciflorus* Morog. was found affected with the pathogen in the month of Jan-Feb. The pathogen is similar to mango mildew pathogen (Prakash, 1980).

2.1.6 Disease cycle

Wagle (1928) studied the life cycle of the causal pathogen. The fungus is disseminated through wind. The wind borne conidia cause infection after germination which takes 5-7 hours. The germ tube grows and within two days produces branched mycelium. The mycelium spreads profusely on the epidermal cells which are killed by the feeding of the fungus and become brown. On the fourth day, several vertical bodies begin to appear from the mycelium. These conidiophores give rise to conidia in five days. Thus, the life cycle from conidia to conidia is completed in about five days. The attack generally begins from the buds at the tip of the inflorescence, as these being more hairy very easily catch the spores. Then it gradually extends on the flower-head, only few infected panicles are sufficient to cause a wide spread epidemic under favourable weather conditions. Mildew also appears naturally on the vegetative shoots of the mango but the life cycle is somewhat longer being about nine days. Conidia retain their vitality for 4-5 days only. If kept in the sun without any moisture, conidia shrivel within 4-5 hours. When conidia germinate at lower humidity, they often lose their ellipsoid shape and become rod shaped. Such conidia are always shorter. The low percentage of germination, obtained at different temperatures, is due to the growth of saprophytic organisms in water cultures.

2.1.7 Epidemiology

The disease is destructive in the plains of northern India and coastal areas during cold and wet season. The fungus is favoured by cloudy weather and heavy morning mist (Kulkarni, 1924, Prakash & Srivastava, 1987). The disease is most severe during cool, dry weather. Spores are wind disseminated and are released on a diurnal basis (Schoeman *et al.*, 1995). Peak spore release, between 1100-1600 h, was positively correlated with hourly temperature and negatively correlated with hourly humidity, vapour pressure deficit and leaf wetness. Mildew pathogen persists for a longer period in UP hills. Minimum, optimum and maximum temperatures for germination are 9°, 22° and 30-32°C, respectively (Uppal *et al.,* 1941). Conidia germinate best at temperature ranging from 9-32°C (23°C is optimal) and at relative humidities as low as 20% .

Gupta (1979, 1989a,b) found that the atmospheric temperature is important for the appearance and development of the disease. Minimum temperature (10-13°C) and

maximum (27-31°C) and RH (82-91%) were most suitable for the development of the disease. He further emphasized that maximum infection occurs at 26°C and 100% RH and rainfall does not play any significant role on disease development but dry weather favours the development of the disease. Conidia attached to conidiophores on mildewed leaves and inflorescences retain their viability upto 40 days as compared to detached conidia which lasted 21-35 days on glass slides and host leaf surfaces. Misra and Prakash (1988) stated that predominance of susceptible cultivar 'Dashehari', high wind velocity for 3-4 days with maximum temperature around 30°C, minimum temperature around 15°C, relative humidity of minimum 23.4-25.5% and maximum 73.3-83.9% are conducive for the rapid spread of mildew pathogen.

Optimal disease development occurs in the diurnal range of 10-31°C and 60-90% relative humidity. Conidia germinate in the absence of water with in 5-7 hr at 23°C and 20% RH . Since conidial germination occurs at a wide range of relative humidities, the development of mildew is usually independent of this weather parameters. Lonsdale and Kotze (1991) investigated the critical infection period for powdery mildew disease of mango. A sharp increase in infection occurred during the period July-Aug. for cv. Zill and between 3rd Aug.-24th Aug. for cv. Keitt. During this period, flowers had begun to open on the panicles. Prior to flowers opening and during fruit set, little infection occurred. Conidia exhibited a diurnal pattern of dispersal and liberated from 12 to 16 hr. Dispersal took 3-4 days to reach pre-rain levels when dry weather followed by rainy period however, rains reduced the dispersal of pathogen (Gupta, 1988).

Joubert (1991) monitered mildew by calculating the area of inflorescence (70) in an unsprayed orchard. The first symptom was seen at the beginning of Aug. approximately one week after the first spores were released and 50% of inflorescences were in full bloom. A peak in disease incidence appeared in the first week of Sept., when spore release was at a maximum full bloom and fruit set to pea size were the most susceptible.

2.1.8 Biochemical changes

There is a continuous decrease in the percentage of moisture and acidity in host tissue, while pH increases with the rise of powdery mildew infection. In infected panicles, the amount of protein is high, whereas those of reducing sugars is low. The contents of total sugars and tannins decrease with the severity of the disease but due to excessive moisture loss in the panicles of 3rd stage, these (total sugars and tannins) register a slight increase over healthy panicles (Prakash *et al.*, 1989).

2.1.9 Management

2.1.9.1 Cultural

Prakash (1998) observed that the first sign of powdery mildew symptom is seen after emergence of panicle at eastern side of the tree top, 2-3 weeks after 25-30% inflorescences are fully expanded and flower starts opening. On this basis, recommendations of fungicides are to be made and repeated after 3 weeks until panicle susceptibility decreases at the end of fruit set. Regular inspection of orchards, removal/pruning of

diseased leaves malformed panicles reduce the load of primary inoculum and improve the control achieved by spraying of mildewcides (Prakash and Misra, 1992, 1993a,b, Prakash and Raoof, 1994). Raisinghani (1945) from Sind reported that the loss could be reduced by 50-75% if branches are shaken after each shower.

2.1.9.2 Chemical

Periodic spraying/dusting of mango trees with wettable Sulphur/Sulphur dust (Prakash and Raoof, 1994), Dinocap (Ruehle, 1956), Benomyl (Mc Millan, 1973) and Mancozeb (Persley *et al.*, 1989) have been found very effective. In Zimbabwe (Rhodesia), four sprays (when flower clusters have expanded, just before the clusters open) have been recommended (Hopkins, 1941). In India, the disease has been controlled by spraying of wettable Sulphur, Dinocap, Carbendazim, Benomyl, Tridemorph, Tridemephon, Bitertanol, Oxythioquinone, Thiophanate Methyl, Vigil etc. (Datar, 1981, 1986, Gupta and Dang, 1981, Lingaraj, 1969, Prakash and Singh, 1982, Rawal and Ullasa, 1989, Prakash, 1979, Prakash and Raoof, 1994, Joshi and Chauhan, 1985). Spray schedule was tested at 3 stages (emergence of panicle, opening of flowers, and fruit set), and Bavistin (0.1%) at 20 days interval was found quite effective in reducing the disease (Prakash and Raoof, 1982, 1985, 1994).

The phytotoxic problem with Sulphur during hot conditions is reported in the dry tropics (Peterson *et al.*, 1991). Probably this may be due to high dose (4.5 g/l) applied over the mango tree. However, wettable sulphur fungicides were evaluated for their repeated sprays on the control of powdery mildew of mango. All the wettable sulphur fungicides tested, significantly controlled the disease without any phytotoxic effect, and reduces the cost of fungicides application (Prakash and Misra, 1986). Modern mildewcides that control powdery mildew, are very effective provided that spraying commences upon the appearance at the first sign of disease and that coverage is thorough. The flowering stage appears to be the most critical for infection, however, little infection occurs before flower opening or during fruit set. Therefore, spraying is utmost important only during flowering and fruit set. Few newer fungicides are viz. Punch C (Flusilazole + Carbendazim) (Brooks, 1991 and Lonsdale and Kotze, 1991) Topas (Periconazole), Afugon (Pyrazophose) (Haq *et al.*, 1994). In some regions, both mildew and hopper occur together. In such case a combined treatment of fungicide and insecticide have also been recommended (Pal and Prakash, 1984).

2.1.9.3 Resistant varieties

Differences in cultivars susceptible to mildew are widely recognized by various workers (Gupta, 1976, Datar, 1983, Prakash and Misra 1986, Prakash and Raoof, 1994, Johnson, 1994), but none of the cvs. has been found immune or potentially suitable for incorporating in the breeding programme. However, out of 90 mango varieties tested, only Neelum, Zardalu, Banagalora, Totapari Khurd and Janardan Pasand were found resistant (Gupta, 1976).

Datar (1983) reported cv. Totapari with some degree of resistance. Mango cvs. Ghanya, Jahangir, Yellamondela Thiamandi, Bablipunasa, Kharbuja and K.O. 7 have

also been reported resistant (Prakash and Raoof, 1994). Zill, Kent, Alphonso and Nom Doc Mai are very susceptible, Haden, Glenn Carrie and Keitt are moderately susceptible, and Sensation, Tommy Atkins and Kensington are slightly susceptible (Johnson, 1994). No differences in resistance were observed among rootstock tested. Grafts were more important for manifestation of resistance. Cultivars viz. Extrema Pahiri and Bourbon were the most susceptible while Oliveira, Imperial and Carlota were more resistant (Simao and Gomes, 1995).

2.2 Anthracnose

Anthracnose on mango was first reported from Puerto Rico in 1903 and subsequently from USA, Cuba, Philippines, British Guiana, Dominican Republic, Mauritius, Fiji, Sierra Leone, Brazil, Columbia, Guatemala, Mozambique, Dutch East Indies, Portugal, Pakistan, Trinidad, Peru, French, Guiana, Taiwan, Uganda, Jamaica, Sri Lanka, Congo, Morocco, South Africa, Malaysia, Australia, Bangladesh, Thailand, Costa Rica and Barbados (Prakash *et al.*, 1996).

In India, the disease was reported by various workers (Prakash and Misra, 1988). Losses due to anthracnose have been estimated to be 2-39% (Prakash *et al.*, 1996). Young plantation of cv. Bombay Green was completely wiped out in Terai region of Uttar Pradesh due to severe wither tip.

2.2.1 Symptoms

Characteristic symptoms appear as oval or irregular vinaceous brown to deep brown spots of various sizes scattered all over the leaf surface. Under damp conditions, the fungus grows rapidly forming elongated brown necrotic areas measuring 20-25 mm in diameter. Later on the lesions get blighted and rupture. Infected leaves often show 'shot hole'. Young leaves are more prone to attack than the older ones. Leaves and young panicles infected with gall midge (insect), stimulate the activity of the fungus resulting into heavy incidence of the disease (Prakash and Srivastava, 1987). Petioles, when affected, turn grey or black (Fig.3). The leaves droop down, slowly drying up and ultimately fall-off, leaving a black scar on the twig. Disease produces elongated black necrotic areas on the twigs. The tip of very young branches start drying from tip downwards. Gummosis is usually the after effect of the disease (Prakash *et al.*, 1996).

The blossom as well as peduncle blight is the most destructive phase of this disease, as it affects fruit set and ultimately the yield. On the inflorescence, the earliest symptoms of the disease are the production of blackish brown specks on the peduncle and flowers. Small black spots appear on the open flowers and panicles, which gradually enlarge and coalesce to cause death of flowers. The infected flowers fall-off, having the more persistent spikes on the peduncles. The severity of the disease may vary according to prevailing weather conditions.

On PDA (Potato-Dextrose-Agar) medium, the fungus (*C. gloeosporioides*) grew well with greyish white to dark grey and may produce aerial mycelium ranging from a thick mat to sparse tufts (Holiday, 1980). Conidia are hyaline, unicellular and either cylindrical with obtuse ends or ellipsoidal with a rounded apex and a narrow truncate

base. They are 7-20 x 2.5-5.0 μm and are formed on hyaline to faintly brown conidio-phores in acervuli that are irregular in shape and about 500 μm in diameter. Setae are 4-8 x 200 μm 1-4 septate, brown and slightly swollen at the base and tapered at the apex.

Fruits may be attacked at any stage of their development. Young infected fruits (1-2 week old) often fall from the tree in large numbers. On older fruits, black spots are produced. Initially the spots are round but after coalescence, they form large ir-regular botches or even cover the entire fruit (Fig.4). The spots have large extensive rotting. Under deep cracks and the fungus penetrates deep into the fruit causing moist conditions, the blackened areas become covered with minute pinkish reproductive bodies of the fungus. Staining, russetting and tear streaking, involving only the skin of the fruit, are attributed to the same fungus.
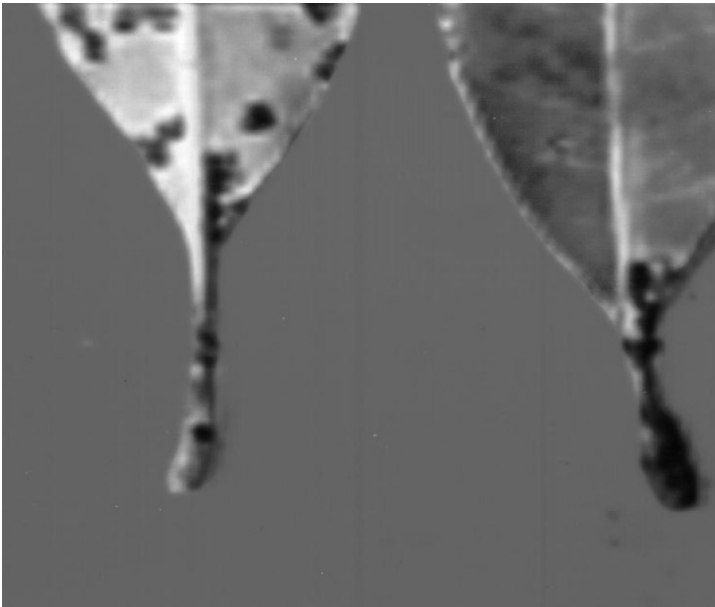
Figure 3: Anthracnose symptoms on leaf lamina and petiole

2.2.2. Causal organism

The disease is caused by *Coll*et *otrichum gloeosporioides* Penz. [*Glomerella cingulata* (Stons.) Spauld & Schrenk]. Seven species of *Coll*et *otrichum* are known to cause fruit rot in Queensland. On the evidence available, it was found desirable to designate a new form of *C. gloeosporioides* as *Glomerella cingulata* var. *minor*. The conidial state of *C. gloeosporioides* var. *minor* causes anthracnose on mango, papaya, avocado, apple and other hosts in Queensland (Simmonds (1965). Fitzell (1979) and Prakash (1996) reported *Coll*et *otrichum acutatum* from New South Wales and India, respectively. It was isolated from leaves, panicles and fruits. Twig blight phase, re-

ported by Hayes (1953) from Uttar Pradesh, is caused by *Gloeosporium mangiferae* P. Henn but it is identical to *C. gloeosporioides* as observed by the author. Variation among the fourteen Indian isolates of *C. gloeosporioides* of mango were recorded on the basis of their growth pattern on different media and pH levels. Isolates also showed differential reaction on four mango cultivars. Isolates also behaved differential reaction to fungicides and were found host specific).

Simmonds (1941) discussed the histological structures involved in latent infection. A fine infection thread penetrates the cuticle directly from the appressorium and forms a hyphal structure adjacent to the cellulose wall of the epidermal cell. This subcuticular hypha is considered to be the form in which the fungus survives its period of latency. The appressorium is considered important as it serves as an anchorage struc-
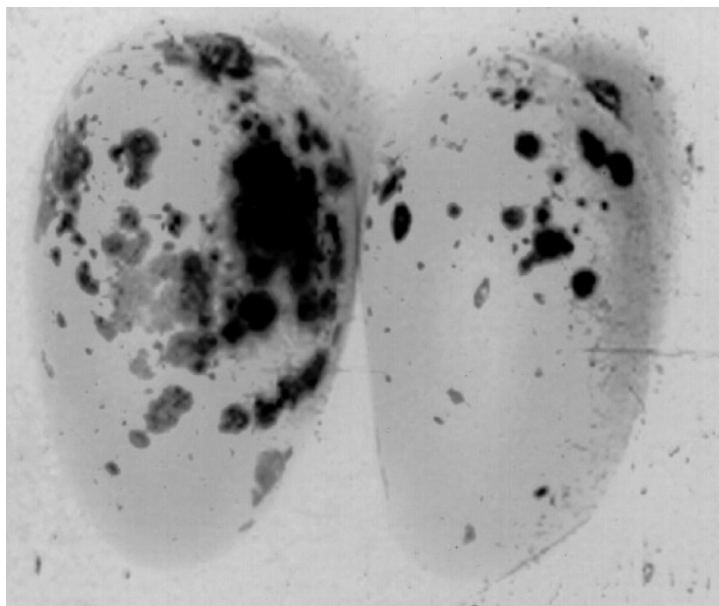
Figure 4: Anthracnose spots on fruits

ture and from which the infection thread may be produced.

2.2.3 Disease cycle

The pathogen survives on the fallen leaves, blighted peduncle, dead stem and diseased twigs attached to the trees. The pathogen produces spores under favourable conditions and these serve as foci of infection for the succeeding bloom. However, under tropical conditions, fresh supplies of spores are being continuously made throughout the year. Studies on the viability of fungus revealed that the 70% spores of the fungus, produced in acervuli on the twigs, were viable. On diseased leaves, the fungus remained viable

for 14 months (Prakash *et al.*, 1996). Spores of *C. gloeosporioides* were found attached to the body of the vector, *H*et *erospilus prosopidis* (Hymenoptera : Branconidae) and dry spores had greater viability (Nemeye *et al.*, 1990).

2.2.4 Epidemiology

The optimum temperature for infection of anthracnose is around 25°C. The injury caused by the anthracnose pathogen is dependent on humidity, rain, misty conditions or heavy dews at the time of blossoming. Continuous wet weather during flowering causes serious blossom blight. Relative humidity above 95% for 12 hrs is essential for infection and development of *C. gloeosporioides* on mango fruit. Infection progresses faster in wounded tissues, and in ripe fruits (Prakash *et al.*, 1996).

2.2.5 Management

2.2.5.1 Cultural

Diseased leaves, twigs and fruits, lying on the floor of the orchard, should be collected and all infected twigs from the tree should be pruned and burnt. Plant vigour plays an important role in keeping the plants free from twig infection. Therefore, proper irrigation and fertilizer application are essential to maintain the tree vigour.

2.2.5.2 Chemical

i. Preharvest : The disease can be controlled effectively by seven sprays of Captan (0.3%) at flowering (Aragaki and Ishii, 1960), Zineb (0.2%) or Bordeaux mixture (4:4:50) twice at flowering and than at 15 day intervals until harvest (Tandon and Singh, 1969), or Bavistin (0.1%) at 15 day intervals (Prakash and Misra, 1988). Micop, Fycol, Blitox, Dithane or Blizene (Lingaraj, 1969), Captan, Blitox and Difolatan (Gadre, 1979) and Copper oxychloride were also found effective. Spraying of Copper oxychloride (0.2%) + Zineb (0.2%) after completion of heavy showers followed by wettable Sulphur (0.2%) before flowering. Carbendazim (0.3%) at pea stage and Zineb (0.2%) before stone hardening reduce the incidence of the anthracnose (Jadeja and Vaishnav, 1984).

ii. Postharvest : The major strategies in controlling anthracnose are regularly scheduled sprays in the field to reduce the latent infection and treatment of the fruit with hot water/fungicides/forced air after harvest to eradicate the left over latent infection. Preharvest sprays of Copper oxychloride, TBZ, Agrimycin, Aureofungin (Raoof and Prakash, 1986), Thiophanate methyl or Carbendazim and subsequent dip (Prakash,1996, Prakash and Misra, 1993b), and spraying of Benomyl combined with antitranspirant (Barmore *et al.*, 1973) and Bavistin + Nufilm have been found effective in controlling the disease. Hot water treatment at 51-51.5°C for 15 min (Pennock and Maldonaldo, 1962), 54.5-55.5°C for 5 min, 50-55°C for 15 min (Hatton and Reeder, 1964, Hunter, 1969), and 46°C for 90-115 min (Tandon and Singh, 1968), 52° for 50 min, 52° for 15 min with 0.17 Carbendazim give good control of anthracnose. Fruit dip at 55°C for 5 min having

Benomyl + TBZ has also been found effective. Heated Benomyl at 55°C for 5 min (Jacob *et al.*, 1973) or hot Benomyl at 52-55°C for 10 min (Dodd *et al.*, 1991) has been found effective. Instantaneous dip in Benomyl (0.05% a.i.) or TBZ (0.09% a.i.) (Sohi *et al.*, 1973) and 30 sec dip in Flusilazole (0.01%) have also been found effective (Pelser and Lesar, 1989). Treatment by forced air at 48°C for 150 min (97) and 51.5^{o}C for freshly harvested and stored fruit reduce the disease. Prochloraz dip (0.025%) for 30 sec and storage at 6% O_2 have been found most effective (Sangchote, 1989).

2.2.5.3 Resistant varieties

Cultivars Edward, Mayaguazano and Elamandi are reported resistant against anthracnose (Pennock and Maldonaldo, 1962, Sohi *et al.*, 1973).

2.2.5.4 Integrated disease management

The management strategies recommended to control anthracnose include cultural practices and tree management, variety selection and protective fungicide spraying, using curative fungicide, Prochloraz and Mango Anthracnose Estimator (MAE). Use of this method saves 25% spray during dry seasons (Fitzell and Peak, 1985).

2.3 Dieback

Lasiodiplodia theobromae (Pat.) Griffon & Moube) is an aggressive and vigorous pathogen and is able to infect exceptionally large number of host plants, causing various types of disease symptoms viz. black root rot, fruit rot, dry rot, wood stain, dieback, stem end rot, seedling rot, graft union blight, twig blight and tip dieback, brown rot of panicle (Ragab *et al.*, 1971, Prakash and Singh, 1976b, Prakash and Raoof, 1979, 1985, Prakash and Srivastava, 1987, Reckhaus, 1987, Prakash, 1996, Ramos *et al.*, 1991, Prakash and Eckert, 1992, 1998, Ploetz and Prakash, 1997, Das Gupta and Zachariah, 1945, Voorhess, 1942, Prakash and Misra, 1992, 1993ab, Rath *et al.*, 1978, Spencer and Kennard, 1955). About 30-40% mango plantation has been found affected with the disease creating a serious problem in the Moradabad region of Uttar Pradesh, India (Prakash & Singh, 1976, Prakash and Srivastava, 1987, Prakash, 1996, Ploetz and Prakash, 1997).

2.3.1 Distribution

The disease is known to be present in India and other mango growing countries of the world viz. Barbados (Bourne, 1921), Puerto Rico (Alvarez Garcia, 1968, Alvarez Garcia and Garcia, 1971), Egypt (Ragab *et al.*, 1971), Dutch East Indies (Indonesia) (Mullar, 1940), El-salvador (Acuna and Waite, 1977), Brazil (Batista, 1947, Ribeiro, 1980), Niger (Reckhaus, 1987), USA (Florida, California, Hawaii) (Ramos *et al.*, 1991, Voorhess, 1942, Prakash and Eckert, 1992, Stevens and Shear, 1929), South Africa (Marloth, 1947). The disease is steadily gaining ground each year, spreading into new orchards and damaging by way of declining many such mango grooves mainly in the tropical and sub-tropical world (Prakash and Raoof, 1979). The disease is prevalent not only in Uttar

Pradesh, Rajasthan but also in other mango growing states of India.

2.3.2 Symptoms

The effect of the disease on the general appearance of the tree is noticeable at any time of the year but it is most conspicuous during October and November (Prakash and Singh, 1976b). The disease is characterised by dying back of twigs from top downwards particularly of the older trees followed by complete defoliation which gives an appearance of fire scorch. Discolouration and darkening of the bark at a certain distance from the tip is the external evidence of the disease. Such dark patches are usually seen on young green twigs and are hardly distinguishable in older branches. The bark is discoloured at several places, when the dark lesions increase in size, dying of young twigs begin, starting at the base affecting the leaf midribs and extending along the veins towards leaf edges. The upper leaves lose their green colour and gradually turn brown. This browning of the whole leaf is accompanied by the upward rolling of the margin. Such leaves shrivel, fall off in a month leaving the shriveled twigs altogether bare, which is the characteristic symptom of the advanced stage of the disease. Internal browning in the wood tissue is observed on slitting open along the long axis. Cracks appear on branches and exudes before they die out. When the graft union of young plant is affected it usually dies. It has also been noticed that the infection occurs at node of variable distance below growing point and the part of the twig on both the sides of infection dies.

2.3.3 Causal organism

In India, die back of mango is caused by *Lasiodiplodia theobromae* (Das Gupta and Zachariah, 1945, Prakash and Raoof, 1979, Rath *et al.*, 1978). In USA (California), twig blight of mango was associated with *Botryosphaeria ribis* (Prakash and Eckert, 1992). In Florida, tip dieback has been recently reported by Ramos *et al.,* (1991) and they also isolated *Botryosphaeria ribis*. Other causal agents of mango dieback, such as *Physalospora rhodina* (Alvarez Garcia and Garcia, 1971), *Ceratocystis fimbriata* (Ribeiro, 1980), *Microphoma* (Fernando *et al.*, 1960), *Hendersonula toruloidea* have been found to be associated with some type of dieback. Similar malady named as Recife sickness caused by *Diplodia recifensis* was reported to be responsible for serious damage (Batista, 1947).

Thus, the causal organism causing dieback disease in India was identified as *Lasiodiplodia theobromae* Pat. (Prakash and Raoof, 1979), whereas, in USA, *Botryosphaeria ribis* has been isolated frequently from the mango twigs (Prakash and Eckert, 1992, Ramos *et al.*, 1991, Stevens and Shear, 1929, Ploetz and Prakash, 1997).

Lasiodiplodia theobromae was isolated frequently from twigs, graft union and branches of mango plants showing symptom of the disease. On OMA (Oat Meal Agar) medium, all isolates were strongly mycelial, regular, greenish white, lumpy with formation of pseudosclerotial bodies, saltation present. Hyphae were septate, hyaline at first, granular, later guttulate, turning dark grey to black. Pycnidia occur singly or in groups on dark stomata, erumpent, short beaked, globose or sub-globose, measuring 140-410

μm in diameter, leathery or carbonaceous. Pycnidiospores liberated through an apical ostiole, hyaline, oval, one celled, thin walled and granular, measuring 22-45 x 10-15 μm. When mature, they are two celled, divided equally, dark brown to black, thick walled, striated and unconstricted at the septum.

Bhatnagar and Singh (1979) studies the effect of auxins namely 2, 4-D, IAA, colchicine and MH, on the growth of the fungus which requires them for good growth, the 2, 4-D at 10 ppm resulted in maximum growth. According to Srivastava (1969), sugar contents decreased greatly while many amino acids decreased, some occasionally increased, due to the proteolysis of host proteins. Srivastava and Tandon (1970) reported that the mango isolate grows better on a mixture of amino acids. They also studied the growth, sporulation and spore germination of the fungus.

2.3.4 Survival and spread

The organism is a wound parasite and capable of causing great damage under favourable conditions. The pathogen penetrates the host through epidermal wounds and lenticels. The organism invades and destroys the epidermal and sub-epidermal cells, then enters the vascular tissues, enabling the mycelium to spread throughout the branch. Entrance of mycelium into the cambium and phloem vessels is thought to occur primarily through stem wounds. Movement through the pith tissues has also been observed. Artificial inoculation experiments have shown that establishment of the fungus requires at least 48 h at the temperature of 27-32°C and RH of 80-85%. The fungus remains in the vascular tissues until tissues die. Diseased twigs bearing fruiting bodies are the main source of perpetuation and survival of the pathogen and thus serve as initial inoculum for next season. Use of infected bud stick is largely responsible for the carry over of the pathogen from one growing season to next and for dissemination to different new areas. Within orchards, the most important means of spread are inoculum already present and contaminated garden tools. The former accounts for the increase in decrease severity and the latter contributes to the survival and spread of the pathogen within an area and from season to season. Trees damaged by gummosis, insects, drought and lack of nutrition favour the disease development. High summer temperature predispose the mango plants to the attack of the pathogen through reducing the vitality of the plant (Das Gupta and Zachariah, 1945). Disease development is favoured by rains, relative humidity (approx. 80%) and maximum and minimum temperatures of 31.5 and 25.9°C. The growth of germ tube of single celled spores was best at 30°C. On exposure to higher temperature (54°C) for 10 minutes, loose spores lost their viability.

2.3.5 Management

Pruning of dead wood and pasting of cut ends and healthy bud wood are the only alternatives to combat the disease in the immediate future. It should be ascertained that the scion wood selected for propagation is free of infection, while multiplying the planting materials for distribution. Every care should be taken to prevent introduction of the disease in the newly planted orchards. The orchard should be inspected regularly for any fresh infection to ensure free of the disease by judicious pruning followed

by spraying/pasting of Bordeaux mixture or Copper oxychloride (Prakash and Raoof, 1979). Cultivar Mohan Bhog appears to be susceptible while Siroli is resistant (Verma and Singh, 1973).

2.3.5.1Chemical

Various fungicides along with different cultural practices like pruning, pruning + spraying, soil and foliar application of fertilizer and fungicides were tested for three consecutive years. It was found that pruning (3" below the infection site) followed by spraying of Bordeaux mixture (5:5:50) was the most effective treatments in controlling the disease (Prakash and Raoof, 1989). Of all the management practices tested, fungicide treatment had the greatest impact on reducing the incidence of disease. Sprays of Bordeaux mixture and pruning reduced external twig infection and to some extent internal discolouration. Until more effective control measures are found, Copper fungicides will continue to play a key role in the management of dieback disease.

2.4 Sooty Mould or Black Mildew

Black mildew is also known as sooty mould or sooty blotch (Plotze and Prakash, 1997). It is very common wherever honey dew or sugary substances secreting insects viz. mango hopper, scales, coccids and mealy bugs are found. Various Ascomycetous fungi produce dark coloured superficial growth on the surface of leaves, stems and fruits of mango. The fungus in the true sense is non-pathogenic, because it does not enter the host tissue and absorb nutrients. It draws the substances not from the host directly but from the sweet substances known as 'honey dew'. The damage by the fungus is not direct but indirect as it interferes with the normal functioning of plant by cutting off the effective leaf area requires for photosynthesis.

2.4.1Symptoms

The disease is characterized by the presence of a black velvety thin membraneous covering on the leaves, stems and fruit. These range from thin, diffuse webs of dark hyphae to opaque felty layers, in severe cases, the tree appear black due to heavy infection of mould on entire surface of leaves and twigs (Fig.5). The affected leaves curl and shrivel under dry conditions. Because of the production of masses of black spores, which stick to the leaf surface due to sticky 'honey dew', the foliage appears black, ugly and hence the name 'sooty mould'. The severity of incidence is dependent upon the sugary secretion by insects. During flowering time if the fungus infects the blossoms, fruit setting is affected and some times even small fruits are fall. Mature fruits having black patches are also detract considerably from the appearance and marketability. Various types of symptoms are produced from different species of fungi. The different species may occur singly or in combination on affected parts. The sooty moulds and black mildew were earlier considered to be the same disease, but their causes are actually distinct (Lim and Khoo, 1985). *Meliola mangiferae* is an obligate parasite, which penetrates in host surfaces. In contrast, the fungi, which cause sooty mould are

saprophytes where growth is restricted to plant surfaces that caused sooty moulds in Malaysia.

2.4.2 Losses

The sooty mould created a history during the year 1984 as the disease was appeared in an epidemic form and gradually engulfed the entire mango belt in Siyana block of district, Bulandshehar, Uttar Pradesh, India. The disease was so much serious in certain pockets, where thick branches of tree died resulting in serious casualties of older plants (Prakash and Srivastava, 1987).

2.4.3 Causal organism
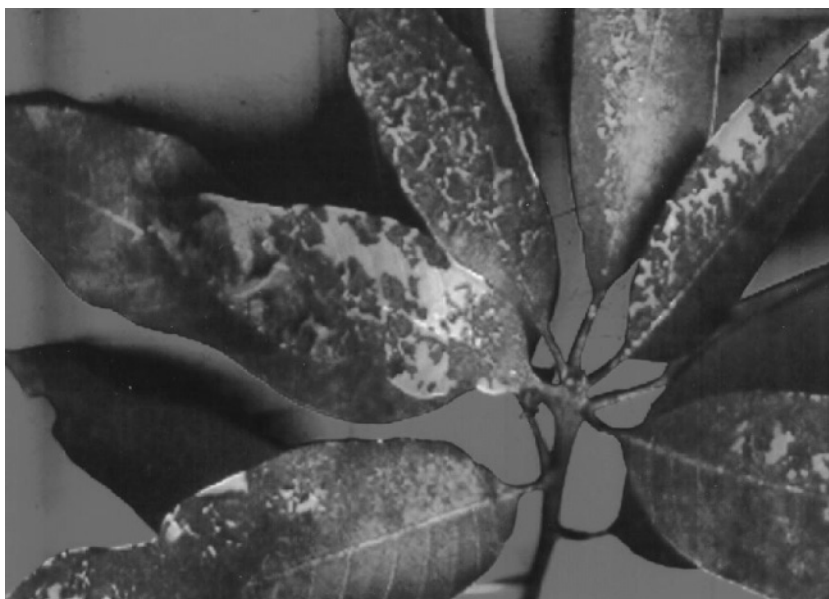
Figure 5: Sooty mould growth on mango covering entire leaves

Sooty mould is caused by the fungus *Meliola mangiferae* Earle (Butler and Bisby, 1931), *Capnodium mangiferae* Cke. and Borwn (Vaheeduddin, 1953), *Capnodium ramosum* Cke. (Butler and Bisby, 1931), *Tripospermum limacinula, Trichopelteca, Cha*et *othyrium, Antennulariella, Capnodendron, Scorias, Scolecoxyphium* and *Tripospermum acerinum* Syd. Speg. (Das and Mohanty, 1972). Since these fungi may not sporulated on the plant and they are often found in combination with one another. It is difficult to identify the specific species involved on a given leaf. In India (Uttar Pradesh), the disease is caused by *Microxyphium columnatum, Leptoxyphium fumago* and *Tripospermum myrti*. The mycelium of the fungus is superficial and does not penetrate the host tissue.

2.4.4 Varietal susceptibility and Biochemcial changes

The screening test was conducted on 29 cultivars of mango cultivar Alphonso was found to be resistant (Peethambharan and Aravindokshan, 1975). Kulkarni and Kulkarni (1978) studied the physiology of sooty mould (*Capnodium mangiferae*) infected leaves and found that there was an increase in the amount of iron and potassium and a decrease in sodium, manganese and calcium as compared with those of healthy leaves. Singh and Singh (1972) reported that the infection on fruits reduced the total soluble solids (TSS) and induces early deterioration and rotting.

2.4.5 Epidemiology

Disease is severe in old and dense orchards where penetration of light intensity is low. Trees exposed to eastern side (sunlight) have less incidence while trees in centre of the orchard, especially those growing dense have 95% incidence. Sugary substance secreted by the insects is stated to be a condition favourable for development of sooty mould. Continuous and heavy rainfall results in continuous washing off such substances. Incidence of insects on the shoot is directly associated with disease severity. High humidity, however, proved to be congenial for growth of the fungus (Singh and Singh, 1972, Misra, and Prakash, 1993).

2.4.6 Management

The remedy for this disease consists in removing the cause by destroying the insects. The mould will die out for want of a suitable growth medium if honey dew secreting insects are killed by suitable insecticides. An application of pesticides should cover both the surfaces of leaves. Spraying of Elosal (900 g/450 lt.) at 10-15 days intervals proved to be quite effective (Singh and Singh, 1972). Spraying of Wettasul (wettable sulphur) + Metacid (Methyl parathion) + Gum Accacia (0.2 + 0.1 + 0.3%) and Indian oil formulation No. 1 & 2 at 15 days interval could control sooty mould (Misra and Prakash, 1993).

2.5 Scab

Scab is found in all mango producing countries on young mango seedlings. The disease is first observed in Cuba and Florida in the 1940s. The leaf specimens collected in Florida in 1937 indicate that the disease may have been in th state earlier. It is now widespread throughout the Western tropics. The disease can cause significant damage on young seedlings in nurseries but it is not a problem in commercial orchards (Bitancourt and Jenkins, 1943, Prakash and Srivastava, 1987, Ploetz and Prakash, 1997)**.**

2.5.1 Symptoms

The scab fungus (*Elsinoe mangiferae* Bitancourt and Jenkins) attacks leaves, twigs, panicles, blossoms and blotches on the bark of stems and spots on the mango fruit

(Fig.6). Young succulent leaves are most susceptible, and generally increase in resistance as they mature. Rainy weather promotes sporulation and infection of the fungus on the host tissues. On these, lesions are formed which differ in size and colour depending upon the age of the plant and athe country in which the disease occurs. These spots in Cuban nursery, are pale to brown and covered with a delicate buff down, which represent the vegetative stage of the fungus but on older foliage, the lesios are bigger in size and are grey in appearance surrounded by dark margin and bear on their surface small dot like structures, which are the ascomata of the *Elsinoe*. In Brazil, spots are chiefly epiphyllous, circular to elongate or irregular, and grey at the centre, with a dark periphery. They are mainly centred on the midrib or are disposed in close proximity to it.

Scab spots in Indian nursery, are present at lower surface, circular, slightly angular, elongate, 2-4 mm in diameter, brown but during rainy season, lesions differ in

Figure 6: Scab spots on mango fruits

size, shape and colour. Symptoms produced by the disease are very much like anthracnose and may be confusing. On leaves, the spots are smaller than anthracnose infection and the surface is covered with a delicate velvety down. Severe attacks cause crinkling and distortion of the leaf, followed by premature shedding. Larger spots on young leaves are greyish in colour surrounded by narrow dark borders and frequently the centres wither away leaving irregular shot holes. The blotches on the stem bark are greyish and irregular in shape. On young fruits, the infection is grey to greyish brown with dark irregular margins. As the fruit attains in size, and spots also enlarge, and the centre may become covered with cracked and fissured, corky tissues. Conidia of the fungus are peoduced on the fruit until it reaches maturity.

2.5.2 Causal organism

Scab is caused by the fungus, *Elsinoe mangiferae* Bitan and Jen. [anamorph : *Sphaceloma mangiferae* (Bitancourt and Jenkins). The ascomata are extra epidermal, pulvinate with a pseudo-parenchymatous, hyaline matrix. Brownish ascocarps (30-48 x 80-160 μm) are produced in the host epidermis. Globular asci, 10-15 μm in width, are dispersed irregularly in ascocarps and contain 1-8 hyaline ascospores, measuring 4-6 x 10-13 μm long, three septate and constricted at the middle septum. The sub apical cell is longitudinally septate. Conidiophores of *S. mangiferae* are erect, sinuous, 2.5-3.5 x 12.35 μm, and wider as the base. Conidia are brown in colour, with or without septa, measuring 2-4 x 6.29 μm (Ploetz and Prakash, 1997).

2.5.3 Disease cycle

Succulent, young leaves are most susceptible, and generally decrease in susceptibility as they mature. No specific information is available on epedemiology of mango scab. The roles played by ascospores and conidia in the infection are also not known but it can be assumed on the basis of disease development by other species of *Elsinoe*, that high humidity and free moisture are needed for the formation of spores and initiation of infection by *E. mangiferae* (Ploetz and Prakash, 1997).

2.5.4 Management

The disease is serious in mango nursery but it is of a minor importance in commercial grooves. Spray application of fungicides for the control of anthracnose have generally been considered effective for scab control. Frequent spray of copper fungicides to protect new flushes of growth are effective for scab control in nurseries (Prakash and Misra, 1988). The disease is also noticed on fruits and needs protection measures for the control (Prakash, 1997, 2000).

2.6 Black Banded

The disease is recorded by Massee from Poona (Saccardo, 1906). Later on, it has been reported from Goa, West Bengal, Karnataka, Maharashtra, Bihar, Orissa, Andhra Pradesh, Kerala, Andaman & Nicobar Islands and Tamil Nadu in mild to severe form (Prakash and Srivastava, 1987, Ploetz and Prakash, 1997). The disease is caused by *Rhinocladium corticolum* Massee imperfect stage of *Peziotrichum corticolum* (Massee) Subramaniam.

2.6.1 Symptoms

The disease is noticed on the midribs and veins of leaves, twigs and branches of mango as black valvety fungal growth. The incidence of dissease is very low on the main branches. It presents a characteristic and conspicuous black banded appearance. It is thus considered appropriate to name it as 'Black banded' disease. The infected por-

tions of the bark usually show the mycelial growth and clusters of conidiophores. The mycelial growth drops off in the summer months leaving light black bands in the affected portions (Fig.7). The fungus is confined to the upper layer of bark (Reddy *et al.*, 1961, Prakash and Srivastava, 1987).

2.6.2 Management

Neelum, Alampur Baneshan, Nawab Pasand, Kovaji Patel and Sambandham were highly susceptible. About 40 varieties were found free from the disease including *Mangifera odorata* (Reddy *et al.*, 1961). Application of Bordeaux paste and spraying of Bordeaux mixture is recommended for the control of the disease followed by gunny rubbing.
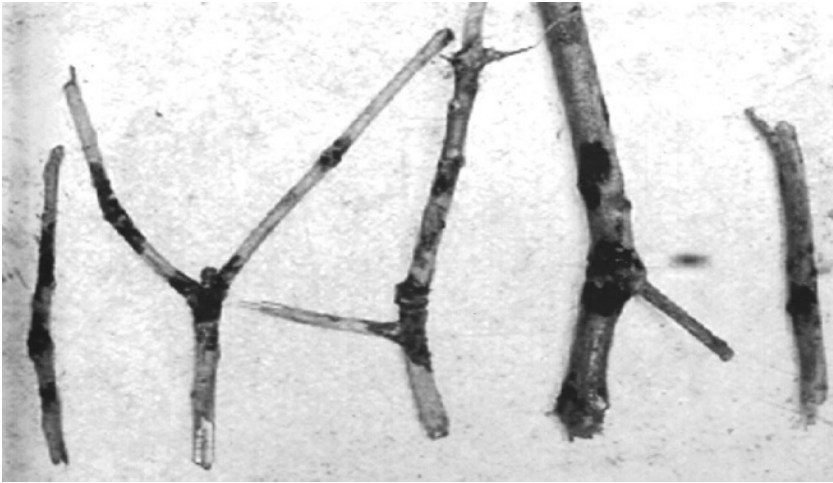
Figure 7: Black banded symptoms on mango twigs

2.7 *Phoma* Blight

It is a widespread disease of mango and has been reported for the first time by Prakash and Singh (1977) from Lucknow (India). Later, the disease has been found to be prevalent in many other mango growing areas of India (Prakash and Srivastava, 1987, Prakash, 1996, Ploetz and Prakash, 1997).

2.7.1 Symptoms

Symptoms of this disease are noticed on old leaves only. Initially the lesions are minute, irregular, yellow to light brown, scattered over the leaf lamina (Fig.8). As the lesions enlarge, their colour changes brown to cinnamon, and these become irregular. Fully developed spots are characterised by dark margin and dull grey necrotic centres. In

severe cases, the spots coalesce to form the patches of 3.5-13.0 cm diameter which result in withering and defoliation of infected twigs. Such plants can be located easily from a distance.

2.7.2 Causal organism

The disease is caused by the fungus *Phoma glomerata* (Corda) Woll. and Hochapf. Its morphological characters have been described in detail (Prakash and Singh, 1977).
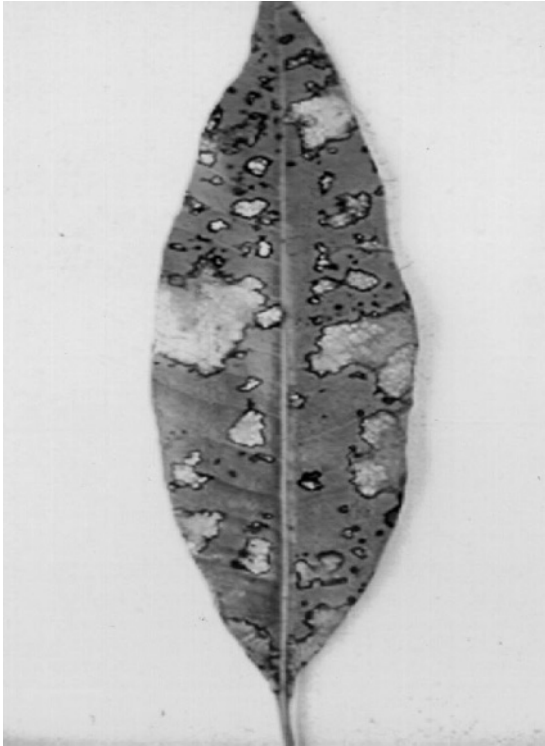
Figure 8: Phoma blight symptoms on old leaf of mango.

Pycnidia light-coloured to black and carbonaceous, mostly globose-ampulliform to obpyriform, sometimes irregularly ovoid-ellipsoid to oblong, ostiolate, occasionally 2-3 ostioles, single or in cluster, neck short, ranging from 25.50-286.50 x 38.12-500.00 μm (averaging 31.56-393.10 μm). Ostioles ranged from 4.8-36.0 μm, often pycnidia coalesce to form irregular large fructifications with many ostioles. In potato-dextrose-agar culture, pycnidia were abundant and contained many pycnidial initials, immature pycnidia were observed. Chlamydospores and dictyochlamydospores are abundant. Pycnidiospres hyaline to dark coloured with two or more guttules, mostly ovoid to

ellipsoid or irregular in shape, continuous or occasionally one-septate, measuring 3.5-13.2 x 1.6-4.9 μm (avg. 8.3 x 3.2 μm). Dictyochlamydospores dark brown to fuliginous, arising in unbranced or branched chains of 3-18 or more elements from aged pycnidia, single chlamydospores and intermediate stages that alternated between chlamydospores and dictyochlamydospres. Later, generally obclavate-ovoid to obpyriform, often fusiform-ellipsoid to ovoid or oblong, 2-8 transverse septa and some longitudinal septa, measuring 17.50-78.20 x 10.10-28.12 μm. The fungus was cultured on PDA medium. Growth was very slow, colony circular, aerial mycelium dense, dark greenish-grey, pycnidia abundant.

2.7.3 Management

Benomyl (0.2%) followed by Copper oxychloride (0.3%) have been found effective against the disease (Prakash, 1978 and 1979).

2.8 *Phoma* Leaf Spot

It is another leaf spot disease caused by *Phoma sorghina* (Sacc.) Boerema. (Doren and Vankest) on mango from Lucknow, India (Prakash and Raoof, 1985b).

2.8.1 Symptoms

Disease manifests itself in the form of small, irregular, oval to roughly circular water soaked spots on young leaves, measuring mere pinhead to 2.5 mm in size. Lesions are brown, later differentiated into brown margin with straw colour. Yellow halo around the brown margin is also observed. The infected leaves become brown and unlimately dry. Lesions near the midrib are elongated and more conspicuous. In severe cases, the spot coalesce to form large spots measuring upto 14 mm. Symptoms produced by this fungus are very much much similar to anthracnose and these may sometimes be confused. In this case, spots are smaller and there is no cracking in the centre as found in anthracnose (Prakash and Raoof, 1985b, Prakash, 1996).

Control measures are the same as reported for phoma blight disease.

2.9 Pink Disease

The disease is widespread and destructive in many tropical and subtropical regions of the world where heavy rain fall occurs. The disease is also known as 'thread blight', 'rubellosis' and 'cobweb' (Prakash and Srivastava, 1987, Prakash, 1996, Ploetz and Prakash, 1997). The disease reduces the vigour and fruit bearing capacity of the tree. Trees having dense canopy, well developed foliage, 6-15 years old trees are prone to the disease (Lim and Khoo, 1985). The disease is very common on inland soils increase with high rainfall in Malaysia. It causes a reduction in leaf canopy which creates large area open through which sunlight can penetrate, scalding inner branching and causing the bark to crack.

2.9.1 Symptoms

The disease appears as white, felty mycelial thread of *Erythricium salmonicolor* on the twigs and branch crotches (Lim and Khoo, 1985). By then, the fungus has invaded the bark to get established in the internal tissues and interferes with the transport of nutrients. Often, the fungal growth spreads to girdle the stem. Severely infected bark gets shredded and the wood exposed. Leaves turn yellow and dry, shoots and branches of the affected plants wilt and dry. Roots are not infected. The pink colour on the tissues represnts profuse conidial production by the fungus and hence, the name 'pink disease' is considered. Under favourable conditions, the mycelial threads coalesce to form a rough, pink crust on the bark surface. This stage usually coincides with the penetration of the bark and wood by the fungus. This stage generally takes one to several months to develop. In advance cases, the fungus may produce pustular or nectar stage. These pustules are orange red and arranged systematically in rows along the stem.

2.9.2 Causal organism

The causal organism is a basidiomycete, *Erythricium salmonicolor* (Berk. and Broome) Burdsall [Syns. *Corticium salmonicolor* Berk. and Broome, *Phanerochaete salmonicolor* (Berk. and Broome) Julich, *Necator decr*et *us* Massee]. Two types of sporulations may occur (Holliday, 1980, Lim and Khoo, 1985). Clammy pinkish-white hymenium over the pink crust is formed on bark by *E. salmonicolor*. Basidiospores are formed on the hymenium and borne on sterigmata on narrowly clavate to cylindrical basidia. Basidiospores are hyaline, ellipsoidal and 8-10 x 5-7 μm. Conidia of *N. decr*et *us*, are produced in reddish-orange sporodochia, are healine, ellipsoidal, thin walled, unicellular and 10-18 x 6-12 μm.

2.9.3 Disease cycle

The disease is seen during or just after rainy season. The anamorph and teleomorph are formed during wet climate. Conidia and basidiospores are dispersed by rain splash or by wind, hence, the 'vernacular malady' name for the disease, "penyakit cendawan angin", which means wind mushroom disease. Wet atmosphere is conducive to the sporulation, dispersal and germination of spores and to colonization of the host by the fungus. The disease persists from one to another through dormant mycelium inside the bark and in the cankerous tissues serve as a potential source of infection in wet season.

2.9.4 Host range

The fungus, *E. salmonicolor* has a very wide host range viz. cocoa (*Theobroma cacao*), coffee (*Coffea arabica, C. liberica*), carambola (*Averrhoa carambola*), citrus (*Citrus* spp.), black pepper (*Piper nigrum*), guava (*Psidium guajava*), jackfruit (*Artocarpus h*et *erphyllus*), langsat (*Lansium domesticum*), mangostein (*Garcinia mangostana*), rubber (*Hevea brasiliensis*), chempedak (*Artocarpus integer*) and rambutan (*Nephelium lappaceum*).

2.9.5 Management

The disease can be kept under control by cutting and removing the affected branches, and thus eliminating the entire infected end. Such ends should be protected with Bordeaux paste. Control of disease relies on early detection and removal of affected tissues from orchards. Disease can also be controlled by lime Sulphur and oil based coppers. Various fungicides viz. Tridemorph, Tridemefon, Flusilazol, Oxycarboxin and several protectant such as Copper oxychloride, Copper oxide, Copper hydroxide including Bordeaux mixture were found effective when applied by spraying or painting the affected parts. Besides this, wide tree spacing and proper free air circulation in the canopies, sunlight penetration help in reducing the disease.

2.10 Grey Blight

The disease was first reported from the Dominican Republic (Cifferi and Gonzalez, 1926), Sri Lanka (Park, 1932). In India, it was reported by Tandon *et al.,* (1955), Sarkar (1960), Prakash (1975,1976, 1996), Prakash & Srivastava (1987), Ploetz and Prakash (1997). Pathogen does not kill the plant entirely but photosynthesis activity is undoubtedly reduced. It is a weak parasite capable of infecting injured tissues, and healthy fruits in contact with the diseased ones. No detailed account of the total amount of loss incurred has been estimated.

2.10.1 Symptoms

The disease is characterized by the presence of brown spots on the lamina of mango leaves. These spots may develop from the margins of tip measuring few milimeters to a few centimeters in diamter. Initially, spots are light brown and minute which gradually increase in size and become dark brown.

Some of the spots enlarge and form large lesions with greyish white or light olive grey centre with tan coloured margins. The spots may coalesce to form larger grey patches on the leaf lamina. At this stage, black spots of acervuli may become visible to the naked eye in the central region and more on upper surface of the leaf and never extend beyond the midrib (Fig.9). If the infection starts from the tip, it advances regularly on either side of the midrib. In final stage, the infected portion gets detached from the leaf.

In case of severe infection, leaves are defoliated. Fungus produces abundant spores in acervuli which develop in grey spot leaf lesions and necrotic areas of infected fruit (Lim and Khoo, 1985). When lesions become old, black columns of spores liberate from epidermis by rupturing. The fungus is capable of attacking healthy full grown mango leaves. Wounding results in quick attack by the fungus and early spreading of lesions. Moist atmosphere helps in increasing and hastening infection.

2.10.2 Causal organism

Pestalotiopsis mangiferae (Henn.) Stey. [Synonym : *Pestalotia mangiferae* Henn., no

teleomorph of the fungus is known] causes grey leaf spot and stem end rot of mango fruit (Johnson, 1994, Lim and Khoo, 1985). *P. mangiferae*, *P. moniifolia* Guba and *P. versicolor* (Speg.) Stey (Prakash, 1974, 1976, 1987, Prakash and Srivastava, 1987, Ploetz and Prakash,1997), *P. glandicola* (Cast.) Stey. (Ullasa and Rawal, 1985) and *P. funerea* var. *mangiferae* (Uppal *et al.*, 1935) have also been reported from various parts of India.

The mycelium consists of narrow anastomosing septate hyphae, thin walled and hyaline when young, thick-walled and light brown at maturity. The cells contains granular protoplasm. Mycelium on the host is intracellular, and is observed both in the
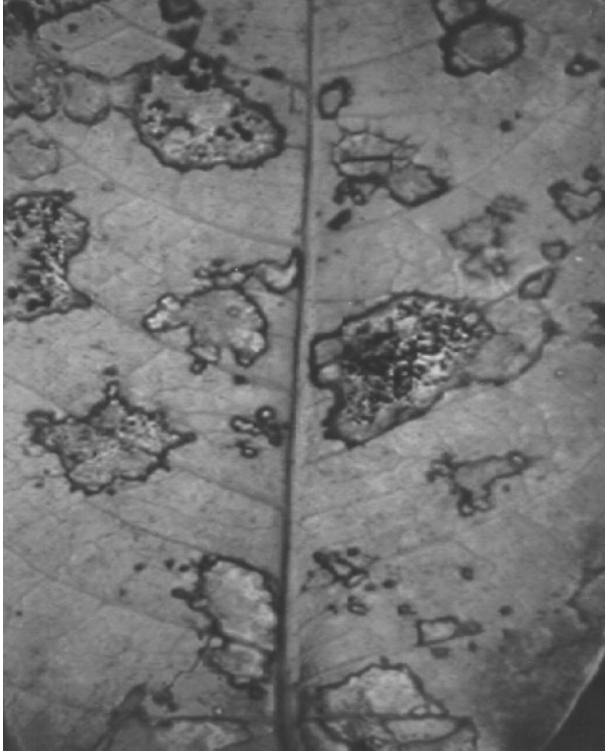
Figure 9: Characteristic brown spots of Grey blight of mango on leaf lamina.

affected necrotic regions and in the adjoining areas of living tissue. Acervuli are dark brown to blackish in colour, and discoid in shape. An acervulous arises from pseudo-parenchymatous stromatic tissue which is arched above by the overlying epidermal layer of the leaf tissue. At maturity, the acervulus erupts through the overlying epidermal layer. Conidium is oblong or ellitic-fusoid, 5 celled, with a short narrow hyaline stalk and three hyaline apical appendages. The two end cells are hyaline or pale olive in colour and conical in shape, while the three median cells are generally brown in colour of which the second cell from the base is very often light brown. The other two are dark brown. Conidia are often constricted at septa. They measure 17.0-28.0 μm x 8.0-12.0 μm

(Indian isolate). The fungus produces conidia having three thick walled brownish, concolorous median cells and thin walled, hyaline apical and basal cells, the apical cells bear three characteristic paraphysis. Conidia are 20 x 5 μm, fusiform and straight to slightly curved (American isolate). Two other species of *Pestalotiopsis* viz. *P. moniifolia* and *P. versicolor* occur on mango but produce larger conidia (Ploetz and Prakash, 1997).

The fungus is capable of growing at temperatures between 10 and 35ºC and the optimum lies between 20-25ºC. The temperature range for spore germination is from 10-34ºC with optimum 30ºC. Best mycelial growth with intensive sporulation takes place between pH 5.5-6.0 and grows well on Potato Dextrose Agar, Czapex Dox Agar, Richard's Agar, Oat Meal Agar and Decoction Agar with abundant conidial growth in the first three media and moderate in the others (Sarkar, 1960). Growth studies have also been conducted in culture media containing various carbohydrates and nitrogen conpounds (Sarkar, 1960, Pandey and Mohammad, 1974-75). Different solid and liquid media for growth and sporulation of the fungus have been tried and maximum growth and sporulation is observed on host extract and Richard's media, and optimum temperature for growth and sporulation is 30ºC (Pandey and Mohammad, 1974-75).

2.10.3 Management

Bombai variety is the most susceptible while Himsagar is moderately susceptible and Langra is the least (Sarkar, 1960). Pandey and Mohammad (1974-75) reported Chausa to be resistant. Jadeja and Vaishnav (1984) recommended first spray of Wettable Sulphur (0.2%), + Zineb (0.2%), after completion of heavy showers. Second spray of Wettable Sulphur (0.2%) before flowering. Third spray of Carbendazim (0.3%) at pea stage and final spray of Zineb (0.2%) before maturation of stone may reduce the incidence of grey blight. Fungicides being used for the control of other leaf spot diseases, such as Copper oxychloride, Dithiocarbamates, will also take care of the disease.

2.11 Leaf Blight

A full account of this disease has been published by Hingorani and Sharma (1956). The disease has been subsequently found to be present throughout the year in Delhi and other parts of India (Prakash and Srivastava, 1987). Heavy incidence is observed during the rainy season (July-September). The disease is considered to be of little economic importance. However, for the last 10 years, the disease has become important (Prakash, 1996).

2.11.1 Symptoms

The disease is characterised by the presence of yellowish pinhead spots on leaves, twigs and rarely on the stems. Soon after, spots enlarge and become light brown to dark brown in colour. Later, these spots become oval to irregular in shape, raised and dark purplish. Tissues become ash due to the appearance of pycnidia on the surface of lamina. Due to complete drying of leaf, the infection travels downwards towards the petiole. The bark of the infected stem/twig turns grey because of appearance of ellipti-

cal lesions which girdle the stem/bark at the point of infection.

2.11.2 Causal organism

The disease is caused by *Macrophoma mangiferae* Hingorani and Sharma. Pycnidia of the fungus are produced in 7 days by exposure of culture to uv radiation (Max. 60 sec.), which takes 40 days under normal conditions. Single spore isolation from irradiated cultures have resulted in a mutant strain which is stable and is different from the parent culture. The mutant isolate appears to be more virulent (Vasudeva, 1954-55, Hingorani *et al.*, 1961a,b). Different sources of Carbon, Nitrogen and Phosphorus have been shown to affect the growth and pycnidial formation of the two isolates. There is no difference in the pH and temperature requirement of the two isolates (Hingorani *et al.*, 1961a).

2.11.3 Host range and varietal susceptibility

In host range studies, besides *M. indica*, the pathogen weakly infects *Eryobotrya japonica, Eugenia jambolana, Ficus* sp., *Carica papaya* and *Vitis vinifera* (Hingorani *et al.*, 1960). Under studies of relative susceptibility of 38 mango cultivars, only Khandeshi Borasio and Asal Dadamio were found to be healthy and they were believed to be resistant (Desai and Patel, 1963).

2.11.4 Management

Removal and destrucion of infected parts is helpful in reducing the disease inoculum. Effect of Burgundy mixture, Perenox, Lime sulphur and Dithane on spore germination have been tested *in vitro*, indicating that these fungicides may be profitably utilized for the control of disease in field too (Hingorani *et al.*, 1960).

2.12 Angular Leaf Spot

2.12.1 Symptoms

On mango leaves in the begining, the spots are minute, irregular and brown in colour. In due course of time, the spots enlarge and turn darker in colour. Spots are distinctively visible on both the surfaces of leaves and are irregularly scattered on the entire leaf surface more towards midrib (Fig.10). The spots are mostly angular in shape and are generally restricted by the midrib or side veins. As the spots turn older, the central area becomes grey to almost white, with distinct dark brown margin, which is the characteristic symptom of the disease. Spots vary in size from 3-11x2-8 mm. Number of black pycnidial bodies are distinctly visible in the grey area on the old spots.

2.12.2 Causal organism

Plagulae epiphyllous, irregularly circular, olivaceous, with black dots, measuring 3-11

x 2-8 mm of diam. Mycelium superficial, hyphae septate, not constricted, abundant, ramified, having arborescent disposition, olivaceous-maroon, having cells of 7.0-14.5 x 2.0-2.5 μm covering the pycniostromata. Septa and hyphopodia are absent. Pycnostromata superficial, membranous, isolated, orbicular, scutellar, dimidiated, meandriform, astomous, of irregular dehiscence at maturity, 320-490 μm of diam., 15-24 μm of height, maroon, glabrous, edges thin, clear maroon, film-like, upto 85 μm of extension, lower wall indistinct. Conidiophores not observed. Hymenium superior and

Figure 10: Angular leaf spot of mango

hence inverted. Pycnidiospores fusoid, continuous, sessile straight or curced with 9.0-1.5 x 1-2.0 μm. Collected on live leaves of *M. indica* L. during July 1976 from Bhira (Lakhimpur Kheri) and Bijnor, U.P., India. Leg. Om Prakash, Central Mango Research Station, Lucknow, India, I.M.I. 202390.

2.12.3 Management

The control was controlled by spraying of Bavistin (Carbendazim, 0.1%) at 20 days' interval before emergence of symptoms.

2.13 *Alternaria* Spot

Alternaria spot or black spot disease is becoming an important disease of mango.

Mukherji and Bhattacharya (1965) reported the disease caused by *Alternaria tenuissima*. Yadava and Udainarain (1970) reported *A. alternata* [*A. tenuis*] from the fallen stem of mango. Anonymous (1959) from Egypt reported *A. tenuissima* affecting mango leaves. Singh and Tandon (1967) and Prakash and Raoof (1985b) isolated *A. alternata* from mango leaves causing leaf spot and fruit rot of mango. Its occurrence on mango is apparently restricted to the eastern hemisphere. To date, it has been reported from Australia, South Africa, Israel (Cronje *et al.*, 1989).

2.13.1 Symptoms

The disease is noticed on leaf, twig and fruit. Symptoms first appear as small, brownish circular spots on the surface of leaves and fruits and as black patches on the twigs. A high concentration of round black spot of 0.5 to 4.5 mm in diameter occurs evenly over the leaf lamina. Symptoms are most prominent on the lower side of the leaves. Minute sunken spots concetrated on midrib and side veins, later coalesce to form dark brown band (Prakash, 1979, 1984). Initially, it beccame apparent on lower surface of the leaf where it shows light brown discolouration. After a few days, the infection is visible on the upper surface of the leaf also. The spots gradually enlarge and become irregular black and form larger patches. The tender leaves are found to be more susceptible than mature ones. On fruit, the fungus produces latent infection which develop after fruit begin to ripen. The pathogen penetrates through lenticels on the surface of mango fruits. Initially, spots are concentrated around the stem end of the fruit where high numbers of lenticels are present. The spots grow in size and caolesce to become a single spot that can cover half of the fruit. The lesion centres are sunken slightly. Below the skin, reddish brown patches can be seen inside the flesh. The pathogen also attacks mango inflorescences resulting in a significant decrease in fruit set.

2.13.2 Causal organisms

The disease is caused by *Alternaria alternata* (Fr.) Keissler and *A. tenuissima* (Fr.) Wiltsh. In *A. alternata*, conidiophores originate solitary or in groups, simple or branched, straight or flexuous, smooth and pale to midolivaceous or golden brown sometimes geniculate. Conidia are obclavate, 20-36 x 9-9.5 µm, 18-44 x 7.5-12.5 µm (Indian isolates) 3 to 5 septate, borne in long chains. The hyphae of *A. tenuissima* are hyaline to dark olive, intercellular 3.4-10.2 µm, septate 1-6 µm wide. The conidiophores are olive-buff to dark olive, septate, with nearly 9.8 µm between the septa, measuring 20-100 µm in length, 3-4.5 µm in width. As a rule, they are unbranched but sometimes branched. Conidia are born at the tip, either singly or in chains of 2-3. They are usually smooth walled. Conidia are beaked, dark olivaceous, 2-9 septate with or without longitudinal septa, elongated oval, linear or obclavate.

The spores measure 13.2-59 µm in length, 6-16.3 µm in width. They have 2-11 transversal septa, 0-10 longitudinal septa. The beak is 1.4-4.4 µm long, 2-4.5 µm wide, sometimes terminally swollen, sometimes presenting two or three scars, and it has 0-8 transversal septa. The colony on potato-dextrose-agar, white cottony, 1-2 mm high growth is produced with loose aerial mycelium, in older isolates, the felt-like growth

appeared in the centre, white to pale smoke grey in colour. The submerged mycelium is grey to dark green, 60-70 mm in diameter. The colour of the colony gradually changes from dark olive-green to black. Abdel-Megid (1971) observed the optimum growth of the fungus at 25°C and 90% RH. Mukherjee and Bhatnagar (1965) reported the growth of the fungus was directly correlated with humidity, maximum grwoth occurred at maximum humidity and fell steadly with decreasing humidity. Singh and Tandon (1967) found that isolates of *A. tenuis* from banana, guava and mango were pathogenic under artificial condition except isolate from papaya. Leaf isolates failed to infect the fruits of their respective hosts. The leaves of *Capcicum annum, Solanum tuberosum, Lycopersicum esculentum, Citrus* and mango were susceptible. Leaves of *Solanum melongena* were also infected by mango isolates.

2.13.3 Disease cycle

Conidia of the fungus are disseminated by air current and in dew from dried or newly infected foliage. Fruit infection has been related to the number of hours with relative humidity over 80% and a minimum of 350 hours is required for quiescent infection on fruits. Fallen leaves on the ground are a source of conidia and also overwintering reservoir.

2.13.4 Management

The disease can be controlled by regular field spray programme by application of Cuprosan super D. followed by Bordeaux mixture (Abdel-Megid, 1971), Merculin followed by Mercurized Copper oxychloride (Mukherji and Bhattacharya, 1965). Three sprays with Mencab, starting 2 weeks after fruitset are most effective. A post harvest treatment with Iprodine or Prochloraz is as effective as three pre-harvest treatments (Prusky, 1987).

2.14 *Phytophthora* Induced Disease

Phytophthora palmivora (E.J. Butler) E.J. Butler causes various types of disease symptoms viz. wilt, crown rot, root rot, leaf blight and the death of nursery trees in several parts of world viz. Philippines, Thailand, India and Arizona (Matheron and Matejka, 1988, Kueprakone *et al.*, 1986, Prakash, 1977 & 1984, Tsao *et al.*, 1994). Unconfirmed reports of damage on mature tree come from Colima and Yucatan, Mexico and Esquintla, Guatemala (Ploetz and Prakash, 1997). In India, leaf blight is caused by *Phytophthora parasitica* (Prakash, 1977 & 1984). Initially water soaked spots on young leaves are visible which later enlarge in size and destroy the entire lamina. Severe outbreaks are associated with prolonged period of wet weather. It was very serious in shady and humid places in the orchard (Prakash, 1977, 1984). Leaves and shoots near the soil are blighted more. Infection of emerging seedling from stones by *Phytophthora* sp. causes damping off. gumming followed by distinct bark lesions developed above ground on mango plants whereas root and crown rots are observed at or below the ground level. Crowding of seedlings in nursery, excessive rains and high humidity, exacerbate

Phytophthora diseases.

In the Ivory Coast, damage has also been noticed on the trunk of mature trees (Lourd and Keuli, 1975) and also infects fruit in Malaysia and West Africa (Chee, 1969). However, mortality of mature trees is not seen but stem cracking and bleeding may occur. *P. parasitica* pruduces papillate sporangia, pear shaped to spherical, measuring 35-56.5 x 29-45 μm which germinate directly with germ tube or with motile zoospores. It also produces oospores, 18.5-30.0 μm in diamter. The abundant chlamydospores are also produced. The optimum temperatures for growth of fungus are 28 to 30°C. Infection usually occurs by means of zoospores, which are released when free moisture is abundant.

2.14.1 Management

Nursery soil should be fumigated with methyl bromide or surface burned. Nursery should be taken in virgin soils. Sanitary measures must be enforced to avoid introduction of pathogen in the nurseries. Overcrowding should be avoided in the nursery and frequency of irrigation be reduced. To avoid run off water from existing nursery/ orchard may help in reducing the disease problem.

2.15 Wilt

Wilt of mango caused by *Verticillium albo-atrum*, Reinke and Berth. was first reported in Florida (USA) (Marlatt *et al.*, 1970). Later on, it has also been reported from India (Prakash, 1982-1984, Saxena and Rawal, 1989, Sharma *et al.*, 1993) but the causal fungus is *Fusarium solani*.

Verticillium wilt is a relatively uncommon disease that is found on land on which susceptible vegetable crops viz. tomato and brinjal, were recently grown (Pohronezney and Marlatt, 1982). The first external symptom of the disease is yellowing of leaves which have wilted slowly in scattered areas. One or more main branches die and the leaves turn brown while remaining attached, causing a burnt appearance. The wood of affected branches shows brown discolouration, and severely diseased plant dies. The incidence was so severe that almost 100 per cent new mango plants grown on old tomato land became infected with the fungus *V. albo-atrum*.

In India, the grafted plant developed symptoms of epinastly and progressive foliar chlorosis often accompanied by transient wilting followed by necrosis and defoliation. The symptoms start developing in the rainy season when the nursery plants are stone grafted. The scion buds sprout and symptoms appear on young leaves. The leaves tend to curl upward and inward from the tip or twist along the midrib. As the disease advances, the leaves are shed, stem does not wilt but remains green until all the leaves are shed. At last, the graft became green, leafless and dried up. The tap root and secondary root of affected plants may contain yellow to orange brown discolouration. The branches wither, resulting in death of the plant. The disease is prevalent in large areas of the Konkan region.

2.15.1 Management

The only treatment which restored vigour to infected trees was injection of Sequestrene 138 (2 oz in 5 gallons water/tree) into the soil at the base of each tree (Goldweber, 1975). Fumigation with Methyl Bromide or other fumigants would not be practical for larger areas. Soil drenching with Carbendazim (0.1%) or Captafol (0.25%) has been found effective in controlling the seedling wilt (Sharma *et al.*, 1993).

2.16 *Sclerotium* Rot

In Philippines and India, about 10 and 18% mango seedlings die due to stem rot and many of the seeds (stones) rot either before or in the course of germination (Palo, 1933).

2.16.1 Symptoms

The disease is characterized by the presence of mycelial weft on the base of the stem at the ground level. Beneath the mycelial growth, a dark brown spot may develop which gradually encircles the base of the stem. At this stage, the succulent top droops and bends towards the ground, tissues loose turgidity and the seedlings dies within a week. When the disease is at peak, the fungus may be seen encircling stem upto the height of 2" or even more above the ground level. The desease also causes severe rotting of the seeds during or before germination. Numerous sclerotia develop on the cotyledons of the rotted seeds.

2.16.2 Causal organism

The disease is caused by *Sclerotium rolfsii* Sacc. in India and *S. delphinii* Welch in Philippines (Prakash, 1996, Ploetz and Prakash, 1997). On sterile agar medium, it produces coarse white threads, which radiate in all directions from the sclerotium. The coarseness of the mycelial growth is due to the development of strands, which consist of a group of more or less parallel hyphae that have grown closely together. The large hyphae develop at certain of the septa, a clamp connectin, opposite which a hyphal bud frequently arises and grows generally parallel to the main thread. A group of hyphae of this kind may anastomose and grew closely together, developing into strands that impart coarseness to the growth. Twenty five days old lactose-beef-agar and dextrose-beef-agar cultures, the sclerotium produced pinkish cinnamon patch consisting of strands of short barrel shaped cells and hyphae with knot-like structure. Also on these two media, the fungus under certain conditions, developed mats of loosely interlaced lobulate hyphae, which were perhaps special feeding stuctures of the fungus. As the sclerotia harden the mycelial growth is gradually depressed to the level of medium. A comparison of the average measurement of the hyphae of the mango sclerotium with *S. rolfsii* isolated from pepper, rice and tomato and four strains of *S. delphinii* showed no marked difference in their diameter. The sclerotia first appear as white tufts of interlaced hyphae on the surface of the fungus growth or at the ends of mycelial strands. Sometimes the sclerotia are formed first as enlarged white bodies on distinct stalks 1 to 5

millimeters in length. In a few plate, cultures on potato-glucose-agar numerous white tufts, some of which gave rise to the formation of stalk-bearing sclerotia, were noted to have developed. However, many of these white tufts did not continue to develop into sclerotia and became inconspicuous with age. At first without a cortical layer of hard coloured tissue. The larger sclerotia exude droplets of clear liquid from several points on their surface. On media rich in food, such as steamed oatmeal, this exudate becomes amber-coloured as the culture ages. The points of exudation are often marked by sunken spots or pits, which are usually round and distinct on larger sclerotia, but generally absent or inconspicuous on smaller ones.

On favourable media, the sclerotia produced are very irregular in shpae and size, some are subglobose, others elongate or nearly spheroidal, but on media lacking in nutriment they assume a globose form and tend to become uniform in size. The larger sclerotia are not only pitted but also often flattened and concave on the ventral sides. Hilum-like scars may be found on the ventral portions of some sclerotia at points where they are attached to the mycelial columns or strands. The development of sclerotia may begin after 6 or 7 days on very poor media and after 8 or more days on media rich in food. As the sclerotia mature on potato-glucose-agar, the surface colour turns to ochraceous-buff, finally to Hay's brown or occasionally tawny. On other media, however, the colour of the sclerotia may differ slightly in shade from those produced on potato-glucose-agar. On nearly all media tried the fungus tends to unite the sclerotia into irregular aggregations 5 to 10 mm in extent. A section of a mature scleriotial body produced in culture shows that it consists of pseudoparenchymatous tissues with intercelluar cavities and inclosed by a hard, compact layer of coloured cells. This layer may be from one to four cells thick. The composite sclerotia, which were sometimes produced on steamed oatmeal, appeared sponge-like in texture, and when sectioned the tissue consisted of loose hyphal wefts, especially at points where the pits were markedly shown.

The sclerotia produced in nature are generally globular and smooth and are smaller and more uniform in size than those produced on artificial media. They develop either from the white tufts of intertwined hyphae on the diseased tissues or laterally from the mycelial strands that creep upon the surface of the soil from the lesion. Upon maturity they turn from white to either cacao brown or chocolate and vary in diameter from 0.6 to 1.6 mm.

2.16.3 Disease cycle

The sclerotia germinate usually by the production of individual mycelial threads from points which come in contact with the medium. Sometimes the large pitted sclerotia send forth a dense fascicle of mycelium form the pits. In infection experiment in the greenhouse, many of the freshly matured pitted sclerotia, which were placed on very moist soil in pots, produced mycelial fascicles that bore new but smaller sclerotia after 6-8 days.As far as observed the mango Sclerotium produces spores neither in culture nor under field conditions. The sclerotia are of pin head size initially white, but at maturity chocolate brown in colour, measuring 1.0-2.6 mm in diameter (Prakash and Singh, 1976a).

The sclerotia remain viable for more than a year under drought conditions while

in moist conditions persist longer or indefinitely, especially if susceptible hosts are present. The fungus passes over adverse conditions by means of sclerotial bodies. The sclerotia kept in dry conditions remained alive for more than a year.

2.16.4 Management

Infected soil should be thoroughly surface burned before the seed beds are prepared. Diseased mango and weeds should be removed and burned. Excessive use of water and close planting should be avoided as the organism is moisture loving. Seed beds should be prepared with sufficient drainage arrangement. Planting of susceptible hosts should be avoided. Two minutes dipping of stones in Agallol / Brassicol / Captan / Thiram and subsequent soil drenching at 10-15 days interval to reduce the intensity of the disease (Gadre, 1979).

2.17 Root Rot and Damping Off

Damping off of mango seedlings is a serious problem in nurseries in Indonesia (Muller, 1940). Root rot and damping off of mango has been reported by Prakash and Singh (1980) from India.

2.17.1 Symptoms

The disease is characterised by sudden dropping of leaves after the emergence of seedlings from the soil. During prolonged rainy and humid weather, infection occurs at/ or below the ground level with circular to irregular water-soaked patches. These patches enlarge and ultimately girdle the entire base of the stem. On account of rotting, the diseased tissues become soft, dark brown or black and the entire seedling collapses and dies. Fungal mycelia and sclerotia are densely present on the severely infected parts. Rotting may spread both above and below the stem down upto roots and roots are disintegrated.

2.17.2 Causal organism

The disease is caused by *Rhizoctonia solani* Kuhn (telemorph : *Thantephorus cucumeris* (Frame and Donk).

2.17.3 Management

Soil treatments with Ceresan is recommended. First application of Ceresan can be given ten days before sowing and the second two days later, after turning over of soil up to a depth of 20 cm. During the growing season, Bordeaux mixture (1.5% should be sprayed on the plants and the soil at weekly intervals (Muller, 1940).

2.18 Gummosis

Surveys in several parts of Uttar Pradesh have shown that 30-40 per cent of young mango trees are affected by the gummosis especially the mango tree planted in sandy soil but its prevalence has also been noticed in other mango growing soils. The disease occurs on stems, branches of seedlings as well as grafted mango. Slowly it is becoming a serious problem in many areas. The disease is noticed after rainy season particularly in winters. The disease is characterized by the presence of profuse oozing of gum on the surface of the affected wood, bark of the trunk and also on larger branches but more common on the cracked branches. In severe cases, droplets of gum trickle down on stem, bark turns dark brown with longitudinal cracks. Bark rots completely and the tree dries up because of cracking, rotting girdling effects (Prakash and Srivastava, 1987, Prakash, 1996).

Studies on the pattern of association of fungi in diseased samples revealed that only *Lasiodiplodia theobramae* (Pat.) Grifton and Mauble (Synonyms : *Botryodiplodia theobromae* Pat.) has been encountered alongwith others fungi. Prakash (1978-79) and Alvarez Garcia and Garcia (1971) reported the gummosis in mango caused by *Physalospora rhodina*, perfect stage of *Botryodiplodia theobromae*.

2.18.1 Management

The disease can be kept under control if proper curative treatments are given at the proper time. The diseased bark/portion should be removed, cleaned and covered with Bordeaux paste, Copperoxychloride paste. Application of Copper sulphate 500 gm/kg (depending upon the age of the tree) in soil around the tree trunk is advocated. However the gummosis is very less in the orchards receiving regular Copper oxychloride sprays for control of leaf spot diseases (Prakash and Srivastava, 1987, Prakash, 1998).

2.19 Black Root Rot

Black root rot is reported to be an uncommon problem on young and old (40 years) mango trees. Canopies of affected plants wilt sudden and subsequently leaves defoliate from the wilted plant. A water soaked, blackened decay having unpleasant putrid odour from the roots of infected tree is obseerved (Lim and Khoo, 1985). Various species of fungi have been recovered from affected trees, including *Lasiodiplodia theobromae, Fusarium oxysporum, F. solani*, but these were thought to be secondary colonizers of roots (Lim and Khoo, 1985). Although mango is generally considered to be a flood intolerant plant species but its flood tolerance may be quite variable. However, considerable variation in the response of individual trees in an orchard to flooding is often evident. Potted plants usually adapted by forming hypertrophied lenticels (intumescence) when they are flooded experimentally (Larson *et al.*, 1993). Those plants which do not form hypertrophies, lenticels succumb fairly rapidly to flooding. In the latter cases, the roots of plants have symptoms of black root rot (Ploetz and Prakash, 1996).

Flood tolerance is known to be an environmentally and biochemically complex

process in mango (Larson *et al.*, 1993), it is conceivable that some of the fungi interact with flood induced stress to cause black root rot disease in mango (noticed by Lim and Khoo, 1985).

2.20 *Cercospora* Leaf Spot

2.20.1 Occurrence and distribution

Leaf spot was recorded on mango leaves from Japan (Yamamoto, 1934), Uganda (Hansford, 1943), Venezuela (Muller and Chupp, 1944), Tanganyika (Wallace and Wallace, 1947), Sierra Leone (Deighton, 1952), French Guinea and Ivory Coast (Frossard, 1959, Kranz, 1963), Congo (Viennot and Comelli, 1959), Thailand (Johnston, 1964a, b), Egypt (Ahmad and Ibrahim, 1969) Malaysia (Lim and Khoo, 1985) and India (Munjal *et al.*, 1961, Prakash and Srivastava, 1987, Ploetz and Prakash, 1997, 1998).

2.20.2 Symptomatology

The disease is characterized by the presence of irregular or round lesions on lower side of the leaf lamina, measuring 3.5-10.0 mm in diameter. However, smaller dark brown spots measuring 1-2.0 and 3-8 mm in diameter are found in Malaysia and Egypt, respectively. Initially, the lesions are light brown but soon after they become brown to black in colour. The lesions are surrounded by yellow halo, and centre somewhat dull in colour. A few to over a hundred spots may be seen on a leaf blade, depending upon the severity of infection. Several spots may coalesce to form large irregular patches. The affected portions of the leaf blade may soon dry off and wither, resulting in a scorched appearance of the foliage. Fungus produces copious conidia on necrotic host tissues especially in leaf debris fallen under the tree. The fungus produced large, olive grey conidia, 30 to 60 x 3.5 to 5.0 μm and usually on the lower surface. Conidia are wider at the base than the apex, straight to slightly curved, 3-7 septate, born in subglobular dark stromata which are 20-60 μm in diameter (Lim and Khoo, 1985).

2.20.3 Causal organism

The disease is caused by *Stigmina mangiferae* (Koorders) Ellis, [Synonym : *Cercospora mangiferae* Koorders, a teleomorph for the fungus is not known] and *Cercospora mangiferaeindicae* Munjal, Lal and Chona.

2.20.4 Management

Kranz (1963) tabulated the susceptibility of 42 mango cultivars against Cercospora leaf spot. None of the varieties was found to be resistant. Removal and burning of leaf debris from orchard is recommended. Benzimidazole fungicides are also found effective against the pathogen. Frossard (1959) recommended Copper fungicide and oil spray, the latter gave excellent control.

2.21 Mango Malformation

Malformation, also known as bunchy top, is a serious threat to the mango growing areas of the world. In recent years, the extent of this malady has taken such as high magnitude that the mango industry is badly threatened in India, particularly in northern India. In spite of a lapse of hundred years since the disease was first reported and a good number of papers published on mango malformation (MM), the etiology of the disease still remains obscure. The complex nature of the malady is obvious by the diverse claims made by different workers from different countries about its cause, i.e., physiological, viral, fungal, acarological and nutritional.

2.21.1 Geographical distribution

Mango malformation was first recognized in 1891 by Maries (Watt, 1891) from Darbhanga, Bihar. Later on the disease was reported from different parts of the country but more attention was given only in mid 1950s when it assumed serious proportions. The disease is mainly restricted to northern India where over 50 percent of the trees are affected. It has also been confirmed in most mango growing countries, viz., Egypt (Hassan, 1944), South Africa (Schwartz, 1968), Israel, Central America, Mexico, USA (Florida) (Malo and Mc Millan, 1972), Sudan, Cuba, Brazil, Australia, Bangladesh, Pakistan and United Arab Emirates (Kumar and Beniwal, 1992, Kumar *et al.*, 1993).

2.21.2 Symptomatology

Three distinct types of symptoms described by the workers are bunchy top of seedlings (BT), vegetative malformation (MV) and floral malformation (MF). Intermediate stages of these types have also been observed in nature (Singh and Chakravarti, 1935). Various types of symptoms can be grouped under two broad categories and accordingly malformation can be either vegetative or floral (Varma, 1983). Recent findings of Kumar and Beniwal (1987) further show that vegetative and floral malformation symptoms are produced by same malformation factor(s) because shoots carrying floral malformation factors(s) produced vegetative malformation symptoms on the scion shoot after the establishment of union and in subsequent years produce malformed panicles.

Bunchy top phase of the disease has been reported by Nirvan (1953). The disease appears on the young plants in the nursery beds when they are 4-5 months old. The characteristic symptom of the disease is the formation of a bunch or occasionally several bunches at the top of a little lower down the main shoot of the young plant. The bunch is formed of numerous thickened small shootlets, clustered together and arising from a leaf axil at the top or lower down the main shoot, and on which are borne many small rudimentary leaves. These shootlets are much thicker than the main axis from which they arise. The disease symptoms are shown by the swelling of several buds in the axil of a leaf or the production of several small shoots at the apical end. The shoots thus produced remains short and stunted. Growth of the plant is stopped, which gives an appearance of 'Bunchy top'. Vegetative malformation (MV) is more pronounced in young seedlings as well as seedling trees than in the grafted plants. The affected

seedlings develop excessive vegetative branches which are of limited growth, swollen and have very short internodes. These dwarf branches form bunches of various seizes which are often produced on the top of the seedlings (Varma, 1983) giving a bunchy top appearance. Such formations are also frequently found on seedling trees. The axillary buds of dwarf and even normal looking branches are usually enlarged indicting disturbance in the apical dominance. Bunchy top and malformation are considered to be the expression of the same disease on the basis of similar symptoms (Tripathi, 1954).

Malformation of inflorescence (MF) as the name implies is a disease of the inflorescence. Variations occur in malformed panicles (Singh *et al.*, 1961). In the early stages of panicle formation, no differentiation between healthy and diseased panicles can be made. The abnormal inflorescence may persist long after the normal one has fallen off the tree and may finally become vegetative. The malformed heads dry up in

Figure 11: Mango malformation of inflorescence

black masses and persist on the tree for a long time, even up to the next flowering season (Mallik, 1959a,b). the most characteristic symptom of MF is the reduction in the length of the primary axis and the secondary branches of the panicle which make the flowers appear in clusters (Fig.11). Frequently, the flower buds are transformed into vegetative buds and large number of small leaves and stems, which are characterised by appreciably reduced internodes and are compacted together, give MF a witche's broom like appearance. In yet other cases, the flower buds seldom open and remain as dull, green unopened buds. In still other cases, the main axis is shortened but the flowers are changed into small leaves. Branches with the diseased inflorescence can produce both malformed as well as healthy panicles in the following bearing season.

The affected inflorescence are of three types, heavy, medium and light (Varma *et al.*, 1969). The panicles of heavy type are very compact due to extreme crowding of flowers and unlike normal panicles keep growing to form large hanging masses of flowers. The panicles of 'medium' type are slightly less compact and their growth is not continuous. Though it may start after sometime like the 'heavy' type and persist on plants longer than the normal panicles (Singh and Chakravarti, 1935). The 'light' type of panicles are difficult and do not persist on plants. Due to larger bracts, malformed panicles give leafy appearance than the normal ones and not due to phyllody. Variations in malformed panicles have been well illustrated by Singh *et al.,* (1961). Sharma (1953) reported both compact and spreading types of malformation. There is a change in the sex ratio of the flowers with a shift from hermaphrodite to staminate (Khan, 1943, Khan and Khan, 1960).

Malformed panicles normally do not bear fruits. Even the fruits set in the season do not grow more than size of the pea. These have been found to yield normal fruit in off season (Mallik, 1963, Jawanda, 1963). The light type in variety Bombay Green also retains fruits even in normal season (Majumdar and Sinha, 1972). Mallik (1963) could not get success in hand pollination of hermaphrodite flowers in malformed panicles with normal pollen, but Varma and his colleagues (Varma *et al.*, 1974a,b) succeeded when they used normal pollen of cv. Dashehari. Infection appears to be localised. However, possibility of systemic infection in branches cannot be ruled out. All the branches produced on a malformed branch may not bear malformed panicles and even in a malformed panicle some secondary rachis may be normal (Mallik, 1963). Conversely, on a healthy panicle some parts may be malformed but such expressions are not very common (Varma, 1983).

2.21.3 Etiology

Four causes have been attributed to the disease, i.e., physiological, nutritional, entomological and pathological. Recent findings have demonstrated that the disease may be of fungal origin. Since fungal etiology of the disease has not been fully confirmed from all the places where this disease exists, possibility of more than one cause cannot be ruled out (Varma, 1983). Only pathological aspect of the disease is being described below.

2.21.3.1 Virus association

Sattar (1946) considered the disease either of viral nature or a physiological disorder. Sharma (1953) reported that the disease could not be attributed to any fungus, bacteria or virus. Viral disease like symptoms of the malady and failure to isolate any pathogenic organism led to speculation (Sattar, 1946, Singh and Jawanda, 1961, Ginai, 1965) that the disease was viral in nature. The disease does not appear to be transmitted through stones (Kausar, 1959, Bindra and Bakhetia, 1971). Mallik (1963) transmitted the disease successfully by grafting or budding, and also by dodder, but no precaution was taken to prevent movement of mites in these studies. Vasudeva (1960) reported the bunchy top symptoms by wedge graft and found that the same virus could cause two types of

symptoms. In his studies, virus was considered to be the main cause of the disease just after the failure of physiological theory, but it soon started losing ground, where Singh *et al.,* (1961) and Prasad *et al.,* (1965) tried to transmit the disease from branches to seedlings, seedlings to scions and seedlings to seedlings by inarching, cleft grafting, bark patch budding, mechanical inoculation or through insects without any success. Even viral and mycoplasma like nature could not be confirmed by Beniwal and Bhatnagar (1975). The results thus obtained from the studies do not suggest that MM is of viral etiology (Varma, 1983).

2.21.3.2 Fungal association

Summanwar *et al.,* (1966) reported, for the first time, a fungus *Fusarium moniliforme* Sheld, associated with malformation (MF and MV) and proved its pathogenicity. Summanwar and Raychaudhuri (1968) investigated that mite carried the fungus on the bodies and irritation caused by the mites paved the way for the fungus. The fungus, *F. moniliforme* has been consistently isolated from various parts of affected malformed plants. Isolations were made from malformed panicles of 392 isolates from 130 trees, 336 were *Fusarium moniliforme* and 36 *Cylindrocarpon mangiferum* (Chowdhary and Varma, 1972, Prasad *et al.*, 1972, Summanwar *et al.*, 1966, Varma *et al.*, 1969, 1971, 1972, 1974a,b, Chadha *et al.*, 1979a).

The fungus was further identified as *F. moniliforme* var. *subglutinans* (Varma *et al.*, 1974b, Chadha *et al.*, 1979a). Aerial mycelium appearing powdery due to microconidia which are 0-1 septate, oval to fusiform and produced on polyphialides, macroconidia are lacking or rarely produced, 1-2 septate, falcate and without chlamydospores. Pigmentation is typical violet. The fungus does not have any special nutritional requirement as reported by Chattopadhyay and Nandi (1977). Varma *et al.,* (1971) reported that the growth of the fungus was inhibited during summer months even at room temperature. Vegetative malformation (Prasad *et al.*, 1972, Summanwar *et al.*, 1966, Varma *et al.*, 1969, Chadha *et al.*, 1979a) and floral malformation (Varma *et al.*, 1974a,b) can be initiated in the healthy test plants by artificial inoculation of aerial branches with the fungus as it is mostly intercellular and occasionally forms intracellular agglomerates in the cortex and phloem regions and the fungus formed globose bodies similar to chlamydospores, particularly in the cortex when inoculated with spore suspension (Varma *et al.*, 1972, 1974b). Cross inoculation studies with strain of *F. moniliforme* further confirmed the host specificity and the mango strains only caused typical disease symptoms and infection (Varma *et al.*, 1974a,b, Chadha *et al.*, 1979a). A definite evidence has revealed that the disease is caused by *F. moniliforme* var. *subglutinans* (Ghosal *et al.*, 1977a,b). Fusarial pathogens in contact with the host species produce the common toxic compounds, namely, fusaric acid, lycomarasmin and 12-13 epoxytrichothecences.

Fusarium oxysporrum is also involved in causing malformation of mango and the fungus has been isolated from all plant parts. Typical BT symptoms can be produced in seedling by inoculating the fungus through soil. The fungus is systemically present in parenchymatous cells of the pith region of malformed tissues (Bhatnagar and Beniwal, 1977). It is just possible that the disease is caused by more than one species of *Fusarium*. Fungal etiology as also behaviour of mango malformation was also studied by Andotra

et al., (1984). A *Fusarium* sp. was isolated from infected tissue. Inoculation of mango seedlings showed that the disease was neither systemic nor completely localized but behaved erratically. Internal spread is always acropetal and is supposed to be facilitated through active cell divisions of terminal growth under environmental conditions favourable to both host and pathogen.

Effect of *F. moniliforme* var. *subglutinans* infection on mangiferin production in the twigs of *Mangifera indica* was studied. Infected twigs contained less mangiferin than twigs of healthy plants. In both cases, mangiferin concentration was high during cooler months and low during hotter months (Chakrabarti and Ghosal, 1985).

2.21.3.3 Natural spread

The annual recurrence of malformation in new seedlings clearly shows its natural spread. Increase in infection has been reported by Nirvan (1953), Singh *et al.,* (1961) and Mallik (1963). Although the fungus *F. moniliforme* does not sporulate *in situ*, it does so on drying malformed panicles (Varmaet *et al.*, 1974a). To study the aerial flight of these fungal spores, the use of rotary traps for six months in places having high incidence of MM could not yield the spores of *Fusarium* (Varma *et al.*, 1971), which clearly indicated the role of some other agency in transmission of the disease. Puttarudiah and Channabasavana (1961), Singh *et al.,* (1961), and Nariani and Seth (1962) successfully reproduced the disease by transferring the mites which have been later reported to carry the fungus *F. moniliforme* on their surface (Summanwar and Raychaudhari, 1968). Varma *et al.,* (1971) explained the possibility of mites in transmission of disease. This finding was supported by the feeding behaviour of the mites (Varma, 1983). The small percentage of mites carrying the fungus and their presence in south-eastern parts of India (Varma *et al.*, 1971), where the disease is sporadic in nature, also indicated the possibility of some additional factors in the movement of the disease (Varma *et al.*, 1974a,b). The propagation and distribution of diseased plant material may cause wide and erratic distribution of the disease (Varma *et al.*, 1971).

2.21.4 Factors responsible for incidence

The severity varies considerably from year to year. A tree once affected connot escape the disease in subsequent years (Mallik, 1963). Majumdar and Sinha (1972), and Varma *et al.,* (1969) studied the seasonal variation at the time of flowering with the prevailing temperature. They observed 60 percent diseased panicles in Neelum during the flowering of February-March, whereas the same plant had only 4.5 percent malformation during off season flowering in June when the average minimum and maximum temperatures were higher than those of February-March which resulted in decrease in disease incidence. Fluctuations in the incidence of malformation in varieties Neelum, Alphonso and seedling trees were investigated by Jagirdar and Shaik (1968). The disease is serious in the north-west region where temperatures lie from 10-15°C during December-January (winter) before flowering. The disease is mild in the areas where temperatures lie from 15-20°C, sporadic from 20-25°C and nil beyond 25°C. Puttarudiah and Channabasavana (1961), Singh *et al.,* (1961) and Chadha *et al.,* (1979b) reported that the occurrence of

malformation differed according to the age of the plants. They observed more disease in young plants than in old ones. About 91 percent incidence in 4-8 years old plants and 9.6 percent in older plants was reported (Singh *et al.*, 1961). Age of the flowering shoot also influences the incidence of MF, as reported by Varma (1983).

2.21.5 Management

In view of the seriousness of the disease, various attempts have been made to correct the plants either by pruning or spraying pesticides or both. Some recovery in plants treated with Phorate and Captan after pruning has been reported by Bindra and Bakhetia (1971).

Varma *et al.,* (1971) realized the need of systemic fungicides for the curative control of disease as the causative fungus is located in the cortex and phloem portion. They advocated the spraying of Benlate in combination with thorough pruning which could reduce the disease from 69 percent to less than 1 percent. Using disease free plant material (Varma *et al.*, 1971), prophylactic sprays with fungicides (Varma *et al.*, 1971, Chattopadhyay and Nandi, 1977) keep the plants healthy and check further spread of the malformation. Sharma and Tewari (1975) found that Bavistin did not appear to work systemically against mango malformation. However, spraying of this fungicide reduced the disease.Foliar spray of 200 ppm Bavistin (Carbendazim) gave maximum disease reduction (95%) in cv. Dashehari and 91.3 percent in cv. S.B. Chausa. Potassium metabisulphite gave maximum disease control in S.B. Chausa (92.5%) and NAA gave 94 percent reduction in Dashehari (Mehta *et al.*, 1986, Siddiqui *et al.*, 1987).

Several other approaches to control the malady were adopted assuming it could be due to pathological, acarological (mite transmission virus) and/or physiological causes. Several control trials were laid out on mango malformation by using fungicides, acaricides, plant growth regulators and de-blossoming and their combination with 37 treatments during the years 1975 to 1978. The results achieved showed the possibility of reducing the malady by spray of NAA 200 ppm followed by de-blossoming at bud burst stage. The economics of mechanical de-blossoming operation was also worked out (Chadha *et al.*, 1979a,b).

Varma *et al.,* (1971) screened 34 fungicides, 17 insecticides and 4 growth regulators for their fungicidal and fungistatic action and found Benlate, Brestan, Busan, Captan, Dithane M-45, Panogen and Thiram most effective. Among the insecticides, Aphiden and Phosphamidon were fungicidal at 1000 ppm but not at lower concentrations. Other insecticides and growth hormones were only fungistatic. Benlate and Aphiden could inactivate the fungus *in vivo*. Dwarf shoots developed into normal vegetative shoots when these chemicals were sprayed on diseased plants and fungus could not be isolated from fresh growth, whereas the fungus was present in the dwarf shoots, indicating their concentration in sprayed shoot to be below fungicidal level.

It is evident from the cited literature that at present, no definite control measures for mango malformation can be advocated. However, the following measures may reduce the incidence of malformation : i. It is advisable to avoid scion stick from trees bearing malformed inflorescence for propagation. Indexing of healthy mango trees be done to serve as material for propagation. ii. Only certified samplings should be used for

propagation. iii. As soon as the disease symptom is noticed, the affected terminals should be pruned alongwith the basal 15-20 cm apparently healthy portion and bunt. iv. Healthy orchards located in disease prone pockets should be sprayed with fungicides/insecticides/as a prophylactic measure to avoid further recurrence of the disease. v. Spraying of 200 ppm NAA in the first week of October is advocated followed by de-blossoming at bud burst stage. vi. Early flowers should be de-blossomed.

2.22 Fungi Causing Diseases of Minor Importance

Various fungi have been reported by different workers to cause minor diseases in mango. Of them, few fungi cause damage to the mango, and are only localized in particular country from where they have been originally investigated. The fungi other than those causing various diseases already discussed earlier are : *Actinodochium* sp. in India, *Actinodochium jenkenssii* in India, *Acrothecium pennis*et *i* in India, *Armillaria mellea* in Ugnada and Gold Coast (Small, 1924, Bates, 1963 and Anonymous, 1926), *Aschersonia lichenoides* in Dominican Republic (Gonzalez and Ciferri, 1928), *Ascochyta mangiferae* Batista in Brazil, S. Leone and India (Batista, 1947, Deighton, 1952, Kamal *et al.*., 1979), *Asterolibertia mangiferae* n. sp. in India (Hansford and Thirumalachar, 1948), *Asteromella* sp. in India (Vala *et al.*, 1985), *Aureobasidium pullalans* in India (Reddy, 1968), *A. nidulans* in India, *Aspergillus niger* in India and Australia (Banerjee *et al.*, 1934, Baker, 1938), *Aspergillus fumigatus* and *A. variecolor* in India (Sinha, 1945), *Botryotrichum piluliferum* in India (Prakash and Raoof, 1985d), *Boothiella t*et *raspora* in India (Tandon and Srivastava, 1974), *Cercosporella* sp. in India (Anonymous, 1981).

     *Cha*et *othyrum mangiferae* in India (Srivastava *et al.*, 1979), *Ceratocystis fimbriata* in Brazil (Viegas, 1960, Piza, 1966, Martins *et al.*, 1974, Ribeiro and Coral, 1968), *Cladosporium oxysporum* in India (Anonymous, 1981),*Cladosporium herbarum* in Dominican Republic (Ciferri and Gonzalez, 1928), *Coniothyriopsis mangiferae* in India (Shreemali and Bilgrami, 1973), *Coniella musaiaensis* in India, *Coll*et *otrichum phomoides* in Philippine Islands (Ocfemia, 1931), *Coll*et *otrichum capsici* in India (Sinha, 1948 and Prakash, 1996), *Calonectria mangiferae* in Madagascar (Sechet, 1953), *Coccomyces vilis* in India (Butler and Bisby, 1931), *Curvularia lunata* in India (Singh, 1971), *Coleophoma mangiferae* in Pakistan (Ahmad, 1956), *Cytospora* sp. in Ceylon (Haigh, 1931), *Cytospora mangiferae* in India (Sharma and Agarwal, 1974, Prakash and Raoof, 1985).

     Daedalea boseii in India (Butler and Bisby, 1931), *Didymella mangiferae* in Brazil (Batista, 1947), *Dimerosporium mangiferum* in India (Butler and Bisby, 1931, Padwick, 1939), *Dirinaria* sp., *Diaporthe citri* in Australia and USA (Baker, 1937,1938, Ruehle and Wolfenbarger, 1949), *Dothiorella* sp. in India (Vala, 1985), *Dothiorella ladharense* and *Dothiorella mangiferae* in Pakistan and India (Ahmad, 1956, Butler and Bisby, 1931, Prakash, 1974-86), *Dothiorella dominicana* in Dominican Republic and India (Petrak and Ciferri, 1930, Butler and Bisby, 1931, Anonymous, 1981), *Drechslera hawaiiensis* and *Drechslera specifera* in India (Pawar, 1981, Prakash, 1974-86), *Fracchiaea h*et *erogenea* in Pakistan (Ahmad, 1956), *Fomes conchatus* in India, *Fumago vagans* in India (Srivastava and Bhargava, 1977), *Funalia leonina* in India (Prakash and Raoof, 1985), *Fusarium* sp. (Crown rot) and *Fusarium solani* in India (Prakash,

1974-86, Purkayastha, 1968), *Ganoderma lucidum* and *Ganoderma applanatum* in Formosa (Sawada, 1934-35), *Gloeodes pamigene* in South Africa (Pole Evans, 1934, Wager, 1937), *Gloeosporium raciborskii* in India (Stevens and Pierce, 1933), *Gloeosporium mangiferae* in Dutch East Indies (Muller, 1940), *Glomerella psidii* in India (Venkatakrishniah, 1952), *Gonatophagmium mangiferae* in Burma (Mulder, 1973), *Gnomonia mangiferae* in India (Malhotra and Mukherji, 1978), *Graphina* sp. (Prakash, 1974-86), *Guignardia bidwellii* in Jamaica (Hansford, 1923), *Guignardia mangiferae* in India (Prakash and Raoof, 1985).

Haplosporella beaumontiana in India (Prakash and Raoof, 1985), *Hendersonia creberrima* in South Africa and India (Brodrick and Westhuizen, 1976, Butler and Bisby, 1931), *Hendersonia* sp. in India (Vala *et al.*, 1985), *Hexagonia tenuis* in India (Prakash and Raoof, 1985), *Hyalotia laurina* in India (Kapoor and Chaudhury, 1977), *Hypocryphalus mangiferae* in Venezuela (Medeiros Rossetto, 1966), *Lasiodiplodia* sp. in India (Vaheeduddin, 1953), *Lophodermium mangiferae* in Dominican Republic and India (Gonzalez and Ciferri, 1928, Tewari and Srivastava, 1977, Singh, 1979, Vala *et al.*, 1985), *Microthyrium mangiferae* in Dominican Republic (Gonzalez and Ciferri, 1928), *Macrophomina phaseolina* in India, *Massarina usambarensis* in India (Butler and Bisby, 1931), *Nodulisporium indicum* in India (Reddy and Bilgrami, 1972), *Omphalia flavida* in Puerto Rico and America (Wagle, 1928, Tucker, 1929), *Phoma* sp. in Nyasaland and India (Leach, 1935, Uppal, 1937), *Phoma sorghina* and *Phoma glomerata* in India (Prakash and Singh, 1976b,1977, Prakash and Raoof, 1985), *Phoma mangiferae* in Pakistan (Ahmad, 1956), *Phomopsis* sp. in Jamaica, West Indies and India (Hansford, 1923, Wardlaw and Leonard, 1936, Cheema and Dani, 1934), *Phomopsis mangiferae* and *Phomopsis perniciosa* in India and Pakistan (Prakash, 1974-86, Prakash and Raoof, 1985, Ahmad, 1956), *Phyllosticta mortonii*, *Phyllosticta mangiferae* and *Phyllosticta mangifericola* in India and Brazil (Uppal *et al.*, 1935, Vala *et al.*, 1985, Prajapati *et al.*, 1985), *Phyllostictina mangiferae* in Brazil and India (Vala *et al.*, 1985), *Plenotrichella* sp. in India (Prakash and Raoof, 1985), *Polyporus tulipiferae* in India (Bakshi *et al.*, 1956), *Polystictus persoonii* and *Polystictus leoninus* in India and Pakistan (Uppal *et al.*, 1935, Ahmad, 1956).

Phytophthora sp. and *Phytophthora parasitica* in India (Prakash, 1974-86), *Phytophthora arecae* in India (Narasimhan, 1927, Butler and Bisby, 1931), *Phytophthora palmivora* and *Phytophthora botryosa* in Malaya and Ivory Coast (Lourd and Keuli, 1975), *Pseudocercospora subsessilis* amd *Pseudocercospora mali* in India (Prakash and Raoof, 1985), *Pythium* sp. in Philippines (Camus, 1935), *Pyrenocha*et *a* sp. in India (Vala *et al.*, 1985), *Rhizoctonia lamellifera* in Rhodesia (Hopkins, 1941), *Robillarda sessilis* in India (Prakash and Singh, 1976b, Prakash and Raoof, 1985).

Scytalidium state of *Hendersonula toruloidea* in India (Pandey *et al.*, 1971), *Schizophyllum commune* in Pakistan and india (Prakash and Raoof, 1985), *Sphaerognomonia mangiferae* in Brazil, *Sirosporium mori* and *Stringula elagans* and *Stringula nemathora* in India (Prakash and Raoof, 1985, Prakash, 1974-86), *Sphaerostilbe repens* in Uganda (Chardon, 1929), *Thyronectria pseudotrichia* in Mauritius (Anonymous, 1962), *Treleutia mangiferae* in Puerto Rico (Chardon, 1929), *Tricharia* sp. (Prakash, 1974-86) and *Verticillium* sp. in India (Prakash, 1974-86).

3. Diseases caused by Bacteria

3.1 Bacterial Canker

The promising mango industry in northern India is threatened by bacterial canker disease. The losses are as high as 100% in certain cultivars. The disease is also known as bacterial spot, leaf spot, black spot, mango blight, bacterial black spot (Prakash and Raoof, 1985) and black blight (Pinkas and Maymon, 1996). In India, it was first observed from Poona and Dharwar as leaf spot disease (Patel *et al.*, 1948a & b). Herbarium specimens of the Forest Botanist, Forest Research Institute, Dehradun collected as early as in 1881 and 1908 from Sabour (Bihar) and Dehradun (U.P.), respectively were found infected with similar lesions as described during 1913-14 by Doidge (1915) from South Africa but the report from India was considered authentic. Now, its prevalence has been reported from many countries of the world (Prakash and Srivastava, 1987, Prakash *et al.*., 1996, Ploetz and Prakash, 1997).

During early sixties, the disease was considered as a minor, but now it is posing a great threat to the commercial (Dashehari, Mallika and Amrapali) as well as seedling cultivars grown in the country. Canker incidence was noticed first time in polyembryonic cultivars of mango at the Experimental Research Station, Rehmankhera at Central Institute for Subtropical Horticulture, Lucknow (formerly known as Central Mango Research Station) during the year 1978 and thereafter, it created an alarming situation in the years 1980, 1982, 1983 and 1988. Its wide spread and severity posed much losses of mango fruits. Recurrence, intensity and spread of the disease has also been observed gradually extending in new areas.

3.1.1 Geographical distribution

Mango bacterial canker disease caused by *Xanthomonas campestris* pv. *mangiferae indicae* became serious in Uttar Pradesh mainly in Lucknow as early as 1978 and thus has been commonly present in most of the Indian states. It is also reported from Karnataka (Bangalore), Maharashtra (Ratnagiri, Nagpur, Dapoli, Raigarh), West Bengal (Malda), Bihar (Dholi, Sabour), Goa (Panaji), Delhi (IARI and Badarpur), Gujarat (Navsari, Junagarh, Gandevi), Andhra Pradesh (Sangareddy), Tamil Nadu (Rameshwaram), Kerala (Thiruvanantpuram), Andaman and Nicobar Islands (Port Blair), Punjab (Gangian), Haryana (Faridabad), Rajasthan (Jaipur), Orissa (Bhubaneshwar) and Madhya Pradesh (Jabalpur). Thus, the disease is spreading fast with low to high magnitude and its occurrence is gradually extending in the new areas. The disease is quite wide spread in mango growing regions of the world, viz. Urasia : France, India, Israel, Pakistan, Africa : Egypt, South Africa, Congo, Tanzania, Gold Coast, Morocco, Mozambique, Zaire, Reunion Island (France), Somalia, Sudan, Kenya, Australia : New South Wales and Queensland, Far East : Japan, Thailand, Malaysia, Philippines, Taiwan, Central America : Cuba, French Guiana, New Mexico, West Indies, South America : Brazil, Paraguay, Venezuela and many other tropical and subtropical countries of the world where, probably its presence could not be sited due to oversight. Now, official recognition for its existence from other mango growing countries of the world is warranted as no such

information is available in the literature (Prakash *et al.*, 1994 and 1996).

3.1.2 Symptomatology

The disease was noticed on leaves, leaf stalks, stem, twigs, branches and fruits, initially producing water soaked lesions, later turning into typical canker. On leaves, minute water soaked irregular stellate to angular raised lesion measuring 1-4 mm in diameter, usually crowded at the apex. These lesions were light yellow in colour initially with yellow halo but with age, enlarge or coalesce to form irregular necrotic cankerous patches with dark brown in colour usually on the lower side but occassionally on both sides. On young leaves, the halos were larger and distinct, while on older leaves, it was narrow and could be observed only against light. Under severe infections, the leaves turn yellow and droped off. Canker on leaf stalks, sometimes progress superficially alongwith midrib. On branches, twigs and stem freshly developed lesions were observed as water soaked, swollen later on turned dark brown, raised with longitudinal fissures exposing the vascular tissues mostly filled with gummy substance which oozes outward. The infection was deep seated, black discolouration of underlying tissues with cracked bark. The disease is not noticed on flowers although, it has been reported from South Africa, resulting serious fall of the flowers.

On fruit, water soaked, dark brown to black colour lesions were observed which gradually developed into cankerous, raised or flat spots. These spots grow bigger usually upto 5 mm in diamter, which covers almost the whole fruit. These spots often, burst extruding gummy substances containing highly contagiouus bacterial cells. Sometimes, the exposed flesh in cankerous spots attracts insects and subsequently involved by secondary micro-organisms which initiates rotting. Fruit dropping was observed to be more when cankers develop near the stalk end. It is, however, added that a typical cankerous growth was noticed in cv. Dashehari from Masauli (Barabanki, U.P.) for the first time during 1978 (Fig.12). Severely infected fruits crack and become brown in colour. In some excessive infected fruits, pulp and stones were also found to be infected (Fig. 13). Recently, a heavy incidence of MBCD has again been recorded on leading commercial cv. Dashehari from Sitapur district of Uttar Pradesh (Anonymous., 1996).

3.1.3 Causal organism

The disease is caused by *Xanthomonas campestris* pv. *mangiferaeindicae* Patel, Moniz & Kulkarni) Robbs, Ribiero & Kimura (Patel *et al.*, 1948b, Robbs *et al.*, 1978). First time Patel *et al.,* (1948a) reported the causal organism of disease and named as *Pseudomonas mangiferaeindicae*. Later on, this pathogen was found similar to *Xanthomonas* spp. in most of its characters except the colour of the colony which was albino (white) in case of *P. mangiferaeindicae* and mucoid yellow in species of *Xanthomonas* on various culture media. It is a gram negative, rod shaped, motile by monotrichous flagellum and measures 0.36-0.54 μm x 0.45-1.44 μm. It is oxidase-negative, does not reduce nitrate to nitrite and can not use asparagine as a sole carbon and nitrogen source. The mother culture of the bacterium can be maintained viable and virulent up to 60 months

in in sterile water (Kishun, 1986). Attempts have been made to find out the existence of pathotypes in *X. campestris* pv. *mangiferaeindicae* (*X.c.m.i.*) by various workers (Kishun, 1994a, Raut and Anahosur, 1994, Venugopal *et al.*, 1991). On the basis of cultural and biochemical characters, host varieties, antibiotic sensitivity and isozymes, 3-4 pathotypes have been identified in *X.c.m.i.*. Gagrevin and Pruvost (1995) gave an indication for the presence of a worldwide population of 139 strains from 14 countries. They also indicated that genetic diversity was greatest in among strains from Southeast Asia, suggesting that this region of host diversity was also a centre of pathogen diversification. Groups of genetically similar strains were usually found in only one or two countries. Thus, populations of the pathogen may be clonal.

An other species *Pseudomonas syringae* pv. *syringae* was isolated from branches and leaves with black blight symptoms in mango from Israel (Pinkas and Maymon,
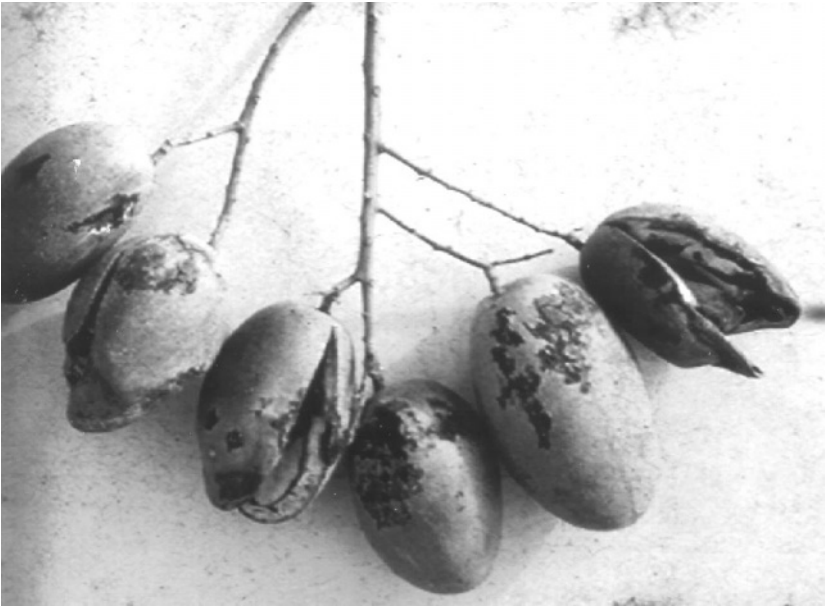
Figure 12: Severe symptoms of canker on Dashehari mango in India

1996).

Since most of the morphological, biochemical characters of *P. mangiferae indicae* were similar to *Xanthomonas* spp., hence Robbs *et al.*, (1973) renamed the pathogen as *Xanthomonas mangiferaeindicae*.

As per new system of nomenclature of phytopathogenic bacteria, Dye *et al.*, (1980) named this bacteria as *Xanthomonas campestris* pv. *mangiferaeindicae* (Patel *et al.*, 1948b, Robbs *et al.*, 1973). Characteristics of the causal bacterium have been published elsewhere (Monicom and Wallis, 1984).

3.1.4 Disease cycle

Xanthomonas campestris pv. *mangiferaeindicae* is an epiphytic colonist of leaves (Monicom, 1986, Pruvost *et al.*, 1990), buds (Pruvost *et al.*, 1993) and fruit (Pruvost and Luisetti, 1991) of mango. Bacterium survives in infected plant parts on the tree. Cankers on mango leaves are reduced by fall of infected leaves but pathogen was found to survive up to 8 months in diseased leaves (Kishun, 1981a,b). Twig canker initiates the infection on fruits. Bacterium is found pathogenic on *Anacardium occidentale, Acanthosperma hispidum, Caesalpinia mimosoides, Ficus glomerata, Lantana camara, Psophocarpus t*et *ragonolobus, Schinus terebenthifolius, Solanum tarvum, Spondias mangifera* and *S. mombin*.

Role of mango stones in the survival of pathogens has been established by
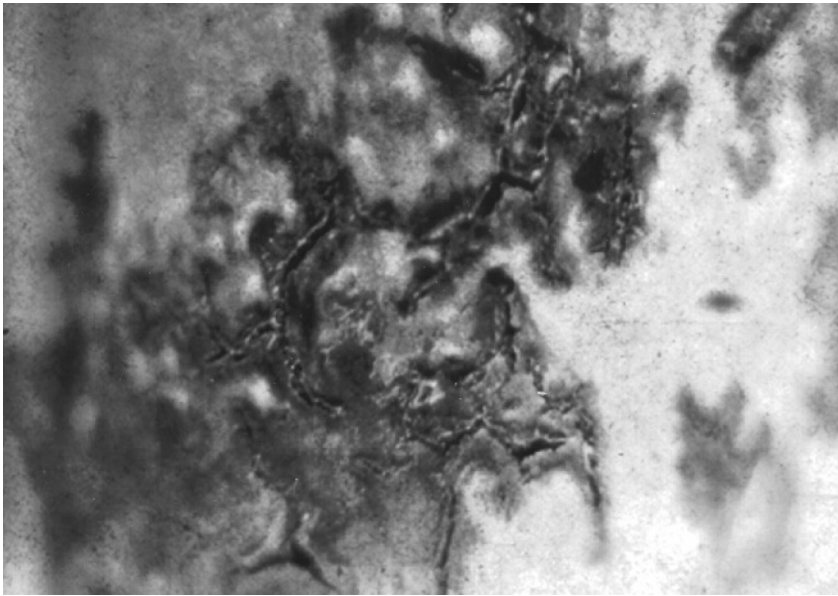
Figure 13: A close view of canker on mango fruit

Prakash *et al.*, (1994). Pathogen also suvives in resident form on weed hosts (Kishun and Chand, 1988, 1994) and mango leaves and fruits (Pruvost, 1990). Disease spread is rapid during rains. In new areas, the desease spreads through infecfted planting material and from diseased to healthy ones through wind splashed rains (Pruvost *et al.*, 1990).

Myllocerus discolor var. *variegata*, *Orthaga euadrusalis* and *Cantheconidia furcellata* (Kishun, 1986), *M. dentifer, M. undecipustulatus* var. *naculosus, Persa lepida, Camponotus compressus, C. sericus, Chrysocaris patricius, Drosicha mangiferae* and *Nezara* sp. (Kishun and Chand, 1989), *Schinus terebenthifolius raddi* (Pruvost *et al.*, 1992) are found associated in the mechanical transmission of the disease. In a bunch,

fruits are more vulnerable at contact points. Leaf and fruit infections are not correlated (Monicom, 1986, Pruvost *et al.*, 1990). The damage caused by MBCD is more on young mango tree while young leaves are resistant. The pathogen usually infects old leaves through wounds and rarely from stomata. There is a direct relationship between the level of disease on tissues and fruits (Manicom, 1986, Pruvost *et al.*, 1990). Thus, the susceptibility of leaves is viewed as an important criterion while selecting the cultivars for reduced disease development on fruit.

3.1.5 Epidemiology

Development of the pathogen in the field is favoured by high relative humidity (above 90%) and temperatures between 25-30ºC (Kishun and Sohi, 1983). Pathogen has been found to be more active under field conditions from July to September than from November to March. Though the temperatures from April onwards remain favourable (28-30ºC), fresh infections do not occur until it rains. However, in trees having infected twigs, infection on fruit starts early in the last week of April and continues to increase during May, when weather is dry (Prakash and Raoof, 1985, Misra and Prakash, 1988, 1992, Prakash *et al.*, 1994). Maximum and minimum temperature between 30-40 and 17.3-26.0ºC, RH 68-100%, evening RH 25-68% and high wind velocity during the month (April-May) have been found favourable for the disease build up (Prakash *et al.*, 1994).

In and around Lucknow, the development of symptoms on fruits was observed when fruits reached near to maturity whereas, Viljoen and Kotze (1972) had observed bacterial canker symptoms during the entire life of the fruit. The most favourable period for infection (on fruit) was May and becomes more severe in the first week of June in polyembryonic varieties. However, leaf infection was more severe during rains (Prakash *et al.*, 1994). During May end, severity of the disease on fruits/leaves varied considerably, as on fruits infection was upto 100%, whereas, leaf infection was 10-25 per cent.

3.1.6 Anatomical changes

Studies on bacterial canker affected parts of Neelum x Langra, Neelam x Dashehari and Bangalora have revealed that the pathogen enters into the mango leaves through stomata in fruits through stomata and lenticels and in twigs through lenticels (Shekhawat and Patel, 1975). It is an exclusively intercellular parenchymatous pathogen. It causes chloronemea, separation, collapse of the cells and forms bacterial pockets. Tanin increases in diseased tissues.

Lignin deposition as slow intercellular wood gum accumulation is profuse in stem and pitted scaby fruit cankers but scanty in raised ones. Kishun and Joshi (1986) while studying histopathology of stem, petiole and leaf of Alphanso and Raspuri, observed that bacterial infection promoted the phellogen activity and the wall of cork cells had a deposition of lignin like substances. Bacterial invasion increased the leaf thickness due to hypertrophy and hyperplasia. The xylem is partially plugged and phloem disintegrates. Several bacterial pockets are formed in the infected zone. Cell contents in the cankerous zone lack insoluble polysaccharides, cytoplasmic RNA and proteins.

3.1.7 Management

Regular inspection of orchards, sanitation and seedling certification are recommended as preventive measures against the disease. Selection of stones from healthy fruits for root stock is advisable (Prakash *et al.*, 1994). Cultivars Jahangir, Fazari and Suvernarekha (Kishun, 1993) are resistant, whereas Langra, Dashehari, Chausa, Bombai, Zardalu, Sunder Langra, Gulabkhas, Kesar and Mankurd (Sinha and Hoda, 1988) are moderately resistant and Siam, Panjang, Karuthkolambban, Apple and Sofinas are tolerant. After evaluating the world germplasm of mango at Central Institute for Subtropical Horticulture, Lucknow, Prakash *et al.,* (1994) classified the North Indian, East Indian and West Indian cvs. in order of their relative resistance. North Indian commercial cvs. viz. Bangalora, Bappakai, Jahangir, East Indian cvs. viz. Bombay Green, Fazari, Kishan Bhog, Scipia and Zardalu were free from canker in field evaluation. Several options are available for the control of canker. In areas with high disease pressure, resistant varieties should be used. In addtion, cultural measures, such as the use of wind breaks to reduce brusing/wounding and reduce inoculum load from the tree canopy management which provides some relief.

Serological identification of *X. campestris* pv. *mangiferaeindicae* was investigated by Sanders *et al.,* (1994). During western blot assays with monocolonal antibiotics (MAGs) raised against isolates of the pathogen. They observed different binding efficiencies between low and high virulence isolates. There was considerable cross reactivity between (MAGs) of *X. campestris* pv. *mangiferaeindicae* and other phytopathogenic bacteria, especially various pathovars of *X. campestris*. Since the probability of observing other than mango pathogens on mango was remote (Prakash, 1992). Agrimycin-100 (250 ppm) proved effective (Rao *et al.*, 1978) when used as fruit dip. Two sprays of streptocycline (200-300 ppm) at 20 days intervals (Bose and Singh, 1980), Streptomycin sulphate (250 ppm) followed by Aureofungin (Prakash and Raoof, 1985) and 3 sprays of Streptocycline (200 ppm) at 10 days intervals (Misra and Prakash, 1992) reduced the fruit infection. Streptocycline (300 ppm) and Copper oxychloride (0.3%) were found more effective in controlling bacterial canker (Prakash *et al.*, 1994). Kishun and Sohi (1984) reported that the disease can be reduced by monthly sprayigs of Bavistin (1000 ppm).

Stem injection of Bavistin (1000 ppm) in 3 to 5 year old mango seedlings has also been found effective (Kishun, 1985, 1988a,b). Kishun and Sohi (1984) have also tried the mixture of 2 chemicals, such as Copper oxychloride (3000 ppm) + Agrimycin-100 (100 ppm) and Bavistin (1000 ppm) + Agrimycin-100 (100 ppm) but Bavistin (1000 ppm) alone has been found better than these combinations. Garg and Kasera (1994) have reported essential oil from *A. occidentale* effective against *X.c.m.i.*

An antagonistic phlloplane bacterium *Bacillus coagulans* has been isolated from mango which has been found very effective agaisnt *X.c.m.i.* strains and may be further utilized in the biocontrol of mango bacterial canker disease (Kishun, 1994b). *B. subtilis* and *B. amyloliquifaciers* have been very effective and may be forther exploited for biocontrol of the pathogen (Pruvost and Luisett, 1989 & 1991). Wu *et al.*, (1980) have isolated the specific bacteriophage of the pathogen which can be tried successfully.

4. Diseases Caused by Algae and Lichens

4.1 Red rust

Red rust 'Algal disease' has been reported from Ceylon (Park, 1932), Florida (Ruehle and Wolfenbarger, 1949, Lynch and Mustard, 1950, Ruehle and Ledin, 1955), East Pakistan (now Bangla Desh), South Africa and Brazil (Batista, 1947) on 448 hosts. Its major distribution in India is the Tarai and other humid regions especially UP, Bihar, Karnataka, West Bengal, Maharashtra, Gujarat, Punjab, Haryana Orissa, Goa, and other states (Prakash and Srivastava, 1987, Prakash, 1996). An alga, *Cephaleuros* sp. is well known part of the lichen, *Strigula*, which is widespread in tropical and subtropical regions. *S. elegans* (Fee.) Mull. Arg. withh *C. virescens* has been found on mango (Prakash and Raoof, 1985). Thirumalachar (1945) reported that *Cephaleuros* may enter into a lichenous association with different fungi and he realized that the fungal component is parasitic on the alga and ultimately destroy the phycobiont.

4.1.1 Distribution

The available information indicates that *Cephaleuros* sp. is limited in occurrence to all Continents and probably all Islands between 32°N and 32°S, provided the temperature and humidity are suitable for their growth and reproduction. To date no studies have been undertaken to ascertain the exact climatic requirement of *Cephaleuros*. Most of the workers believe that warm and moist conditions favour infection and spread. More detailed information on the climatic factors that are conducive to algal development and spread, is needed to clarify these facts. The disease appeared in an epidemic form in the Tarai region of UP in 1956 and was reported to cause reduction in photo-synthetic activity and defoliation resulting in lowering the vitality of the plant (Prakash and Srivastava, 1987). Various aspects of disease have comprehensively been reviewed by Lele (1976). The disease causes the reduction in photsynthetic activity and defoliation which results in lowering the vitality of the plant.

4.1.2 Symptoms

The disease is readily recognised by the presence of the rusty red fructification of the alga on the surface of the leaves, veins, petiole and young twigs. Initially the spots are greenish grey in colour and velvety in textureand finally turn reddish brown. Spots are circular to irregular in shape, erumpent, measuring 2 mm in diameter when coalesce, they may be as long as 1 cm (Fig.14). After shedding of spores, the algal matrix remains attached to the leaf surface, leaving a creamy white mark at the original rust spot.

The upper surface of the spot consists of numerous, unbranched filaments in which some of the filaments are sterile hairs while others are fertile ones. The latter bear cluster of spores at the top. Such fruiting bodies are formed in moist atmosphere, that is why, the disease is more common on closely planted orchards. The parasite can only make headway when plants grow slowly. The alga is generally shed off by exfoliation of outer tissues when plants are vigorous. Thus, the disease is rare on newly emerging

shoots.

4.1.3 Causal organism

The disease is caused by the alga *Cephaleuros virescens* Kunze (syn. *C. parasiticus* Karst, *C. mycoidea* Karst) (formerly *Trentepohliaceae*, division Chlorophyta). The thallus is a pseudo-parenchymatous tissue, one to several cells in thickness, in which the cells are arranged radially. The upper surface of the algal mass bears numerous unbranched filaments which project through the cuticle. Some of these erect filaments are sterile
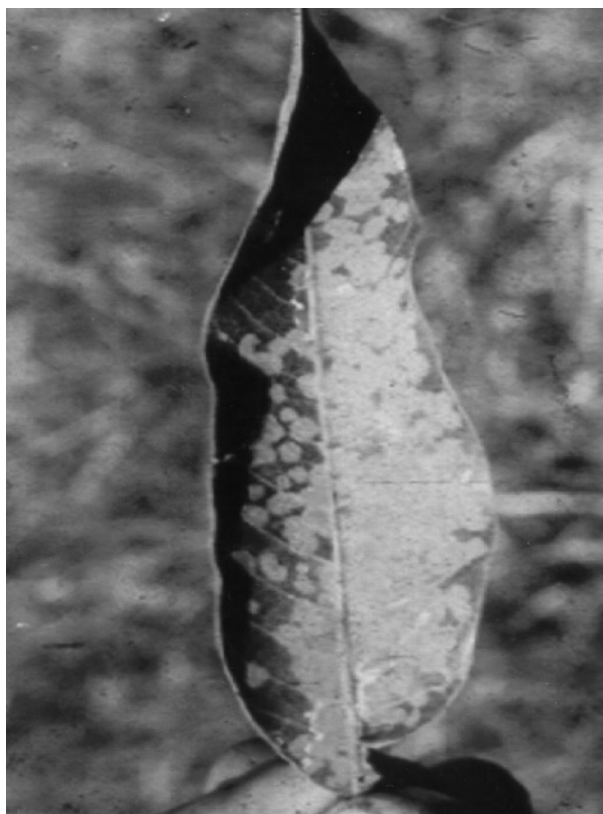
Figure 14: Typical red rust symptoms on mango leaf.

hairs while others bear a cluster of sporangia or gametangia at their apex. Asexual reproduction is by means of zoospores which are formed in sporangia produced at athe apex of fertile hairs. The thallus of the alga is a compact stratum and could penetrate upto the upper most layer of mesophyll tissue of the host. The sporangia are borne in clusters and each lies at the end of short stalk-cell. Mature sporangia break away and its dispersal is by wind. Under moist conditions, these sporangia produce biflagellate

zoospores. As soon as the sporangium falls on the leaf or twig, the zoospores germinate, produces a new thallus after it ceases swarming. In certain cases, the biflagellate gametes are formed within a gametangium resulting enlargement of certain cells in the pseudo-parenchymatous portion of the thallus.

4.1.4 Physiological changes in host

Vidhyasekaran and Parambaramani (1971b) studied the physiology of the disease infected leaf tissues and realized that both glucose and sucrose were less while fructose was more. Starch, cellulose and pectin were more in the infected tissues while lignin content was unaffected. They found the reduction in the total proteins, ammoniacal nitrite, amino and amide nitrogen content whereas, nitrates accumulated in the infected leaves. Glutamic acid and alanine increased markedly but glycine decreased sharply and valine content was not affected by the algal infection.

4.1.5 Spread of the disease

Environmental conditions have an important bearing on the infection process. Stem entry of zoospores, after germination of thier incipient thalli, is achieved by way of cracks in the newly formed bark of young hardening wood. Rain water has been found to be a source for spread of the alga infection and it has been found on increase during rainy season (Thrimurthy *et al.*, 1981). Growth and spread of *C. virescens* has been studied by Prakash and Misra (1988). Maximum temperature above 30ºC, minimum being around 25ºC with high RH and frequent moderate rains with high wind velocity are conducive for the growth and spread.

4.1.6 Management

The main stress for controlling alga is laid on correcting cultural malpractices and alleviating nutritional deficiencies. The direct link between host vigour and damage caused by the alga has been noticed.Avoidance of close planting is helpful. Thus, pruning the canopy, mowing beneath trees and using wider row spacing which increase air circulation and sunlight penetration help reduce conditions which favour the pathogen. Pruning and manuring of host trees is also be beneficial. Prakash and Singh (1979) recommended Bordeaux mixture (5:5:50) followed by Copper oxychloride for the control of algal disease. Sprays with fungicides viz. Difolatan, Bordeaux mixture and Copper fungicides (Thrimurthy *et al.*, 1981) algicides such as Fentin acetate (Lim and Khoo, 1985) have also been reported effective in managing the algal infection. Control of insect pests, mites and other foliar diseases, all increase the tree ability to cope with algal leaf spot.

4.2 Lichens

Lichens are found on full grown trees of mango, mainly on trunks, branches and twigs in areas of high humidity, heavy rainfall and poorly managed orchards especially Malda

region of West Bengal where 60-70 per cent incidence has been recorded on trees located nearby Farrakha embankment during 1980 (Prakash, 1980- report submitted to the Secretary, Agriculture, Govt. of West Bengal, India, Prakash and Srivastava, 1987, Prakash, 1996). Lichens are also observed in Tarai region of UP and other states viz. Goa, Maharashtra, Kerala, Andhra Pradesh, Orissa, Gujarat, Bihar, Karnataka, Tamil Nadu and Andaman & Nicobar Islands (Prakash, unpublished report). In general, trees with severe lichen intensity showed, poor growth. The trunk and branches be completely covered with their growth in old and reglected orchards. Lichens do not damage directely but give them an ugly look and unhealthy appearance.

Lichens are seen in the form of whitish, pinkish, superficial coverings of different shapes on the main trunk, branches and twigs of the trees. Association of lichens, *Strigula elegans* (Fee.) Mull. Arg. with alga *C. virescens* have been found on mango leaves (Prakash and Raoof, 1985) in Lucknow (India). Lichen intensity was very high on mango varieties viz. Paharpur, Sinduria, Puttu, Mundappa, Kalapady, Pairi, Madras-Apoos, Sukul, Langra, Bombai, Zarda and Zardalu (Chakravarti *et al.*, 1972). The lichens can be controlled by gunny rubbing followed by spraying the trunk, branches, twigs with commercial Caustic Soda (1.0%) without adversely affecting the trees (Chakravarti *et al.*, 1972).

5. Diseases of Unknown Etiology

5.1 Stem Bleeding

Mango plantation situated near Farrakka embankment in the Malda region of India are subjected to heavy water logging during rainy season. Stem bleeding type sysmptoms have been observed in varieties Kishan Bhog, Gopal Bhog and Himsagar (Prakash and Srivastava, 1987, Ploetz and Prakash, 1997). Reddish brown substance oozes from the stem cracks located approximately 30 cm above the ground level which dries up and form black crust. On scrapping, the infected tissues are yellowish brown in colour and discolouration can be seen. Large cavities are formed due to disintegration of infected tissues.

Thielaviopsis paradoxa, a strain of the pathogen isolated from sugarcane, infects mango when inoculated artificially (Sundararaman *et al.*, 1928). *Ceratocystis fimbriata* Ellis and Halst. [anamorph : *Chalara paradoxa* (DeSeyn.) Sacc., Synonym : *Thielaviopsis paradoxa* (DeSeyn.) Hohn.] Viegas, 1963, Piza, 1966, Ribiero, 1980) causing blight in mango has been reported from Brazil. Also called "Seca" or "Murcha" has been recognized since the late 1930s in Brazil. Similarly *Ceratostomella paradoxa* causing soft rot of pineapple is also found to be pathogenic on mango (Campacci, 1946).

Above pathogen has been reported to be associated with stem bleeding disease in coconut and Arecanut and might be involved with stem bleeding disease of mango too. In India, The cause of the disease is unknown. However, as a precautionary measure, growers have been advised to paste the tree trunk with Bordeaux paste or Copper oxychloride thrice in a year.

5.2 Bark Cracking

The disease is very common on old trees at Bijnore (U.P.) and Malda (W. Bengal). Younger trees less than 5-6 years of age are not affected. Unlike gummosis, bark cracking is characterized by the development of deep longitudinal cracks in the main branches of the tree trunk but without much exudation, measuring 15-46 cm in length. Rotting is not associated with the cracks but the underlying wood is severely pitted. Gum pockets are also noticed alongwith the cracks. Later, bark gets dried and pulled off resulting in girdling effects such as yellowing and shedding of leaves, and die back of branches. These symptoms have been noticed in mango cvs. Fazli, Himsagar, Lakhan Bhog, Ashina, Kuwan Pahar, Dashehari and Bombay Green (Prakash and Srivastava, 1987). The cause of the disease is unknown. Mango bark cracking disorder has been reported by Rio-Castano and Reuther (1967-68) in 16.6 % of the indigenous selection and 61.1 % of the imported varieties. Smith (1973) reported 93% of treesin someorchards affected by the disorder.

5.2.1 Management

Spraying of Copper fungicide on trees is recommended. Soil application of Copper 250-500 gm/tree may reduce the incidence of cracking. Removal of dead bark and pasting with Bordeaux paste is advocated in the orchards where the disorder is more common (Prakash and Srivastava, 1987).

5.3 Bark Scaling

Scaling of bark is observed at many places but more serious incidence of this disorder is seen at the Central Mango Research Station, Rehmankhera, Lucknow (India) in the rootstock trial where polyembryonic varieties were used as mother plant (Prakash and Srivastava, 1987, Ploetz and Prakash, 1997). In Colombia, a scaly bark disorder, "Cuarteado" was reported on four or more years old trees. The symptoms of deeply furrowed and cracked bark resembled those described by Cook *et al.*,. (1971). The cause of the disease is unknown. Micro-organism has not been isolated from such infected parts. Longitudinal scattered, cracks in the entire rootstock portion of trunk are observed resulting into scaling of bark (Fig.15). The outer layer of affected bark often curl and exudation of gum takes place. Such cracks are quite deep penetrating inside. The phloem tissues are affected and show necrosis, hypertrophy with reduced foliage. Plant may die if such condition prevails for longer period. The literature revealed that there is only one documentation on scaly bark of mango seedling from Islands of Hawaii and Oahu (Cook *et al.*, 1971). They could not isolate any micro-organism from affected parts. Mechanical transmission tests to common virus indicator, plants have been found negative. There is need to carry out intensive research on this malady.

5.3.1 Management

Pasting of rootstock (trunk) with Bordeaux paste or Copper oxychloride is advocated

(Prakash and Srivastava, 1987).

5.4 Woody Gall

In India, woody gall disorder is a problem in the cvs. Langra, Pairi, Gulabjamun in Malda (West Bengal), Hessarghatta (Bangalore), Uttar Pradesh (India) (Prakash and Srivastava, 1987). It has been reported from other countries viz. USA (Hawaii, Miami) (Cook *et al.*, 1971, Ploetz and Prakash, 1997), Mexico (Angulo and Villapudua, 1982), Venezuela

Figure 15: Longitudinal scattered, cracks on rootstock portion showing scaling of bark

(Malaguti and Reyes, 1964) and Puerto Rico (Rodriquez, 1995). On main branches, trunks, sizable galls occur, whereas, on the secondary branches, small galls are found. They are of various sizes, globose to elongate or irregular in shape. They are usually 25-40 cm in diameter with rough surface (Fig.16). A single branch/trunk may have more than 10-15 galls (Prakash and Srivastava, 1987). The bark from the soil line to the first branches was rough and scaly and 5-6 mm long xylem pegs, were evident when the bark

was removed around leaf scars and secondary branches (Ploetz and Prakash, 1997). Angulo and Villapudua (1982) reported a mango disorder known as "Nanahuak", "Bolas" or "Buba of mango", the disease caused small galls 5-10 cm in diameter. Galls were intially light green but became dark borwn with age resembled like cauliflower. The galls remained attached to branches for many years and heavy affected branches died slowly.Similar type of woody galls have been reported to be present in Hawaii mangoes (Cook *et al.*, 1971). They observed xylem pegs 5-6 mm long which resemble those associated with other virus infected woody plants. Such pegs are often found in the

Figure 16 : Woody gall symptoms on mango tree trunk

area of leaf or twig scars on secondary branches. They could not able to transmit the disorder on any virus incator plants. In Venezuela, Archibald (1961) studied the gall disease and found that mango galls are transmitted by inoculation of the axillary buds of 3 months old cacao seedling with tissue extract. Malaguti and Reyes, De (1964) isolated *Calonectria rigidiuscula* from trees/ seedlings of cacao and mango causing gall disease. In inoculation tests, gall formation is slower in mango than in cacao. A causal agent for gall disorder has been identified in Mexico. Angulo and Villapudua (1982) isolated *Nectria rigidiuscula* Berk and Broome (Synonym : *Calonectria*

rigidiuscula, Fusarium decemcellulare, C. Brick, synonym : *F. rigidiuscula* (Brick) Snyd. and Hans) from galls, and demonstrated the pathogenicity. The same fungus causes an important disease of cacao, (*Theobromae cacao*), the cushion gall disorder. It is wound pathogen and is associated with other canker and die back diseases on woody hosts in the subtropical and tropics (Farr *et al.*, 1989). In Homestead (USA), Agrobacterium tumefaciens strain T 37 caused galls on stems and leaves of mango after artificial inoculation (Ploetz and Prakash, 1997).

5.4.1 Management

Removal of galls from the tree trunk/ branch is advocated to the orchardists followed by pasting of Copper oxychloride paste mixed with compatible insecticide (Prakash and Srivastava, 1987). In India, so far no work has been done on woody gall disorder. More studies are warrented to understand the cause, etiology and to evolve effective control measures.

5.5 Crinkle Disease

Based on some unusual symptoms on mango plants, the disease has been named "Mango Crinkle Disease" (MCD). The symptoms observed on 4-5 years old grafted mango plants of different North and East Indian varieties initially suffered from gummosis.

5.5.1 Symptoms

Disease resembling to those caused by virusus included tall lanky side twigs arising from below the cut ends on the new flushes. Petiole of the tender reddish leaves got elongated. The leaves are narrow, develop various deformations including crinkling and curling (Fig.17). Observation on the leaves from the underside revealed swollen primary, secondary and tertiary veins which appear to be the cause of crinkling and curling of the leaves. Acute stage, the leaves developed necrotic patches of variable size. The twigs showed an arrest in apical growth leading to extreme stunting. Very often the deformations and reduction of the leaf lamina reached to extreme giving an appearance of "fern leaf" (Prakash *et al.*, 1985, Prakash ad Srivastava, 1987).

5.5.2 Transmission studies

Attempts were made to transmit the pathogen through sap inoculation on a few indicator hosts of viruses. The inoculum was prepared in conventional way with following modifications : (a) clarification of inoculuam by treatment with charcoal and celite (b) addition of chelating agents in the inoculuam *e.g.* 2-mercaptoethanol and sodium diethyl dithiocarbamate(c) clarification of the inoculum through organic solvents. Experiments with all the above type of modification in the inocula were unsuccessful to initiate disease symptoms on Nicotinana glutinosa, Chenopodium amaranticolor, Phaseolus vulgaris, Cyamopsis tetragonoloba, Cucumis sativus, Datura stramonium

and Gomphrena globosa.

Purification : With a veiw to concentrate and isolate the pathogen, 500 g of infected leaf material was macerated into pulp in a buffered solution followed by addition of butanol (8%) for clarification. Clarified samples were processed for purification either by differential centrifugation or through polyethylene glycol precipitation followed by differential centrifugation. Material so obtained by either of the procedures gave a UV spectrum typical of nucleo-proteins with an absorption maxima at 260 nm and minima at 242 nm. Preparations, however, could not be visualized under electron microscope to reveal the presence of virus particles (Prakash, 1982).

Fig. 17 : Mango crinkle disease showing crinkling and curling of leaves

5.6 Flat Limb

Soon after germination of mango seedling, a flattened stem with ridges and furrows produced needle like leaves giving an appearance of a broom. Occasionally symptoms appear after a few normal leaves have come out. Seedling does not grow further. Small outgrowths and necrosis of flattened stem is yet another expression associated with the disease (Prakash *et al.*, 1985). Affected leaves are thickened, curl downwards and occasionally exhibit dark green islands. The malady is also prominent in bigger trees as compared to seedlings. In this, the apical growth of the twig is restricted and twig are flattened and fleshy with ridges and scars. The apical end of the flattened stem is wavy and clusters of normal leaves emerged from the scattered edges. Interestingly above

one flattened twig another flattened portion also emerges as its branch.

5.6.1 Management

Removal and destruction of such branches are advocated. Pasting of Copper oxychloride at cut ends is essential to avoid the secondary infection of pathogens.

5.7 Fruit Tumour

Symptom of this disease is confined to fruits of polyembryonic cvs. of mango only, except that the tree showedgummosis. Symptoms on fruits included protuberate growths, starting near the tip and occasionally near the shoulder extending up-downwards. They increase in size and become pale in colour with the advancement of disease. Surface of such affected area is scaberulous which gives an ugly look. At acute stages, the protuberances (outgrowths) became necrotic, followed by deep cracks in the skin. Exudation of gum from the cracks is common phenomenon of the disease. Such necrotic skin turns brittle and dark brown and sloughs off easily in small chunks exposing the mesocarpic tissues. The flesh becomes musty, decayed and drops off prematurely (Prakash *et al.*, 1985).

6. Phanerogamic Parasites and Epiphytes

6.1 Parasites

The mango tree is the prey of many parasites and the most interesting of these are so called 'Phanerogamic parasites and Epiphytes'. Mango trees are commonly affected with these parasites particularly in the neglected orchards. They are commonly present on the trunk or branches of the tree, at times more than 100 plants parasitising a single tree especially in Malda region of W. Bengal, thus making the tree weak. In North India, more than 60-90 per cent of mango trees are affected by these parasites. The foliage infected host plant is sparse, reduced in size and its bearing capacity and quality of fruit is considerable lowered (Prakash and Srivastava, 1987).

It has been found to occur from tropical Africa in the west to the Solomon Islands in the east, Sri Lanka, Malaysia (Danser, 1935). In Malaysia, *Dendrophthoe* [formerly *Loranthus*] *pentandra* Linn. is the most important species (Lim and Khoo, 1985), other species in *Dendrophthoe, Elytranthe* and *Viscum* are also known in Malaysia, but less important. In India, *D. falcata* [formerly *Loranthus lingiflorus*] is most common and other less frequently encountered species include *Macrosolen cochinchinensis, Helicanthes elasticus* and *Elytranthe capitellata* (Ploetz and Prakash, 1997).

Since the appearance of parasitic plants is quite distinct from the mango host, they can be easily distinguished in infected trees. The points at which the mango host is penetrated are ausually characterized by swollen growths called burrs. The burrs aid the identification of sites at which the parasite has entered the host, an important feature while controlling these plants. The phanerogamic parasites are present through-

out, both in the hills and plains, but the epiphytes are confined to cold places, mostly at the foot hills except *Ficus* species which are reported to be present even in the plains.

The family, Loranthaceae contains several species of parasitic plants which affect mango tree. *Dendrophthoe falcata* (L.F.) Ettings, a member of the family Loranthaceae is most destructive and common pest of the mango in India, which is distributed in wide areas. Besides this, five other species of the Loranthaceae viz. *Macrosolen cochinchinensis* Van Teigh, *Elytranthe capitellata* Engl., *Helicanthes elasticus* Dans, *Viscum articulatum* Burm. and *Viscum monocium* Roxb. are reported to parasitize the mango (Fischer, 1926). Of the two genera, *Dendrophthoe* [*Loranthus*] and *Viscum*, the former is a very common parasites on mango.

In India, Hooker (1980), Gamble (1922), Singh (1952) reported its occurrence from river Jhalum eastwards in the Himalayan tracts in Kumaun to a height of 1,500 ft. above sea level and attacks 256 different species of hosts in India (Singh, 1956). In India, it has been found in East and West Bengal, Madhya Pradesh, Uttar Pradesh, Orissa, Andhra Pradesh, Tamil Nadu, Maharashtra, Gujarat, Goa, Bihar, Haryana, Punjab and Kerala (Ryan, 1989, Singh, 1956, 1960, Prakash and Srivastava, 1987).

Among other species, *M. cochinchinensis* is reported from Southern and Eastern parts of India but it is also encountered in other parts of the world viz. Borneo, Sumatra, Java, Malaysia, Philippine Islands, southern China and Myanmar (Danser, 1935) and rest of the parasites are very common in Myanmar, Malaysia, India, Tropical Africa and Philippine Islands.

In Australia, only two parasites viz. *Viscum articulatum* and *V. monocium* are very common and involved as parasites of mango (Singh, 1960). The fruits of Loranthaceae are baccate, with a viscous mesocarp and can be easily dispersed by the agencies like animals, birds (honey birds), squirrels and by explosion of their fruits (Singh, 1954, Ryan, 1899, Sahani, 1933). These seeds germinate and produce haustoria. The penetrated host tissues sometime swell to form tumours.

6.2 Epiphytes

In areas of high rainfall, numerous epiphytes, flowering plants, orchids, ferns, mosses and several species of *Ficus* are reported to grow on the stems and branches of mango, both on seedlings and grafted plants. *Ficus* species are mostly restricted to neglected and very old trees. But orchids do not harm the host plant. Among these, *Ficus* species are reported to be common in South-East Asian countries including Myanmar, Sri Lanka and India and they do not have any respect to their host plant. The host is needed by them only just for support in the beginning of their growth, as they require organic matter and soil which are easily available in the crevices, cavities and forks of the host plants (Singh, 1960). *Ficus* frutis are liked by the birds and seeds of *Ficus* pass out in their droppings uninjured which can germinate easily on the host plants. Later on, aerial roots arise from these seedlings and form a network of thick woody roots enclosing the trunk of the parent tree in the mass of *Ficus* props. The host dies due to heavy pressure of the outer covering.

Trees killed in this manner are acalled 'nyaughat' in Myanmar (Brandis, 1874). Among the *Ficus* species reported, some are very common and most destructive.They

are *Ficus parasitica* Koem, *F. lacor* Buch-Ham, *F. rumphii* Blume, *F. bengalensis* L. and *F. religiosa* L. (Singh, 1960).

6.2.1 Management

All epiphytic plants can be removed easily in early stages, before their aerial roots penetrate the ground and surround the host trunk. The affected branches should be cut suffciently to eradicate the haustoria. In early stages, it can be removed easily. If small bit of haustorium is left in the host it regrows with renewed vigour and soon starts damaging the host as before. Lim and Khoo (1985) indicated that control is best achieved by cutting out affected portions of tree far enough below burrs to remove haustoria of the parasite. Cut surface is treated with creosote or wound or other wound dressings. The cut ends should be protected with Bordeaux paste or copper oxychloride spray to prevent the secondary pathogens infecting through wounds (Prakash and Srivastava, 1987). Injection of copper sulphate and Feronoxone and 2-4 D (2%) in the affected branches are quite effective (Kadambi, 1954). The efficacy of chemical injected inside the host also depends on the depth, number of bores, age and size of the host tree (Singh, 1960). Nair (1964) reported that 2-4 D (0.5%) was effective in killing the parasite by two applications at 10 days interval. However, herbicide treatments may be lethal to the host plant, their effective dosage needs to be investigated. Spraying of emulsion of diesel (30-40%) in soap water should be done as it was found effective in eradication of the parasite found on mango (Singh, 1960).

7. Mango Disorders

Exposure of mango fruits to various environmental and cultural situations often leads to tissue abnormalities, which are quite distinct from those induced by pathological and entomological agents. These types of aberrations are generally termed 'physiological disorders'. They are mostly the results of some form of physical damage or disturbances in physiology. Physiological disorders are essentially the results of imbalances in metabolism induced by some factors in the preharvest or post harvest environment that leads to cell collapse and the appearance of water soaked or brown areas on some part of the fruit outside/inside. Preharvest factors that have been found to predispose mango to physiological disorders include growing location, orchard condition, tree nutrition, and condition at harvest while post harvest storage conditions such as temperature, oxygen and carbondioxide levels, packaging and surface coating treatments are contributing factors to the occurrence of the disorders. Important physiological disorders recorded on mango are discussed hereunder :

7.1 Black Tip

The black tip disorder was first published in 1908 by Woodhouse (1909). The then Economic Botanist to the Bihar Govt., he wrote, "Round Bhagalpur, mangoes do not seem to be much affected by the disease but this year much fruit is said to have been spoilt at the Sabour farm by the smoke from the brick kilns which apparently blackens

the apex of mango and interferes with its development". Indeed, this disorder affects the developing fruits rendering them unfit for marketing and thus causes considerable economic loss to the orchardists. Subsequently, the disorder was reported by Naik (1934), Allan (1936) and Pal *et al.,* (1937). In Uttar Pradesh, the disorder was noticed in 1923 by P.K. Dey in the name of "Kueli" (Das Gupta and Verma, 1939a,b, Das Gupta, 1940-51, 1957). The malady has generally been reported to occur in West Bengal, Uttar Pradesh, Punjab, Haryana, Delhi and Madhya Pradesh in the orchards situated in close proximity to a brick kiln. Luckily, South India is free from this malady which may be due to the fact that modern kilns (Bull's Kiln) do not exist (Sen, 1943). Recently, the disease has also been reported from China (Zhang *et al.*, 1995). Researches were carried out by Das Gupta and his associates on the disorder (Das Gupta and Verma, 1939a,b, 1940, Das Gupta and Asthana, 1944, Das Gupta and Sinha, 1944, Das Gupta and Agrawal, 1947, Das Gupta *et al.*, 1950, Das Gupta and Sen, 1958 and Prakash, 1975-76, 1978-79, 1996). It was concluded by the studies that the malady (Black tip=BT) was not associated with any pathogen but it was closely associated with nearness to brick kilns and gases emanating from the kiln as fumes. Recently, the causative factors of BT disorder in mango has been studied in China by dipping fruits in 150 or 600 mg fluoride/kg solution induced symptoms similar to that of the black tip disorder (Zhang *et al.*, 1995).

7.1.1 Damage

The brick kilns smoke is believed to be the cause of 'black tip' disorder of mango, where the damage can be traced, if a brick kiln is found in the vicinity (Sen, 1941, 1943). He reported the beautiful orchards producing healthy fruits before a kiln existed in the area and observed as soon as the kiln started functioning in the vicinity. Again the same orchards recover when the kiln is abandoned. The injury takes place from the exterior and not through the root as a medium, and it is made to manifest by causing plasmolysis, derangement of chloroplast, cell disintegration and a deficiency in food supply (Blakke, 1913). The damage caused by this disorder has assumed alarming situation in recent years in North India especially around the town and industrial areas. An attempt was made to ascertain the total percentage of mango crop damaged annually in all the mango growing provinces in India by inviting figures from departments but no figures were available because of lack of organised survey in the affected provinces. The extent of damage in the affected orchards varied. Dashehari suffered most, the damage being 100 per cent (Das Gupta and Verma, 1939a, Prakash, 1978-79). The extent of damage in orchard depends on the varieties grown and the existence of resistant varieties which lower it to minimum.

7.1.2 Losses

The financial loss, it should be remembered, does not depend solely upon the amount of the crop damage but also upon various other factors, namely the total production, demand etc. of the mango for the year under consideration. Before the incidence of the disorder, the mango crop in different orchards used to be sold at a higher price. Since the advent of the disorder, there was a marked drop *i.e.* 35 percent or more in the income

of the orchardists which compelled the orchardists to abandoned the orchards (Das Gupta and Verma, 1939b). Verma (1950) estimated the loss due to this disease was 25 per cent. Fruit specialist from Punjab reported that in some trees, there was almost cent per cent loss of fruit due to disease in Jagadhari. Losses due to BT disease, during the year 1978, created a history in Malihabad and Kakori areas of Uttar Pradesh, where about 50-60 per cent mango crop was destroyed (Prakash and Srivastava, 1987, Prakash, 1996).

7.1.3 Symptoms

Figure 18: Black tip disorder of mango fruits

Symptoms on the mango are found to become apparent when the fruit reaches certain size. The disorder is noted earliest by April-May, when the fruit is about 1 cm long. The first symptoms of the disorder is the development of a small aetiolated area at the distal end of the fruit against the general green colour of the skin, which gradually spreads, turns nearly black and covers the tip completely. Before the aetiolation is complete, isolated greyish spots appear, which become dark brown, enlarge and coalesce into a continuous necrotic area. At this stage, the affected mango fruit shows three distinct regions externally : (a) the healthy green part which constitutes the major portion of the fruit, towards the stalk end, (b) the necrotic part, a much smaller area than former, at the distal ends, (c) a narrow zone between the two major areas, which consists of the

aetiolated skin. Finally, the tissues decay and necrotic portion collapses. The tip is pulpy (although rest of the fruit is compact, unripe and hard) and slightly sweet, but far different from the usual taste of the ripe fruit. The necrotic area is always restricted to the tip of the fruit. The pericarp and mesocarp disintegrated exposing dark brown flesh beyond which the stone protrudes with a layer of collapsed tissues over it, the thickness of the layer depending upon disintegration of the mesocarp tissue (Fig.18). Although the whole mango may not become necrotic, the apparently healthy portion - the portion which is not directly affected, looses the taste, do not ripen in storage and becomes unfit for human consumption. The disease is accompanied by gummosis (exudation of drops of gums from the necrotic region). Gummosis is most copious in the 'Gola Mohanbhog' variety. Prakash and Srivastava (1987) studied the effect of fumes on the number of days taken from one stage of symptom to another stage, which differed considerably, hence one could know the number of the days taken from one stage of symptom to another stage of BT. Zhang *et al.,* (!995) observed symptoms in the vascular bundles in the pedicel of BT fruits. The freshy picked affected fruits secreted no milky sap from the cut zone of the pedicel. Further in cases, the vascular bundles had turned brown and decayed. In contrast, healthy fruit did secrete sap when picked on prematurely and the vascular bundles looked fresh.

The metabolic changes and the quality of BT affected fruits were also investigated (Das Gupta and Agarwal, 1947, Agrawal *et al.*, 1960, 1961, 1962). The catalase and peroxidase activities were more in apical portion than other region of the fruits. Significantly decreased ascorbic acid content was recorded in BT affected part of the fruit. BT affected fruit part contains more of Ca, Mg, P, Mn in Safeda Malihabadi and K, Fe, Cu in Taimuria mangoes than other part of the fruit. P and Mn were particularly high in apical portion. No definite relation in nutrient elements of apical portion of healthy and BT affected fruit was found. Different strains/variants of this disorder was also reported by Das Gupta and his associates, namely, taper tip (Das Gupta and Verma, 1939a), tip pulp (Verma, 1950) and girdle necrosis (Rai, 1958).

7.1.3.1 Taper tip

The taper tip occurs at the distal end of the fruit showing an intensification of the normal green colour, tapering abruptly and often curved, causing the affected fruits to remain smaller than the normal ones and are easily detachable from the stalk. It is more common in Dashehari mango and almost 100 per cent fruits are affected in some of the orchards (Das Gupta and Verma, 1939a, Prakash and Srivastava, 1987). It is also found in the cultivars Bombay Yellow, Bombay Green, Gola Mohanbhog and Lucknow Safeda. The necrosis may occur in fruit already affected with taper tip. The aetiolation of the epicarp associated with necrosis makes its appearance at the distal end already affected with taper tip which is removed by the formation of brown patches and ultimately disintegration of the tissues.

7.1.3.2 Tip pulp

The first visual symptom is the yellowing of the fruit tip, turning greyish later on. The

tip becomes pulpy while remaining part of the fruit remains compact, unripe and hard. The affected fruits are slightly sweet but have different taste than the normal ripe fruit. The affected fruits do not ripen in storage and cause a complete loss to the grower. The fruits turn prematurely pulpy and darker brown. About 25 per cent loss of fruits nearing in maturity in cultivar Lucknow Safeda has been reported (Verma, 1950).

7.1.3.3 Girdle necrosis

The earliest symptoms appear when fruits are of very small size (5-10 cm) (Rai, 1958) with disfiguring of their lower halves having small aetiolated spots and appearance of brown dotted aetiolated area. Brown dot-like portion collapses and enlarges to form necrotic lesions. The lesions further extent to form necrotic girdles of tissues around sinus region of the fruit leaving the green tip healthy in the initial stages. At advance stage, the tip also turns necrotic and there would hardly be any difference from the symptoms of black tip and girdle necrosis at this stage (Rai, 1958). The disorder is named as girdle necrosis because necrotic areas make more or less complete green tip region of the fruit. The disorder may not pass though, all the stages described above. The seed is protruding beyond the flesh with layers of collapsed necrotic tissues over it. In rare cases, at advance stages, necrotic regions of the fruit may ooze whitish brown gummy substances before the cell of epicarp show discolouration. The mesocarpic tissues are already disintegrated and form a cavity underneath necrotic spots. Thus, in girdle necrosis, mesocarpic tissues are affected prior to epicarpic tissues. The mesocarpic cavity enlarges, and becomes continuous below the epicarp in sinus region of the fruit which eventually collapses exposing the seed covered by a thin layer of necrotic tissue. Rai (1958) reported that the girdle necrosis differs from black tip as girdle necrosis may occur at any place in the sinus region separating the non-necrotic girdle extends to the tip. At advanced stage, it can not be differentiated from BT. Besides these differences, (Rai, 1958) believed that the girdle necrosis and BT were also caused by Brick kiln fumes because he observed girdle necrosis in orchards close to brick kilns as histopathological changes were similar in fruits affected by girdle necrosis or BT without any involvement of pathogen in such fruits.

7.1.4 Factors responsible for disorder

7.1.4.1 Brick kiln fumes

Investigations have shown that the brick kiln fumes are responsible for black tip disorder (Sen, 1941, 1942, 1943, Das Gupta and Verma, 1939a Ranjan and Jha, 1940, Srivastava, 1964, Prasad and Singh, 1965, Pal and Chadha, 1980, 1993, Ram, 1989, Zhang *et al.*, 1995, Prakash, 1996), since, it has been reported from orchards near brick kilns. Sen (1943) found damage as soon as the kiln started operating in the vicinity of the orchards which previously used to produce healthy fruits. These orchards recovered when the kilns were abandoned. An inverse relation between incidence of the malady and the distance from brick kilns was also reported. The maximum distance for the damage has been given as 630 m but cases of damage upto 1600 m are also reported (Singh, 1960). Of the

coal fume gases, sulphur dioxide, ethylene, carbon monooxide and fluoride are especially toxic to the fruits but sulphur dioxide causes the maximum damage (Ranjan and Jha, 1940, Zhang *et al.*, 1995).

During surveys conducted in 1978, 1980 (Prakash, Pal and Sahay, unpublished) covering 150 orchards in Kakori, Rehmankhera, Malihabad, Mall, Rahimabad, Chinhat, Barabanki, Alamnagar, Kanpur Road, Rae Bareli Road, Sitapur Road and Kursi Road, brick kiln fumes were found to be the major cause of this disorder. Its incidence was more in east-west as compared to north-south direction of the brick kilns.

Khader *et al.,* (1988) worked out index to quantity the intensity of BT in a given orchard or locality, considering the degree of damage on individual trees. The disorder was found to be severe within 2 km of the brick kiln which decreases with increase in the distance. The orchards within 1 km distance from the kiln in east or west were most vulnerable (75%) whereas those in north and south showed less than 15 percent incidence. Similar conclusions were also drawn earlier by Sen (1943). The height of the chimney also affects the extent of damage. It is observed that shorter the chimney, larger the damage.

7.1.4.2 Climate

The direction and velocity of the wind also plays an important role in degree of incidence of the disorder. Srivastava (1963a, b, 1964) noticed that in years of severe westernly winds at the time of fruit development, the fumes caused damage even to the orchards situated about 1.6 to 2.4 km from the kiln although severe damage was noticed up to 150 m. Sen (1943) found that the damage was more severe in orchards situated on the eastern and western sides of the operating brick kilns.

7.1.4.3 Tree vigour

The brick kiln fumes do not seem to have much effect on the vegetative growth of the mango trees. However, in the trees situated near the periphery of the brick kiln, foliage showed twisting and epinasty. But their productive vigour was not affected (Srivastava, 1964).

7.1.4.4 Histopathology

The histopathological changes brought about during the incidence and spread of the BT disorder has been studied by comparing healthy and affected tissues (Das Gupta and Sinha, 1944, Das Gupta and Asthana, 1944). A deposition of brown substance in lumen of vessels of outer mesocarp at distal end of the fruit was seen. The brown discolouration spreads to the neighbouring parenchyma, while the deposits also appears in the ducts. Generally the browning and the deposits extend throughout the mesocarp, the affected cells disintegrating and coalescing to form a dead tissue. The necrosis in badly affected cases, extends to the endocarp. The epidermis breaks at certain points, the break continuing in the neighbouring mesocarp forming fissures in the fruit, through which the brown deposited substances oozes and later harden to a

dry mass.

Fresh healthy fruits from healthy orchards have not revealed the presence of deposits. Neither have any deposits been observed in preserved healthy fruits. The unpreserved (fresh) infected fruits showing early stages of necrosis and show deposits only in rare cases but in later stages of necrosis, deposits are found in large quantities in the necrotic region and in ducts and vessels slightly around the region. Preserved diseased fruits show copious deposits both in ducts and xylem elements. In normal fruits, the tannins which formed are used up by the fruit itself. But when there are some metabolic disturbances due to changes in atmospheric condition caused by fumes emanating from a kiln or other factors such as deficiency of an important element, these tannins are stored up in fruits in the form of an unutilized tannin compounds.

The appearance of deposit is the first internal index of the disease long before the aetiolation at the distal end becomes externally visible. As the deposits increase, the vessels become clogged and the supply to the distal end of the fruit is partially cut off and disease starts. The deposits are translocated to the distal end where they accumulate and eventually burst the tissues. Thus the lack of supply, accumulation of deposits and consequent metabolic and histological changes bring about the disease condition in the mango fruit. In earlier stages of necrosis, deposits occur only in vessels, in later stages, they occur both in vessels and ducts, while in very advanced stages, deposits occur in the parenchyma. Maximum deposit concentration occurs in the tip region of mango when the symptoms of necrosis first appear. They are less copious in the upper shoulder (base) and relatively poor in the lower shoulder and trunk regions and vessels and vessels of stalk of necrotic mango.

7.1.4.5 Chemical nature of deposits

The deposits are found not to be reacted upon by concentrated sulphuric, hydrochloric or nitric acids. Alkalites also have no effect on them even on boiling. They are not soluble in any of the organic solvents like acetone, ether and chloroform. From the chemical nature of the deposits, it appears that probably some kinds of tannins, phlobatnnins or phlobaphones or their derivatives are responsible. Deposits are light yellow fluid, and they pass from viscous-deep yellow to semi solid and solid, brick red types. In final stages of disease, solid vascular deposits become dark brown. Ductular deposits in fresh mangoes are stratified solid, bright brown, occasionally reddish but never yellow. In formalin preserved mangoes deposits are found oily, shining red (in ducts only) and solid lump like in various stages of aggregation both in vessels and ducts.

Pal *et al.,* (1937) chemically examined the infected fruits and reported them physiologically more ripe than uninfected ones. Such ripening process before the maturity of the fruit causes early senescence and consequent shedding.

7.1.4.6 Biochemical studies

Agarwala *et al.,* (1960) investigated the effect of black tip disease on the catalase and peroxidase activity of 7 to 8 week old fruits of cultivars. Dashehari, Malihabadi Safeda

and Tamboori. In general, the peroxidase activity of the three varieties was almost in the same order, the catalase activity of Tamboori and Dashehari varieties was appreciably lower than that of the Safeda variety. The catalase and peroxidase activity of the upper (proximal 2/3) and the apical (diseases or its corresponding) part of the healthy and diseased fruits were measured. In the diseased fruits higher activity of catalase and peroxidase was found in the apical (necrotic) portion than in upper portion. No significant difference in the activity of the two enzymes was found in the upper and apical parts of the healthy fruits. The activity of catalase in Tamboori and peroxidase in both Safeda and Tamboori varieties was significantly higher in the apical part of the diseased fruits of advanced stage of necrosis than in the fruits showing necrosis of the extreme apical end. As in case of tannins, carbohydrates and total titrable acids (Agarwala, 1947), ascorbic acid and respiratory enzymes (Agarwala *et al.*, 1961), the effect of the disease on the two tron prophyrin enzymes, catalase and peroxidase, is largely confined to the apical (necrotic) part of the fruits, the increase in the enzyme activity appears to be related to the severity of the disease (necrosis). Increase in the activity of the two enzymes would suggest either an enhanced rate of synthesis or these enzymes in the diseased tissue or the destruction in diseased tissue by some inhibitor, normally present in the healthy fruits. The effect of ethylene and sulphur dioxide gases on the respiratory rate, sugar and acid content of the mango fruits was studied by Ranjan and Jha (1940). They reported that ethylene air mixture accelerated (after an induction period of 15 hr. or more depending on the age of the fruit), the respiratory rate and increased sugar content but it did not affect the total acid content of the fruits.

7.1.4.7 Nutritional studies

The length wise distribution of potassium, calcium, magnesium, phosphorus, iron, manganese, copper, molybdenum and boron in the healthy and diseased fruits of Malihabadi Safeda and Tamboori varieties of mango has been studied by Agarwala *et al.,* (1962). The tissue concentration of potassium, magnesium, phosphorus, manganese, copper and boron in the two varieties of mango was of almost the same order. The concentration of calcium, iron and molybdenum, particularly that of calcium, was appreciably high in the Safeda variety. There was an accumulation of calcium, magnesium, phosphorus and manganese in the apical part of the mango fruits of both Safeda and Tamboori varieties and of potassium, iron and copper in the Tamboori variety only. The tissue concentration of phosphorus and manganese in the apical part of the two varieties of fruits was particularly high. The molybdenum and boron content of the apical part of Safeda and Tamboori fruits was lower than the upper part. Though the mineral nutrient element composition of the apical part of the two varieties of fruits studied was generally appreciable and at times significantly different from the upper part of the fruits. Yet the absolute tissue concentration of none of the elements studied appears to be indicative of a mineral nutrient toxicity or deficiency being the direct cause of black tip disease.

On the other hand, it is not unlikely that the accumulation or a lower concentration of particular nutrient element in the apical end of the fruit might be a result of some other metabolic derangement similar to or identical with the one causing the disease. In

general pattern of mineral nutrient element distribution in the mango fruits was gener-
ally not markedly, affected by the disease.

7.1.4.8 Varietal susceptibility

Marked differences in varietal susceptibility have been studied by Sen (1943) who
suggested that the differences are connected with the number of fruit lenticels per unit
area of the skin. Varieties with a high proportion of these structures are more suscep-
tible to the disorder than the others. Further, lenticels per unit area of the skin, are
always more preponderant in the apical region of the fruit than elsewhere, as the cells in
that part show relatively smaller enlargement in size. It is not, however, considered
likely that preponderance of lenticel at the apex is the cause of initiation of the black tip
affect at the point, although it may supplement to some extent, a corsory examination of
the fruits suggested that the varieties which were more susceptible to black tip had less
hardly appearance. Work on the thickness of skin of the fruit of seven varieties revealed
that Calcutta-Bombai shows the thinnest skin and Langra the thickest. He further re-
ported that the thickness of skin of the fruit of different varieties do not show any
concommitant relation to their susceptibility to black tip damage. The rate of increase in
size of the fruit and the presence of relatively larger number of lenticels at its lower end
may also have to play some part in the process of development of the disorder (Sen,
1943). Das Gupta and Verma (1939a,b) observed that the black tip occurred in all the
orchards near Lucknow, India except Malihabad proper mango belt area. Similar results
have been obtained when survey was made in 44 orchards around Lucknow. Black tip
intensity was very less in Malihabad block (Prakash and Srivastava, 1987, Prakash,
1996). Dashehari variety suffered most (100%). Of other varieties, Gola Mohanbhog,
Fazli and Langra came next in order, with an estimated loss of 90 per cent. Khajuri shows
a loss of 75 percent and Amin Safeda (Lucknow) and a certain Bombai variety about 50
percent. Loss in the Malda variety was only 10 per cent. there was no immune variety in
the orchard. In other orchard, there were two completely immune varieties namely,
Safeda (Narma) and Taimuria. Among the varietal differences in the resistance, sub-
varietal differences were also observed by Das Gupta and Verma (1939a). In the Safeda,
different sub-varieties were found susceptible to varying degree. Thus, Safeda
(Lucknow) showed about 50 per cent. Safeda (Malihabad) 10 per cent while Safeda
(Narma) was completely resistant. Similarly among three sub-varieties of the Khajuri,
the loss was 75 per cent in two sub-varieties and 10 per cent in another. There was a
varietal difference in the resistance of the Tukhmi (Tukhum=seed persian, growing from
seed) mangoes. Among the Tukhmi varieties, Pautahia and Lambauri were observed
susceptible and four other varieties viz. Baheli, Sahinia, Basindha and Paharia were
almost completely free from the disease. They further reported that the Tukhmi trees in
the garden grown in pairs in the two members of each pair belong to a different variety
viz. Pautahia grows with Basindha and Lambauri grow with Baheli. The first mentioned
member of pair is in each case susceptible. It is a conclusive proof of the varietal
resistance of the necrosis, since in both the cases, the members are growing in the
identical edaphic and climatic conditions. Singh (1961-62) examined 7 varieties of mango
for black tip disease and found that Dashehari was the most susceptible and Safeda the

least. Naurial *et al.,* (1969) found Langra, Dashehari and Gola to be highly susceptible while Fazli was moderately susceptible. Prasad *et al.,* (1969-71)and Anonymous (1960) reported Dashehari variety was most susceptible while Lucknow Safeda was not at all or least affected. Prakash and Srivastava (1987) found Dashehari to be the most susceptible variety whereas Lucknow Safeda, Taimuria, Mallika and Langra were found less susceptible.

Damage of black tip could be traced on Bombai, Langra, Fazli, Zardalu, Himsagar, Gulabkhas, Bharat Bhog, Kumar Pahar, Shukul, Darma, Jalibandha, Jalsain, Kadua Lilkalmi, Golbhadatya, Kaitki, Kapuria, Banka, Arhulwa, Lanbabhadri and Mohanbhog varieties of mango (Sen, 1943).

7.1.5 Causes

Das Gupta and Co-workers (1939, 1940, 1941), Verma (1950, 1952 and Prakash and Srivastava (1987) confirmed that no parasitic organism like bacteria or fungi are casually related to black tip of mango. They did not find the virus connected with it too. Neither do they think that vigour of the tree or soil condition is responsible for the damage. Trees otherwise perfectly healthy show black tip on their fruits. As regard soil, the damage is recorded in diverse localities where the soil conditions are different, but the disorder is quite common in the immediate vicinity of brick kilns.

It is popular belief that the disorder is due to deleterious effect of fumes arising from brick kilns operating in the vicinity of mango orchards. This view is also held by many prominent workers (Woodhouse, 1909, Naik, 1934 and Allan, 1936). Now it has been confirmed by the majority of the workers that brick kiln fumes emitted from the burning of coal are harmful to the mango fruit and are responsible for causing black tip disease (Das Gupta and Verma, 1939a,b, Sen *et al.*, 1943, Sen, 1941, 1942, 1943, Pal *et al.*, 1937, Verma, 1950). This is further supported by the fact that the disorder is not reported from the South India where modern kilns of type used in Northern India are not in use. Of the gases in coal fumes, sulphur dioxide, ethylene and carbon monooxide are especially toxic to the plants and of these sulphur dioxide caused the maximum damage (Ranjan and Jha, 1940). The effect of Sulphur dioxide increases with increasing concentrations of gas and time of exposure (Sen *et al.*, 1943, Das Gupta *et al.*, 1941). In China, the bricks, mainly made of clay, emit about 70 percent of their hydrogen fluoride (HF) and silicon tetrafluoride into the atmosphere while drying in the sun and baking in the kilns. The fluoride content in coal used as fuel is rather high (generally 40-300 mg kg^{-1} upto 1400 mg kg^{-1}) and releases 78-100 percent of its fluoride (F) content when burnt (Zhang, 1984, Zhang *et al.*, 1995).

The ethylene at strong concentration (1:100) produced characteristic symptom, while in greater dilution, it causes more rapid respiration, increase sugar content and softening of tissues. The damage is found to be in inverse rates to the distance of the orchards from the kilns. The maximum distance for the damage has been observed to be 630 meter but cases of damage upto 1500 meter are also reported (Singh, 1960, Sen, 1943) when more than one kiln work at one place and wind blows in one direction for a number of days. The volume of smoke emitting from one place, the course and velocity of wind, level on which kilns and orchards are situated and presence or absence of

vegetation or other objects that may act as screen between kilns and orchards are the factors likely to determine the distance upto which the deleterious effect of kilns fumes on mango can reach. Smoke injury is known to extend upto several miles away from the source of the smoke (Blakke, 1913, Hiksch, 1934, Zimmerman and Crocker, 1934) but about a mile away from brick kilns would appear to be quite safe.

Wind direction and velocity plays an important role in increasing the incidence of black tip. In years of severe westernly winds, it was noticed that the fumes caused damage even to the orchards situated at about 1.5 to 2.25 km. in the eastern direction of the kiln. The foliage of the trees situated near the perimeter of the kiln showed twisting and epinasty of leaves, however, the productive vigour was not affected and could harvest good crop next on year (Srivastava, 1963a, b and 1964). The height of the chimney is also an important factor. The shorter the chimney, the greater the damage. Sen (1943) traced the damage of black tip upto 1700 ft in Bombai, Fazli upto 1050 ft and Langra 700 ft. from the kiln. Black tip was traced upto a maximum distance of 2050 ft in Himsagar. Occurrence of black tip in mango varieties, degree of damage, distance and direction from operating kiln was reported by Sen (1943). Prakash and Srivastava (1987) studied the black tip in relation to its incidence, effect of wind directions and effect of wind at the periphery and shady portions of the tree. The maximum incidence was recorded at the end of May and in the southern direction followed by north. Periphery (exposed to sun) of the tree showed maximum incidence while in shady portion (inside the canopy) of the tree, It was less. The fruits exposed to outer side had more black tip than unexposed. Studies conducted in China provided evidence that fluoride in fumes emitted from adjacent brick kiln was the direct causative factor of mango black tip disorder (Zhang *et al.*, 1995).

7.1.6 Reproducibility

Sen *et al.,* (1943) succeeded in reproducing the typical black tip disorder by burning coal in improved ovens in a mango orchard, so that the smoke emitting from them is circulated around the trees. However, there was no brick kiln within 3 miles of orchard on any side. Black tip symptoms produced during the stages of active development in size of the fruits. Exposure of the coal fumes after the fruit reached maximum size, though yet green, did not produced the black tip effect. It, however, showed an adverse effect to the extent that the fruits tend to ripen pre-maturely and developed toughness of flesh. Das Gupta and Verma (1939a,b) have experimentally reproduced the black tip effect on mango fruits by burning sulphur under a tree bearing healthy fruits and thus, exposing the sulphur dioxide gas when fruits were about an inch in length (still growing). Yellowing of the fruit was visible on the fifth day and blackening appeared on the seventh day. Das Gupta *et al.,* (1941) treated mango fruits with SO_2 gas in fumigation chambers. They could not reproduce black tip effect but found small brick red coloured areas around lenticles all over the skin. In the affected region, the epidermal cells loose chlorophyll, the starch grains become less in the mesocarpic cells, the cell walls become brown with light brown deposits taking place in the cell cavities. The affected area is marked off from the healthy tissue by the development of a cambium which produced new cells in which suberisation takes place. Such suberisation is evidently developed

in response to the injurious effect of the gas. Ranjan and Jha (1940) exposed plucked mango fruits to currents of ethylene air mixture, sulphur dioxide mixture. Sulphur dioxide mixture did not show black tip effect, the skin becomes whitish, through bleaching, but ethylene air and ethylene sulphur dioxide- air mixture produced light brown patches which finally turned darker. This they took to be black tip effect and thought the ethylene was its cause. They did not, however, described the symptoms in sufficient detail, particularly weather or not the effect initiated at the apex, as is characteristic of the black tip disorder. Sen (1942) carried out the experiments, on the effect of ethylene air mixture on mango fruits. Ethylene air mixture was found to cause yellowish brown patches here and there on the skin, but there was no indication of the effects initiating only at the apex of the fruit. The patches gradually enlarged and also become darker but they did not resemble the typical black tip. The typical black tip resemble a dry rot but these patches developed wet rotting. It remains, however, yet to be definitely determined which combustion product or products of coal cause black tip of the mango and what is the mechanism of this effect.

Das Gupta and Verma (1939a), Das Gupta et al., (1950) succeeded in inducing necrosis in healthy Dashehari and Safeda mango fruits by the injection of sterile mango juice exposed for sometime in contact with brick kiln fumes. Injection succeeded in fruits of size 3.3 cm and 9.0 cm and as many as 50 per cent developed black tip disorder. Later, a crystalline substance was isolated from brick kiln fumes which induced typical symptoms of necrosis on injection (Das Gupta et al., 1956) irrespective of the position of the hanging fruits.

The microscopic appearance, chemical behaviour and reactivity on mangoes of the ether-soluble constituents obtained from the juice of necrotic fruits seem to be similar to those of the ether-soluble constituents of brick kiln fumes. How exactly the constituents of the brick kiln fumes cause black tip is not known. There seem to be two possibilities. It may be absorbed directly by the fruits or by region other than the fruits and a subsequently translocated to the fruits. The later is the more likely. Verma (1952) emphasized that brick kiln fumes had some causal relationship to the black tip. It could be reproduced in any appreciable degree by burning coal in small improvised ovens in mango orchards during two consecutive seasons (Das Gupta and Verma, 1939a, Das Gupta et al., 1941). This is at variance with the results of Sen (1941, 1942, 1943) and Sen et al., (1943) who claimed to have reproduced the black tip by burning coal in ovens. In China, dipping fruits in 150 or 600 mg fluoride/kg solutions induced symptoms of black tip.

The fluoride content of the fruits with artificially induced symptoms was about 25 per cent higher than the control fruits. The fluoride content from black tip fruits from an affected orchard was twice as high as that of the normal fruit, from unaffected orchard. The leaf fluoride content of affected trees was about 6 times as much as that of the control (Zhang et al., 1995).Das Gupta and Sen (1960a, b) concluded that the black tip resulted from a disturbance of boron metabolism induced by the interaction of some constituent of brick kiln fumes with cell metabolites of mango leaves and fruits. Zhang et al., (1995) established that fluorine in fumes emitted from adjacent brick kiln was the causative factor of mango black tip disorder in China.

Table-1 : Summary of work done by different workers for controlling black tip disorder of mango

Chemical	Period of spray	Test chemical	Cultivar	References
Borax 6 lb/100 gallons of water	3 sprays (before flowering,during flowering and after.	Borax 6 lb/100 gallons of water	Dashehari, Safeda, Khajari	Das Gupta & Sen, (1958)
Borax 6 lb/100 gallon of water	Once & twice at full bloom and pea stage	Borax 6 lb/100 gallons of water	Dashehari	Das Gupta & Sen (1960a,b), Singh (1961)
Gelatine solution 1% + $CuSO_4$ 0.01%,$CuSO_4$ (alone)0.05% or manganesesulphate 0.05% and borax 6 lb/ 100gallons of water	Yellow tip stage	---do---	Dashehari	
Washing soda 0.5%, caustic soda 0.2% and borax 0.7%	One, time of application not reported.	Washing soda 0.5%	Dashehari	Prasad & Singh, (1965).
Borax 0.6% + sticker	Three sprays (flower bud stage, full bloom stage and fruit setting stage)	Borax 0.6%	Dashehari	Reddy & Kapoor, (1965,1966)
Washing soda 0.5%, caustic soda 0.2% and borax 0.6%	Three sprays (before flowering full bloom and at pea stage)	Washing soda 0.5%	Langra, Dashehari	Prasad & Saran, (1968) Prasad *et al.* (1969-71)
Borax, washing soda & caustic soda (tried all at 0.4, 0.6, 0.8%)	Twice (last week of March and 3rd week of April)	Caustic soda 0.8%	Dashehari Langra	Rajput *et al.*, (1971)
Sodium hyroxide 0.4, 0.6, 0.8%, sodium carbonate 0.6% and borax 0.6, 0.8%	Not reported	NaOH 0.8% (in Langra & Dashehari) 0.6% in Fazri	Langra Dashehari Fazri	Nauriyal *et al.*, (1972)
Sodium carbonate 2% and borax 0.6%	Marble size	Borax 0.6% sodium carbonate 2%	Dashehari	Chandra & Yamdagni (1984), Yamdagni & Chandra (1985)

7.1.7 Management

The control of mango black tip has two distinct aspects (a) cure of disease, (b) prevention of disease. Attempts have been made by several workers to cure the disease by spraying chemicals during the active fruit growth stage (Table 1).

7.1.7.1 Prevention of black tip disorder

(a) Restricting sites of new brick kilns at safe distance from valuable orchards about 1500 meter east and west and 750 meter on north and south directions (Sen, 1943, Srivastava, 1963a, b, Pal and Chadha, 1980, Prakash, 1996). (b) The kiln should not be allowed to operate from the first week of March to third week of May, preferably for the entire mango season. (c) The chimney should be risen to atleast 40 to 50 ft or preferably more, which will dilute the effect of deleterious gases of the smoke coming forth to a harmless concentration during the flowering and fruit bearing season. (d) Brick kilns should be made practically smokeless by the installation of certain fixtures to them (Blakke, 1913). However, it may be feasible to employ such fixture to the brick kilns used in this country is a matter which needs investigation.

The effectiveness of borax, though not consistent (Prasad and Singh, 1965) has been attributed to its ability to supply boron (Das Gupta, 1959, Das Gupta and Sen, 1960a, b). However, no direct evidence has been adduced in support of this contention. Various alkaline substances viz. caustic soda, borax and washing soda have been used with good success in the control of black tip. Since borax undergoes alkaline hydrolysis in water, it could also function as a buffer against the harmless constituents of kiln fumes which are generally acidic in nature. perhaps, the presence of alkalinity on the fruits surface prevents entry of the deleterious fumes emanating from brick kilns in the form they are generated or changed or neutralized into a form which is less toxic.

7.2 Soft Nose

A breakdown of the flesh on the ventral side and towards the apex in mango fruits, even when on the tree prior to harvest, has been termed 'Soft Nose' (SN) in Florida (Young, 1957). In Malaysia, the condition known as 'Yeasty fruit rot' or 'Insidious fruit rot' is thought to be a physiological disorder probably identical with soft nose (Kwee and Chong, 1985).

A physiological breakdown in Kent and Haden cvs. of mango described as soft nose, has been engaging the attention of commerce and research workers. The problem was first observed in fruit on the tree by orchardists in 1950 in Florida. Soon after, it was reported by Young (1957) from all the mango growing regions of Florida. The Kent cultivar is considered one of the most popular variety in Florida for the fresh trade and processing. In mango, physiological breakdown like 'spongy tissue' or 'soft centre' and 'tip pulp' are reported by Krishnamurthy (1980). Subramanyam *et al.*, (1971) and Verma (1950) but these are distinctly different disorders. Investigation revealed that the disorder is of physiological nature but cure is not known.

7.2.1 Symptoms

Disease was found to occur in fruits which are allowed to start ripening on the tree and does not develop after the fruit is harvested (Young, 1957). In case of spongy tissue, symptoms are not apparent either at the time of picking or at ripe stage. The affected tissue is visible only when ripe fruit is cut into two halves. Such flesh tissue is differentiated from the healthy one by its pale yellow colour, soft or spongy nature with or without air pockets and accompanied by off flavour (Subramanyam *et al.*, 1971). There is slight discolouration in the centre of the tissue surrounded by a soft hole. The 'soft nose' is characterised by yellowing of the green skin in the area between the apex and the stigmal point. A lack of firmness in the diseased fruit, one can also feel with some soft nose fruits merely appears to be over ripe, while that around the shoulders and on the dorsal side is unripe (Young, 1957). This is the condition most frequently found in Haden and evidently occurs during ripening which follows full maturity. The over ripe flesh surrounds a mass of yellowish to brown tissue which is of firmer texture than the surrounding affected tissue and is bitter in taste but in advance cases, tissues are greyish black, spongy and extend further. The present disorder differs from the spongy tissue occurring in India in both external and internal symptoms of the diseased fruit (Subramanyam *et al.*, 1971).

7.2.2 Varietal susceptibility

In Florida, the disease has been observed first in Indian varieties viz. Mulgoa and varieties originating at first and second generation seedlings of Indian varieties including Haden, Kent, Sensation, Keitt, Davis-Haden, Springfels, Zill, Irwin and Brooks but Haden and Kent appear to be the most commonly affected including Sensation (Young, 1957). Cultivars Ah Pingh, Ameeri, Anderson, Fasali, Couveta, Kent, Lippens, Osteen, Sensation, Smith Tolbert, Tommy Atkins in Canary Islands were also found susceptible (Sauco *et al.*, 1984).

7.2.3 Incidence and losses

As reported by orchardists and shippers, the incidence varies widely with localities and there is a tendency in some areas to harvest fruit less mature than in other areas. Fruit harvested at semi-ripe stage (less mature) enough to ripe to fair quality will show much less incidence of soft nose than fruit left on the tree to start ripening before picking. Five to twenty per cent fruits harvested from Haden and Kent cultivars are found affected, but in severe cases, it may go upto 50 per cent (Young, 1957). Young *et al.,* (1962. 1965) reported the loss as only few per cent of fruits harvested from trees grown on calcareous rock soil, but on acidic, sandy soil, it is common for 15-20 per cent of the crop to be culled because of disorder.

7.2.4 Causes

The causal factors for the onset of this disorder are not known. Several attempts have

been made to isolate the organism, if any, responsible for the soft nose disorder. It was recognised that micro-organism might gain entrance into the flesh through natural weak spots in the skin, cracks resulting from disease such as scab, mechanical injuries or insect stings, but could not find any weak spot or break in the skin. Isolation from all infected tissues were done but no organism could be isolated consistently and concluded that the disorder did not seem to be due to micro-organism (Young, 1957).

In case of 'spongy tissue', Chhatpar *et al.,* (1968-69) isolated various species of Bacillus from these tissues and identified them as *Bacillus mocerous, B. cereus, B. subtilis* and *B. megatereum.* These bacteria have been reported to utilize ascorbic acid, citric acid and pectin to a considerable extent and there is decrease in the level of free hexose, esucrose, ascorbic acid, pectin and carotenoids, no change in protein content, and a shift in pH from 1.6 to 3.2. They have related some of these changes to increase in the activity of invertase and ascorbic acid oxidase and hinted that excretory bacterial metabolites are responsible for this abnormality. Assnani *et al.,* (1971) suggested that the breakdown due to bacterial infection (*Bacillus* sp.) appears to be doubtful. A *Bacillus* sp. has been isolated from the diseased tissue but pathogenicity of this organism has not been successful (Subramanyam *et al.*, 1971). Very little information is available on the relationship of environmental factors to soft nose. The problem was noticed on all soil types used, in warm or cold and wet or dry season on young and old trees, on semi-neglected trees, on well fertilized and adequately sprayed trees (Young, 1957). He further reported that the disorder was less prevalent on the rocky alkaline soils than on the acidic soils but proof is inconclusive.

Young (1957, 1958) reported that the fertilizer application in the mango was responsible for the disorder, but there have been no observable difference among different treatments in the incidence of soft nose. According to Young *et al.,* (1965), soft nose disorder in mango increased with nitrogen fertilization but was alleviated to a great extent by increasing calcium level of the soil. Application of nitrogen fertilizers in sandy acid soil increased the incidence of soft nose in mango, however, the same had no effect on calcareous rock soil (Young and Miner, 1961).

7.2.5 Management

It appears from the studies, that a tendency towards soft nose disorder in Kent mango fruits is aggravated by high nitrogen level in the tree and that high calcium level in the tree may alleviate this tendency. In such cases thus, (i) high nitrogen level should not be allowed in the soil, (ii) proper calcium level should also be maintained in soil, (iii) and early picking or growing of varieties which may be found less inclined to the trouble (Young and Miner, 1961) is recommended.

7.3 Internal Necrosis

A browning of the flesh in mango fruit while still on the trees has caused considerable loss to growers around Lucknow and other parts of UP. The trouble, now very commonly and rather descriptively called 'Internal Necrosis' (IN) or 'Galta rog' apparently was first recognised by Prakash (1975-79) and Ram (1976) in Dashehari mangoes. The

disorder was found in mature fruits while on the tree and the affected fruits dropped before attaining maturity.

7.3.1 Losses

The loss was estimated varying from 0.35-5.00 per cent in different varieties of various orchards irrespective of nearness to brick kilns. But incidence may go high upto 15-20 percent if mango trees planted in sandy soil.

7.3.2 Symptoms

The first external symptom of the disease is the appearance of small isolated water soaked greyish areas in the lower half of the fruit having the tip apparently healthy. Soon after, in each of these greyish areas brown spots develop, which later on coalesce to form a dark brown necrotic area on the fruits, delimiting green parts with indefinite outline from April end to second week of May before endocarp hardening. The brown tissues then turn into brownish black necrotic lesions which later extended towards the epicarp. At this stage, water soaked isolated necrotic areas appear on the surface of the fruits exuding gummy substances on the fruit surface below the green tip. The brown areas developed into dark brown gummy cavities in the mesocarp. In advance cases, the affected tissues of the entire lower half of fruit turned necrotic and collapsed which resulted in longitudinal fruit cracking exposing the seed of the necrotic region. The next phase in the development of the disease is complete disintgegration of the pericarp and the mesocarp of the necrotic area exposing the flesh which is dark brown in colour. Exudation of gum is also seen along with the diseased portion. Tissues of the lower part of the fruits are brown-black, corky with gummy pockets. The browning of seed is also very common feature of the disorder. Some of the mildly affected fruits without any external symptoms, have necrotic seeds and cracking of fruits. The disorder ceases when fruit attains maturity (at the time of stone formation). Such fruits do not last longer on the tree, easily detachable from the stalk, and falls before it ripens. In case of internal necrosis, fruits are easily detachable contrary to black tip infected fruits which are firmly attached to the tree and are not easily detachable.

7.3.3 Causes

Internal necrosis is caused by boron deficiency in the soil while the black tip is caused by deleterious effects of brick kiln fumes. In some cases, the disorder did not show all the stages described above. The growth of fruit was restricted even at the earliest stage of necrosis. The affected parts of the fruit were generally hard and dry. Some fruits developed seed necrosis without presenting any external symptoms. Some of the mildly affected fruits also developed taper tip and deformed shape (Ram, 1989). The other cultivars of mango, viz. Chausa, Bombay Green, Lucknow Safeda and Fazri showed symptoms similar to that in Dashehari but the degree varied, however, cultivar Langra is found free from this disorder.

The degree of susceptibility to internal necrosis in mango cultivars varied con-

siderably in the same orchard. The internal necrosis was found to occur in orchards located away from brick kilns (5-7 km). There was no relationship between incidence of internal necrosis and closeness to brick kilns in mango cvs. grown at different locations. Internal necrosis occurs even in orchards which are at a distance of 30 km from the operating brick kilns. The magnitude of IN mango cultivars varied from one orchard to another. However, boron level is found to be lower in affected fruits. Leaves and fruits of Langra contained lower level of boron than those of Dashehari and Bombay Green. The susceptibility of Dashehari and Bombay Green to internal fruit necrosis may be because of their higher boron requirement than Langra and this may be the reason of absence of necrosis in Langra. Inverse linear relationship was found between boron contents in the leaf and fruit with per cent fruit necrosis. Since application of boron to soil or by injection technique increases its foliar content to the level in healthy trees resulted in effective control of necrosis (Ram *et al.*, 1978, 1989, Prakash, 1975-1979).

7.3.4 Control

Application of borax in soil at the rate of 500 g per tree or foliar spray (1%)or injection (1%) increased significantly the boron content of leaves and fruits and controlled the disorder (Ram, 1989). Prakash (1975-79) screened several chemicals and found that the borax (0.5%) could correct the disorder. Other chemicals did not show consistent result in controlling the disease. Foliar application was found to be more effective than soil application, when sprayed at the marble size fruit.

7.4 Spongy Tissue

A ripening disorder of Alphonso mangoes is known as 'spongy tissue' or 'soft centre' which involves internal flesh breakdown in the ripe fruits. This disorder renders the fruit unfit for human consumption. Losses due to the disorder was estimated about 30 percent (Subramanyam *et al.*, 1971) but it varied between 35-55 per cent (Anonymous, 1968-70) in cv. Alphonso, depending on location, age of tree, variety, season, time of picking, weight and maturity of fruit, orchard soil and environmental conditions. The disorder is mostly common in the states of India viz. Maharashtra, Gujarat, Karnataka and Andhra Pradesh. Cultivars Fernandin, Goamankurad, Olour, Jamadar, Swarnarekha and Vanraj are also reported to be susceptible (Rane *et al.*, 1976). It is also reported from Australia, Philippines and Malaysia (Chaplin, 1986, Nuevo *et al.*, 1984 and Kwee and Chong, 1985).

7.4.1 Symptoms

The disorder was first noted in 1932 in cv. Alphonso fruits (Cheema and Dani, 1934). External symptoms are not visible at harvesting or when ripe, but when cut in half, the flesh is pale yellow in colour, soft or spongy with or without air cavity and has an off flavour (Subramanyam *et al.*, 1971). Three kinds of abnormality symptoms were noticed : (i) blemishes on all parts of the fruits, the flesh of the badly blemished one being abnormal (hard but rubbery in texture, pale in colour, less juicy, acidic in taste) which

could be easily separated from the surrounding normal flesh, (ii) browning of the tissue around the stone commencing in the vascular tissue close to the stone and gradually spreading outwards and in sever cases, the brown colour of the vascular tissue becoming distinct, half of the total flesh being affected and (iii) breakdown of tissue around the stone, somewhat abnormal, being continued to the fruit kept in cold storage. Amin (1967) observed as white sponge like tissue, slightly desiccated in nature in the pulp between the skin and the stone of the ripe fruit. Chhatpar *et al.,* (1968-69) described as slight desiccation in the centre of the tissue surrounded by a soft halo between skin and stone of the fruit. In 1971, Subramanyam *et al.,* noticed the disorder as internal breakdown which is apparent either at the time of picking or at the ripe stage. The

Figure 19: Spongy tissue in mango fruit cv. Dashehari

affected flesh is pale yellow and soft leathery or spongy in nature, with or without air pocket emit off flavour. Katrodia (1979) described the symptoms in which the fruit pulp remains unripe because of the unhydrolysed starch due to physiological and biochemical changes caused by heat in mature fruits. He also noticed that the disorder invariably starts from the surface of the stone. Similar disorder was also noticed in cvs Dashehari and Mallika from Lucknow (Fig. 19).

7.4.2 Damage

Damage due to spongy tissue was maximum (65.1%) in lower part and the least (4.7%) in upper part of the fruit in Ratnagiri district. The damage near the stone was 55.6 per cent, while in middle and near skin, it was 33.3 and 11.1 per cent, respectively (Tare,

1977). Spongy tissue affected fruits were maximum (57.9%) in the lower part as compared to centre (26.3%) and upper part (15.8%) (Katrodia and Rane, 1989).

7.4.3 Distribution

The distribution of spongy tissue varied in different districts and states (Subramanyam *et al.*, 1971). Fruits from Belgaum (Karnataka), Valsad (Gujarat) and Chittur (Andhra Pradesh) recorded 57, 50 and 47 per cent spongy tissue, whereas, from Mysore, Bangalore, Dharwar, Ratnagiri and Kolar recorded 9, 26, 2, 24 and 5 per cent, respectively. Thus, it was higher (41%) in coastal areas in Belgaum, Ratnagiri, Valsad than in the inland area (22%). The occurrence was greater (56.7%) in trees at the base of the hill (Joshi, 1975) than those on slopes (46.6%) or on the top of the hill (35.6%).

7.4.4 Causes

7.4.4.1 Pathological

Chhatpar *et al.,* (1968-69) isolated *Bacillus* spp. from spongy tissue infected fruit on inoculation in healthy mango fruits of cvs. Alphonso, Neelam, Pairi and Fazli, produced spongy tissue symptoms except a local variety Pachhatto. The isolates were identified as *Bacillus macerans, B. cereus, B. subtitis* and *B. megatertum*. Microscopic examination of the sections of spongy tissue by other workers, however, did not find anything but starch. Desai (1966), Subramanyam *et al.,* (1971) and Katrodia (1979) also ruled out the possibility of such association in the absence of pathogenicity test and owing to unfavourable conditions (low pH, high acidity) of ripe fruit for microbial growth. Insect pests do not seem to play any significant roles in spongy tissue development in Alphonso fruits (Anonymous, 1970, Subramanyam *et al.*, 1971).

7.4.4.2 Varietal susceptibility

The disorder was found both in grafted and seedling mango varieties in Gujarat. Kesar was found less susceptible to the disorder (Dasai, 1966). The spongy tissue development was not restricted in Alphonso cv. alone, but Limaye *et al.,* (1975) observed its occurrence to the extent of 36.7, 26.7, 13.3, 10.0, 6.7 and 3.3 per cent in Vanraj, Olour, Goamankurad, Vellaikolamban, Swarnrekha and Fernandin cvs., respectively. The cvs. Pairi, Kesar, Dodhpeda, Neelam and Dashehari were free from this disorder. Amin (1967) found that Jamadar cv. was susceptible. The disorder in Rajapuri variety could not be induced even by 4 hr sun exposure (11.30 to 15.30 hr) at post-harvest stage but the cultivars Vanraj, Madrasi Apus, Kesar, hybrid (Alphonso x Baneshan) and Jamadar showed different degrees of susceptibility (Katrodia, 1979). The variations may perhaps, be due to differential physio-chemical properties of the fruit skin which acts as insulating layer against heat. Katrodia and Rane (1981) reported that spongy tissue was found more (50%) in fruits harvested from vigorous than those from weak trees (9%). Cvs. Carabao and Kensington were also reported susceptible to spongy tissue (soft centre) from Philippines and Australia (Nuevo *et al.*, 1984, Chapliln, 1986). Kwee and

Chong (1985) reported spongy tissue on cvs. Alphonso, Fan Siamese and Harumannis.

7.4.4.3 Biochemical changes

As we have noticed that the spongy tissue develops at maturity stage, attempts have been made to study the chemical composition of mango fruits after ripening :

The acidity : Healthy and diseased fruit have been found to contain higher acid content than the pulp of the healthy fruits (Amin, 1967, Rangwala, 1975, Patkar, 1978, Katrodia, 1979). Ascorbic acid : Subramanyam *et al.,* (1971) observed higher content of ascorbic acid in the spongy than in the healthy tissue. Reduction in ascorbic acid content in the affected / healthy tissue has been reported (Amin, 1967, Chhatpar *et al.*, 1968-69, Rangwala, 1975, Katrodia, 1979). Chhatpar *et al.,* (1968-69) attributed this reduction to the utilization by bacterial isolates. Katrodia (1979) assigned it to the losses by oxidation owing to high temperature in the affected pulp.

Carotenoid pigments: Significant decrease in carotenoid pigments in spongy tissue as compared to healthy tissue of ripe fruits have been observed by Chhatpar *et al.,* (1968-69), Subramanyam *et al.,* (1971), Rangwala (1975) and Katrodia (1979). However, Katrodia (1979) attributed the reduction of beta-carotene to its lesser production or its degradation probably due to higher fruit temperature.

Enzyme activity: An amylase activity in spongy tissue decreased whereas those of invertase and ascorbic acid oxidase increased (Chhatpar *et al.*, 1968-69). Katrodia (1979) also noticed that amylase activity from as high as 3.21 was decreased to 0.39 in spongy tissue and to 0.50 in sun-desiccated tissue. It seems that the accumulation of heat near the stone in the fruit pulp at pre- and post-harvest stages retarded the enzyme activity resulting in retention of more unhydrolysed starch in the affected pulp. The invertase activity was also lower in sun-desiccated tissue (0.19) and spongy tissue (0.46) than in healthy pulp (1.79) owing to the heat. Amylase activity in spongy tissue was about three times lower compared to healthy pulp (Anonymous, 1983).

Starch and sugars: Rangwala (1975) noticed 1.47 per cent starch in spongy tissue and no starch in healthy ripe pulp. Patkar (1978) found 8.25 per cent starch in spongy tissue and 13.80 per cent in the mature fruit. Katrodia (1979) observed 7.96 per cent starch in spongy tissue and 8.0 per cent in sun-desiccated tissue, 0.25 per cent in healthy ripe pulp and 12.87 per cent in mature fruit. The retention of starch in affected tissue was attributed to low amylase activity. Joshi and Roy (1989) also made similar observations. Appreciable reduction of non-reducing sugars has been noticed in the affected fruit (Amin, 1967, Chhatpar *et al.*, 1968-69, Subramanyam *et al.*, 1971, Rangwala, 1975, Patkar, 1978, Katrodia, 1979). The lower content of sugars in spongy tissue seems to be as a result of retention of starch in the affected pulp owing to lower activities of invertase and amylase.

7.4.5 Nutritional aspects

7.4.5.1 Nutritional status of orchard soil and tree

N, P and K content in the leaves of affected Alphonso trees were higher, and Ca and Mg

were lower than in the healthy ones (Rangwala, 1975). Calcium content in Alphonso leaf increased from 1.63 per cent at fruit set to 1.86 per cent at harvest (Tare, 1977). But Katrodia (1979) could not find any difference in macro-and micro-elements content in leaf from healthy and affected Alphonso trees. Subramanyam *et al.,* (1971) observed 0.074 per cent Ca and 0.12 percent P in spongy tissue affected pulp as compared to 0.085 per cent Ca and 0.096 per cent in P in healthy pulp. Higher contents of N, P, K and Mg were found in tissues of affected pulp as compared to healthy ones (Rangwala, 1975).

7.4.5.2 Nutrients application

An application of NPK fertilizers and micro-nutrients like boron in soil separately and in combined form under irrigated and in irrigated conditions did not help to control the disorder in Alphonso (Amin, 1967, Katrodia, 1979 and Joshi, 1975). Desai (1966) noticed both healthy and affected fruits not only on the same tree or on the branch but even in the same bunch.

7.4.5.3 Sprays and dips in nutrients

Single and double pre-harvest dips of fruits in Ca solution significantly reduced the spongy tissue and this increased Ca content in the fruits (Gunjate *et al.*, 1979). Sprays of shoots with chemicals like zinc sulphate, borax and GA or with micro-nutrients like Zn, Cu, Fe and Mn (in sulphate form) alone or in combination with Zn had no significant effect. Spray with Zn alone or in combination with Fe or Cu caused blackening and resetting on the fruit skin. The results, therefore, indicate that the disorder in not related to nutritional imbalances.

7.4.6 Other aspects

7.4.6.1 Soil moisture content

Occurrence of rain, one or two weeks prior to harvest, rendered the fruits more susceptible to spongy tissue (Subramanyam *et al.*, 1971). According to Joshi (1975), high soil moisture content increased susceptibility of fruits. The reason for more damage in irrigated trees was higher sensible heat owing to the high relative humidity under the tree canopy (Katrodia, 1979).

7.4.6.2 Sunlight and temperature

In Gujarat, sponginess in most of the grafted and country mangoes was noticed, when atmospheric temperature was high. Fruits exposed to direct sunlight were found affected by this disorder, particularly those on western side of the tree which get direct sun rays for longer period. The disorder was observed to develop when temperature rose above 40.5^0C (Desai, 1966). Direction of sunlight had no effect on the occurrence and intensity of the disorder since most of the fruits under shade were affected, but those exposed to direct sunlight hardly had spongy tissue (Anonymous, 1970 and

Katrodia, 1979).

7.4.6.3 Convective heat

Convective heat arising from soil was the main cause of damage to the Alphonso fruit at maturity stage. During April-May, the soil temperature rises to above 55.8^0C as a result of solar radiation. The resultant convective heat flux first touches the apex and the fruit hanging down the tree, enters the mesocarp up to the surface of the stone and pulp gets heated resulting in spongy or desiccated tissue. The spongy tissue could be produced artificially by exposure of mature fruits to solar heat, infra-red rays and incubation at post-harvest stages (Katrodia and Rane, 1989 and Sheth, 1981).

7.4.6.4 Effect of rootstock

The fruit of Alphonso on Alphonso seedling rootstock showed the maximum percentage of damage (57.4%) followed by Shahabuddin (55.9%), Pairi (48.6%), Neelam (46.2%) mixed seedling (46.1%), Peshwa (43.1%) and Totapuri (43.1%) (Joshi, 1975).

7.4.6.5 Stage of maturity and harvest

To some extent, early picked fruits escape the incidence (Cheema and Dani, 1934, Katrodia, 1979) but Cheema and Dani (1934) reported internal breakdown in the first picked fruits at full maturity. Increased spongy tissue with advancing fruit maturity and that the tendency for internal breakdown was more at the end of the season (Joshi and Roy, 1989). The disorder was more in late harvested fruits than in mid season and early picked fruits. Early harvest helped to escape the damage, but the quality of ripe fruits from this harvest was inferior (Subramanyam *et al.*, 1971, Katrodia, 1979, Rangwala, 1975, Limaye *et al.*, 1976 and Patkar, 1978).

7.4.6.6 Specific gravity and size of fruit

Fruits having less than 1.0 specific gravity (SG), had no spongy tissue but the occurrence of damage was more (45%) in fruits having above 1.02 SG (Krishnamurthy, 1980). Alphonso fruits weighing around 200 g or less showed 19 per cent damage, whereas those weighing more than 300 g showed 47 per cent damage (Subramanyam *et al.*, 1971). In general, the incidence of damage increased with the increase in weight of the fruits (Joshi, 1975).

7.4.6.7 Mulching

Sod culture having natural vegetation of 'Dhorth' (*Desmostachya binnata* L.) controlled the spongy tissue development in Alphonso fruit to the extent of cent per cent (Katrodia and Rane, 1979). The mulches of dry leaves of mango and paddy straw were also effective in protecting the orchard soil from excessive heating by solar radiation (Katrodia and Sheth, 1989).

7.5 Clustering *or 'Jhumka'*

During the year 1984, there was heavy flowering in mango around Malihabad, giving hope of a record crop. The initial fruit set was also good, however, it did not develop after a certain stage and most of the fruits dropped down. The set was also peculiar in the sense that near the tip of panicle, there was bunchy appearance locally named 'Jhumka' (Prakash and Srivastava, 1987). Even though Jhumka formation is not known to the growers, it is a very rare phenomenon and had not occurred on such a large scale any time earlier, although this disorder was observed in a very low proportion during 1982. Progressive growers are unable to recall the 'cluster' formation on such a mass scale during their life time.

7.5.1 Losses

In most of the orchards, the cluster formation was almost 80 per cent while in some, there was no set at all with barren panicles.

7.5.2 Symptoms

The disorder is characterized by the formation of a bunch of fruitlets of the size of marble at the tip of the panicle. The fruitlets are dark green in colour and their shape is much more curved than the normal shape of a developing fruit. This shape resembles that of unfertilized fruits in a normal season which drop out very quickly after turning yellow. The fruit growth on the dorsal side is so much in these fruitlets that a pressure exerted on the sinus region causes a split of the fruit longitudinally. The dark green colour after attaining the size of marble, further growth ceases and thus remain in the panicle for a considerable period. The retention of these fruitlets for a longer period gives a general impression that there will be a good set. However, these fruitlets do not grow more, though they stay on the panicles for a longer period.

7.5.3 Causes

The incidence of clustering was exhibited only in the panicles which did not carry fruits in the primary rachis (which generally carry fruits to harvest) were devoid of clustering. Although an attempt has been made to ascribe the following reasons, the disorder needs thorough investigations. (i). Lack of pollination and fertilization owing to abberant weather conditions. Some of these factors may be operating simultaneously or one after the other. (ii). Simultaneous production of vegetative growth associated with the disorder must have resulted in massive diversion of photo-assimilates to the growing vegetative points diverted the supply of metabolites from the fruits having a aborted embryo resulting in their drop.

The Dashehari cultivar, being highly sensitive to temperature fluctuations with regard to flowering and fruit setting, could have been highly affected by the abnormal weather conditions. The detailed examination of these fruits showed to have aborted embryos at various stage of development. Under normal cropping season, one could

see rarely some small fruit with aborted embryos. The occurrence of such large number of fruits with aborted embryos was a clear cut indication of the harmful affect of fluctuating temperature during the month of February and March on fruit development in the region would have lead to the phenomenon of clustering in the tip where hermaphrodite flowers are more in number (Singh, 1954). These fruits grow parthenocarpically to a size of marble and remain in the panicles, probably, due to channelization of more assimilates and growth hormones facilitating initial development and retention. Studies showed that 92 per cent of the fruits possessed aborted and shriveled embryo and could not develop further, signifying the role of normal embryo growth in development of fruits (Khader, 1988, 1989).

In countries like Israel and USA (Florida), it has been observed that low temperature during flowering, fruit set and development cause extensive damage to the crop (Young, 1942). Studies in these countries have shown that minimum temperature below 7^{0}C results in poor germination of pollen grains and pollen tube growth. Further, it has also been found that low temperature during fruit setting period could result in seedless or jelly fruits in large scale particularly due to embryo abortion.

7.5.4 Varietal susceptibility

Studies conducted at Central Institute for Subtropical Horticulture, Lucknow, revealed that the clustered panicles and clustered panicles with normal fruits ranged from 4-84 and 8-96 per cent, respectively. The highest percentage of clustered panicles and clustered fruits/panicles were in Nisar Pasand while they were least in cv. Neelam, out of 27 cvs. screened (Anonymous, 1995-96).

Twenty four cvs. screened for clustering disorder in 1994-95, showed that clustered panicles were highest in cv. Lucknow Safeda (60%) followed by Rataul (58.8%), Bombay Green (48.4%) and Gulab Khas (48%). Barren panicles were maximum (65.4%) in Benishan and least (5%) in Kishanbhog, while the number of clustered fruits per panicle were maximum (13.5%) in cv. Rataul.

7.5.5 Management

Studies conducted during the year 1987 and 1994-95, revealed that there was no effect of fungicide/insecticide on Jhumka disorder but double spraying of insecticides at pre and full bloom stages, had significant effect on Jhumka formation in cv. Dashehari (Anonymous, 1995-96). Application of NAA (300 ppm) in the month of November on cv. Dashehari reduced the clustered (Jhumka) panicles from 20 per cent (control) to 5 per cent.

Lower concentrations viz. 100-200 ppm of NAA also reduced the clustered panicles up to 13 and 10 per cent, respectively. The clustered fruit panicles (7%) and barren panicles (5%) were minimum with treatment NAA (300 ppm), while in control, these were 25 and 45 per cent, respectively (Anonymous, 1994-95 and 1995-96).Less use of pesticides in the mango orchards during full bloom reduces the Jhumka and enhances good fruit set.

7.6 Leaf Scorch

Leaf scorch in mango, caused by a nutritional disorder, has been found to limit the vigour and production of trees in Delhi, Haryana, Punjab, Central and Western U.P., Bihar and Rajasthan (Prakash and Srivastava, 1987). Although this disorder causes a great loss to the mango industry, yet no attempt has been made to assess the extent of damage, the possible causes, and remedies. Both seedlings and mature trees are affected by this disorder. The symptoms of this disorder are so distinct that severely affected trees can be spotted out very easily even from a distance due to the burnt look of the foliage. In the cultivar Chausa, on which this observation has been made, the symptoms starts from the tip generally 8 to 10 months after the leaf emergence. In the beginning, only the tip of the leaf is affected. As the leaf gets older, the symptom develops along the margins, which turn brick-red and slowly, covers the major portion of the lamina. Nearly all the affected leaves which emerge in March start dropping off in April of the next year. From January to April, the tree appears to be adversely affected by this malady. It presents a partially naked look from the middle of March due to sparseness of foliage from March onward the young leaves again emerge and get affected by January. In this way, the cycle continues. This has a lot of impact on the flowering and fruiting behaviour of the tree as foliage is necessary for the supply of adequate food materials to the trees. If the symptoms continues for a few years, the young twigs start drying giving the tree a more sickly look (Pandey *et al.*, 1974).

Experiments conducted by the authors, have indicated that this disorder is due to excessive accumulation of 'chloride ions' in the leaf tissue. Leaf analysis data have been shown that the amount of chloride in the leaves of diseased tree is about four times more as compared to the leaves of healthy ones. But potassium showed a different trend. Its content in the diseased leaves was lower as compared to the healthy leaves. Other elements like sodium and calcium showed the similar trend as that of chloride. To arrive at a better understanding, a separate experiment was conducted by Pandey *et al.,* (1974) in which sprays of 2 per cent sodium chloride, sodium bicarbonate and sodium sulphate were given to induce artificial scorching in leaves. It was found that symptoms of scorched leaves produced by 2 per cent sodium chloride spray resembled the symptoms of naturally occurring scorched leaves. This fact indicated that scorching of leaves in mango was a function of chloride toxicity rather than an effect of potassium deficiency.

Knowing that potassium deficiency is a consequence and not the cause of this disease another experiment was conducted in which leaves were sprayed four times at frequent intervals with 5 per cent solution of potassium sulphate since their emergence. It was observed that although leaves sprayed with 5 per cent solution of potassium sulphate showed some symptoms of scorching, the degree was much reduced as compared to untreated leaves. This was further substantiated by higher level of potassium (0.82%) in sprayed leaves as compared to the untreated ones (0.65%). Soil application of potassium sulphate at the rate of 2.5 kg per tree (20 year old) has also given promising result. At present, the probable recommendations that can be given to the orchardists are as follows :

(i). Application of fertilizers containing chloride should be stopped. The dose of potash

application should be increased and instead of murate of potash, potassium sulphate should be used. (ii). Affected leaves which drop off under the tree should be removed immediately to avoid any further absorption of chloride ions released from the leaves during their decay in the soil. (iii).Mango plantation in saline soils should be avoided. (iv).Irrigation water having higher content of chloride ions should not be used.

8. Nutritional Deficiencies

The essentiality of various trace elements for growth and development of higher plants is now widely recognised. Some of the elements which were formerly considered to be traced, are now taken as mega, such as megnesium etc. The recent advances in the study of physiology of nutrition may prove the essentiality of all the 92 elements for the normal growth of the plants. A great deal of the work has been done on mango by Sen *et al.,* (1994). Mallik and Singh (1959) and Smith and Scudder (1995) who defined the deficiency symptoms of nitrogen, phosphorus, calcium, potass, manganese, zinc, boron, sulphur, iron, copper and megnesium. Deficiency of zinc cause little leaf of mango (Lynch and Ruehle, 1940, Oppenheimer and Gazit, 1961). Such symptoms have also been noticed on young seedling by Nirvan (1953) and on vegetative shoots of mango (Tripathi, 1954) but mite was found to be the cause. Disease symptoms of little leaf, copper deficiency, mango decline, and tip burn are described hereunder.

8.1 Little leaf of zinc deficiency

The characteristic symptoms are first seen on terminal flushes in the upper portion of the plants. The leaf blade begins to thickens and fails to reach normal size, in the pinkish early stage of growth. The margins of mature leaf are either bent downwards, which causes the leaf apex to curve down, or the margins are bent upwards causing the apical portion of the leaf to bend upward. Leaf may show recurvature of the apical portion in certain causes. The veins may develop a yellow colour and stand out quite prominently on the upper leaf surface, while the interveinal area usually develops a normal green colour, although in certain cases, a regular yellow pockets appear between the veins, giving the leaf a mottled appearance. The length and breadth of the leaf are drastically reduced as compared to the normal leaf on the same tree. The blade is quite brittle, turgid and thickened. Due to shortening of internodes, nodes, the leaves are crowded at the twig apex and give a rosette appearance (Lynch and Rohle, 1940). Almost similar symptoms were noticed on mango at the Experimental Research Farm, Rehmankhera, Lucknow (Prakash and Srivastava, 1987) and Punjab (Nijjar *et al.*, 1976).

All the new growth is not affected in moderate causes but it does in advance cases and subsequently flushes of the growth may altogether fail to appear and at this stage some die back of twigs are observed. If such condition continues, the tree may untimately dies.

Young tree in the nursery are usually affected on all the growing points. Such plants remain stunted with weak limbs. Bloom spikes developing on affected tree showing little leaf symptoms are usually small irregular in shape, and often include multiple deformed, dropping, spikes, such trees remain unthrifty and unproductive until the

condition is corrected.

The thickened, turgid, little leaves with veinal chlorosis, are the symptoms of zinc deficiency in citrus (Camp and Reuter, 1937, Camp and Fudge, 1939), especially on plants grown in calcareous soil (Peech, 1939). It has been noticed that the disease is present pre-dominently in Florida on trees growing on colitic lime stone soil, overlaid with a shallow top sandy soil having very high pH value, and that orange tree in close proximity to the affected mangoes also showed considerable "Frenching". This has led to the blief that the little leaf of mango is caused by zinc deficiency in the soil (Lynch and Ruehle, 1940). Zinc is fixed the rendered unavailable by lime in soils above a certain pH value (Peech, 1939).

Zinc deficiency symptoms can be corrected by application of zinc spray consists of 5 lb. zinc sulphate, 2.5 lb. lime and 100 gal. of water during autumn and spring (Lynch and Ruehle, 1940). The zinc salt may be added to Bordeaux mixture without the addition of extra lime in one of the anthracnose sprays. If the zinc salt is added to one of the fixed copper, it is advisable to add the lime to prevent zinc burn (Ruehle and Wolfenbarger, 1948). For correcting zinc deficiency, Oppenheimer and Gazit (1961) recommended sprays either 1% zinc sulphate (with or without lime) or 0.2% zinc oxide. Dusting the trees, application of pillets into the holes bored in the trunk and soil application have not been found effective. The effect of sprays strictly local and for this reason, all parts of the tree must be thoroughly wetted to get optimal results.

8.2 Copper deficiency

Copper deficiency symptoms frequently develop in young trees forced into growth by heavy nitrogenous fertilization and may accompany, zinc deficiency sumptoms. Such deficiency symptoms are seen in trees planted in sandy soil (Prakash and Srivastava, 1987). The appearance of weak terminal shoots followed by defoliation and die back of the branches, on the top of long dropping or S-shaped branches of the preceding cycle of growth is usually evident that copper is needed (Lynch and Mustard, 1950). The deficiency remedied by the application of copper fungicides and is seldom observed in orchards receiving copper spray for control of anthracnose and other leaf spot diseases (Prakash and Srivastava, 1987).

8.3 Mango decline

Mango decline is becoming a serious problem in Northern India (Pandey *et al.*, 1971, Prakash and Srivastava, 1987). Prakash (1984) observed sporadic decline symptoms in U.P. especially in the districts Agra, Aligarh, Etawah, Etah, Mainpuri and Farrukhabad. Old plants showed the symptoms of declining trend invariably. Initially the symptoms appear on older leaves with the collapsing of tissues and clearly defined marginal areas of brick red colour towards the tip. Later, with increasing severity almost the entire lamina gets affected. The younger leaves remain unaffected upto 2-3 months of their emergence. Such affected plants were thoroughly examined but the involvement of any micro-organism was completely rulled out (Prakash and Srivastava, 1987).

8.3.1 Causes

The amount of chloride in scorched leaves was found to be more than that of the healthy leaves in cvs. Chausa and Dashehari. In Chausa scorched leaves, it was about four times more whereas in the case of Dashehari, it was twice that of control. The accumulation pattern of calcium and sodium in the healthy and scorched leaves was similar to that of chloride but potassium showed a peculiar trend in both the cultivars, the amount of potassium in scorched leaves being less as compared to control in both the cases. However, it was difficult to say whether the scorching was due to the toxicity of chloride ions or due to the effect of cationic imbalances. To arrive at a better understanding of the actual process, sprays of 2% NaCl, NaHCO$_3$ and Na$_2$SO$_4$ were given to induce artificial scorching of leaves. Natural symptoms of scorching of leaves resembled with the artificially induced symptoms by 2% spray of NaCl. The amount of chloride in the artificially induced scorched leaves by 2% spray of NaCl increased approximately three times, while the content of potassium was reduced to half as compared to the healthy leaves of the same tree. These observations suggest that Potassium deficiency with which the symptoms of this disease resembles is not the cause but a consequence of chloride ion toxicity. Jha and Siddiqii (1965) reported the death of mango tree in the Kosi flood areas of Bihar due to manganese toxicity but the symptoms are quite different form those reported by Pandey *et al.,* (1971).

8.3.2 Tip burn

The tip burn refers to the dying back of the leaf tip and areas adjacent to the centre of the leaf, forming an irregular areas of dead, brown, dry tissue which may sometimes involve maximum one half of the area, which are subsequently attacked by anthracnose and other fungi. The cause of tip burn is not definitely known but salt spray and salt in the water used for overhead irrigation causes one form of tip burn, other factors viz. deficiency of moisture due to drought, high contents of salts in the soil, drying the winds and damage to root systems have been considered the contributing factors (Ruehle and Ledin, 1955).

9. Injuries caused by abiotic factors

9.1 Frost injury

Frost injury damages the young mango plants (upto 10-15 year old). Such damages are usually considered as 'Act of God' and are beyond the control of man. In Tarai, plains of UP and other states especially in Northern India, mangoes are subject to occasional frost injury. Although, occasional minor frost injury has been reported from time to time (Hartless, 1914, Singh and Singh, 1955) but a severe frost was also experienced at the Experimental Research Station, Rehmankhera in the year 1985 (Prakash and Srivastava, 1987) due to sudden fall in temperature which resulted in the death of young mango plants (5-10 years of age). Most of the varieties under trials were adversely affected. The damage occurs most at or near ground level where the frost settles longer. Top of

the young trees are found to be affected. Young trees are easily injured by low temperatures and should be protected during the severe winter for several years after planting.

Symptoms are acharacterized in the form of spiliting of bark, which may not be visible in intial stages but one can only visualize when the plant withers away. Exudation of gum from the stem bark, death of new shoots, charred appearance of leaves, burning of growing fruit buds and panicles, complete death of plants, are the symptoms which have been noticed as indicative of frost injury to a young mango plant (Prakash and Srivastava, 1987, Prakash, 1998). To protect the mango plants from the frost, plants should be thatched with dried grass. The plants are covered on all sides except east to allow penetration of sunlight and air. The same material can also be utilized in summer to protect plants from hot winds and scalding heat. Besides grass, other protective material viz. 'sisal craft' has also been treid in Florida (Sturrock, 1951).

Irrigation, particularly sprinkler irrigation has replaced all other methods, for both old and young mango trees. Over head irrigation is the best but a fair amount of protection is reported to be obtained by under the tree application during cold spells. Grove heaters are effective for protecting the tops of both old and young trees during cold spells (Young and Sauls, 1979). Affected branches, twigs should be pruned and pasted with Bordeaux paste if plants have not died completely (Prakash and Srivastava, 1987).

9.2 Hails

Hails when they strike also damage the mango crop as experienced during the year 1978 and 1983 in Lucknow region (Prakash and Srivastava, 1987). They are of 0.5 to 4.5" or even more in size, irregular in shape, many vary with respect to sharp cutting edges. It injured small fruits, torn the bark of trunk and twigs of mango upto depth of about 0.5 to 1.5" lingthwise. Soft tissues received more wounds and frequently xylem tissue was bleached. Such wounds provided the site for secondary infection for various microorganisms which cause further rotting in the tissues.

9.3 Rain/storms/wind

Hurricane storm/wind followed by rains were occasionally experienced in Lucknow during the flowering and when fruits attain maturity. The unusual amount of high velocity wind driven rains affect the fruit set and huge loss due to dropping of marble size mangoes were noticed in area, thus resulting in about 30-40 per cent loss of the crop in Malihabad region (Prakash and Srivastava, 1987).

10. References

Acuna, H.E. and Waite, B.H. 1977. La muerte regresiva del mango (*Mangifera indica* L.) en El-Salvador. Proc. Ame. Soc. Hort. Sci. Region, 21 : 15-16.

Agarwala, S.C. 1947. Chemical studies in the physiology of mangoes. Ph.D. Thesis, Lucknow Univ., Lucknow.

Agarwala, S.C., Sharma, C.P. and Kumar, A. 1960. The effect of black tip disease on the catalase

and peroxidase activities of mango fruits. Current Science, 29: 195.

Agrawal, S.C., Sharma, C.P. and Kumar, A. 1961. The effect of the black tip disease on the ascorbic acid content of the mango fruit. Journal of Indian Botanical Society, 40 : 397-403.

Agarwala, S.C., Sharma, C.P. and Kumar, A. 1962. The mineral nutrient element composition of the mango fruit with particular reference to the black tip disease. Journal of Indian Botanical Society, 41: 16-23.

Allan, R.G. 1936. Modern Cultivation. Bulletin Department of Agriculture UP., India. No. 13 (Fruit Series).

Alvarez Garcia, L.A. 1968. *Physalospora rhodina* (Berk. and Curt.) Cooke, the perfect stage of *Diplodia* responsible for a die back of mango in Puerto Rico. *J. Agric. Univ. Res.*, Puerto Rico, 52: 260.

Alvarez Garcia, L.A. and Lopez Garcia, L. 1971. Gummosis, die back and fruit rot disease of mango (*Mangifera indica* L.) caused by *Physalospora rhodina* (B. & C.) Cke. in Puerto Rico. J. Agric. Univ. Res., Puerto Rico, 55: 435-450.

Amin, H.D. 1967. Development of white corky tissue in a mango fruit (*Mangtfera indica*). Navsari Agricultural College Magazine, 6: 14-17.

Andotra, P.S., Chib, H.S. and Gupta, B.R. 1984. Studies on the fungal etiology and behaviour of mango malformation under Jammu conditions. Indian Journal Mycology & Plant. Pathology, 14: 90-92.

Angulo, S.M. and Villapudua, J.R. 1982. Buba of mango (*Mangifera indica* L.) in the state of Sinalow, Mexico. (Abs.) Phytopathology, 72: 171.

Anonymous, 1930. Mango hopper and mildew and their control. *Bombay Dep. Agric. Leafl*et 1930 **6** : 4.

Anonymous, 1960. Black tip disease of mango. Annual Report of Horticultural Research Institute, Saharanpur, UP. : p. 19.

Anonymous, 1968. Control of powdery mildew of mango. Nat. Agri. Serv. Skill Agriculture 5: 9-10.

Anonymous, 1968-70. Annual Report, CFTRI, Mysore, India.

Anonymous, 1970. Report of Spongy Tissue Committee. Department of Agriculture, Gujarat State : pp 1-5.

Anonymous, 1983. A Report of the Horticulture Sub-committee. Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri, India.

Anonymous, 1986. Annual research report, Central Institute of Horticulutre for Northern Plains, Lucknow, p. 92-93.

Anonymous, 1987. Annual Research Report of CIHNP, Lucknow on Jhumka malady. p. 103.

Anonymous, 1994-95 and 1995-96. Annual Research Report of CIHNP, Lucknow on causes and control of clustering (Jhumka disorder in mango). pp. 47 & 65.

Assnani, M.B., Chhatpar, H.S., Kadri, M.H., Mattoo, A.K. and Modi, V.V. 1971. Microbial Spoilage of mangoes. Journal of Food Science and Technology 8: 208-209.

Batista, A.C.A. 1947. Serious disease of mango. Thesis, Pernambuco College of Agric. p. 106.

Beniwal, S.P.S. and Bhatnagar, S.S. 1975. Annual Research Report, G.B. Pant Univ. Agric. Tech., Pantnagar, 1974-75.

Berthet, I.A. 1914. Molestia de Mangueira. Boletin de Agric. (Sao Palo) XV, 8-10: 818-819.

Bhatnagar, M.K. and Singh, R.R. 1979. Effect of auxins on the growing of *Pestalotia psidii* and *B. theobramae.* Indian Journal Mycology and Plant. Pathology,. 8: 126-128.

Bhatnagar, S.S. and Beniwal, S.P.S. 1977. Involvement of *Fusarium oxysporum* in causation of mango malformation. Plant Dis. Reptr. 61: 894-898.

Bindra, O.S. and Bakhetia, D.R.C. 1971. Investigations on the etiology and control of mango malformation. Indian Journal of Horticulture. 28: 80.

Birth, G.S. and Norris, K.H. 1965. The difference meter for measuring interior quality foods and

pigment in biological tissues. Technical Bulletin No. 1341, USDA, Washington.

Blakke, A.L. 1913. Iowa Agriculture Experimental Station Bulletin : 145.

Bose, P.C. and Singh, C. 1980. Chemical control of bacterial canker of mango. Pesticide 14: 30-31.

Bourne, B.A. 1921. Fungoid attacks reported or observed. Rept. Dept. Agric., Barbados, 1920-21, p. 10-11.

Brooks, W.H. 1991. Mango powdery mildew: Increased yield with improved mildew control. Year Book-South African Mango Grower's Association 19: 33-34.

Butler, E.J. and Bisby, G.R. 1931. The fungi of India. Sci. Monograph No. **1**. The Imperial Council of Agric. Res. India, Calcutta.

Camp, A.F. and Fudgi, B.R. 1939. Some symptoms of malnutrition in Florida. Fla. Agric. Exp. Sta. Bull. p. 335.

Camp, A.F. and Walter Reuter 1937. Studies on the effect of zinc and other unusual mineral suppliments on the growth of horticultural crops. Fla. Agric. Exp. Sta. Ann. Rep., p. 132-135.

Campacci, C.A. 1946. Pine apple soft rot. Biologico 12: 70-71.

Chadha, K.L., Pal, R.N., Om Prakash, Tandon, P.L., Singh, H., Singh, N.P., Rao, M.R.K. and Lal, B. 1979a. Studies on mango malformation - Its causes and control. Indian Journal of Horticulture, 36: 359-368.

Chadha, K.L., Sahay, R.K. and Pal, R.N. 1979b. Induction of floral malformation like symptoms in mango by a morphactin. Inidan J. Hort. 36: 220-221.

Chakrabarti, D.K. and Ghosal, S. 1985. Effect of *Fusarium moniliforme* var. *subglutinans* infection on mangiferin production in the twigs of *Mangifera indica*. Phytopath. Z., 113: 47-50.

Chakravarti, B.P., Singh, R.K. and Mallik, P.C. 1972. Prevalence and control of lichens on mango and litchi in Bihar. (Abs.) In: "3rd International Symposium on Subtropical and Tropical Horticulture", Bangalore, p. 112.

Chandra, A. and Yamdagni, R. 1984. A note on the effect of borax and sodium carbonate sprays on the incidence of black tip disorder in mango. Punjab Horticultural Journal, 24 : 17-18.

Chaplin, G.R. 1986. Post harvest physiology of mango fruit. In: First Australian Mango Research Workshop, Nov., 1984.

Chattopadhyay, N.C. and Nandi, B. 1977. Nutrition in *Fusarium moniliforme* var. *subgltinans* causing mango malformation. Mycologia, 73: 407.

Chee, K.H. 1969. Hosts of *Phytophthora palmivora.* Review of Applied Mycology, 48: 337-344.

Cheema, G.S. and Dani, P.G. 1934. Report on the Export of Mangoes to Europe in 1932 and 1933. Department of Agriculture, Bombay, Bulletin No. 170: pp. 1-31.

Chhatpar, H.S., Modi, V.V. and Vasavada, P.C. 1968-69. Bacterial production of spongy tissue in mango. M.S. Univ., Baroda 12 & 18.

Chowdhary, P.N. and Varma, A. 1972. *Cylindrocarpon mangiferum* sp. nov.- A new fungus isolated from mango (*Mangifera indica*) affected with vegetative malformation. Current Science., 55: 1077-1078.

Cook, A.A., Milbrath, G.M. and Hamilton, R.A. 1971. Woody gall and scaly bark of *Mangifera indica* in Hawaii. Phytopathology, 61: 1320.

Das Gupta, S.N. 1940-51. Studies on mango necrosis. Report submitted to Indian Council of Agricultural Research, New Delhi, pp. 257 (unpublished).

Das Gupta, S.N. 1957. Air pollution in relation to plant diseases. Presidential Address, 44th Indian Science Congress, Section of Botany, Calcutta.

Das Gupta, S.N. 1959. A boron deficiency disease. Presidential Address, 28th Annual, Session, National Academy Sciences, Section of Biological Sciences, Feb. 6-8, 1959, Agra.

Das Gupta, S.N. and Agrawal, S.C. 1947. Metabolic changes in Dashehari mangoes due to necrosis, chemical studies in the physiology of mangoes. Ph.D. Thesis of S.C. Agrawal,

Lucknow Univ., Lucknow.

Das Gupta, S.N. and Asthana, S.N. 1944. Histopathology of necrotic mango fruits. Current Science 13: 77.

Das Gupta, S.N. and Sen, C. 1958. On the prevention of mango necrosis. Current Science 27: 446-447.

Das Gupta, S.N. and Sen, C. 1960a. Studies in the disease of *Mangifera indica* Linn. XI. The effect of boron on mango necrosis. Phytopathology 50: 431-433.

Das Gupta, S.N. and Sen, C. 1960b. Studies in the disease of *Mangifera indica* Linn. XII. Further studies in the effect of boron on mango necrosis. Proceedings of National Institute of Sciences, India, B-26: 80-87.

Das Gupta, S.N. and Sinha, S. 1944. Studies in the disease of *Mangifera indica* Linn. IV. Investigations into the pathological histology of fruit affected with black tip disease with note on the anatomy of fruits. Proceedings of National Academy of Sciences, India 14: 102-108.

Das Gupta, S.N. and Verma, G.S. 1939a. Studies in the disease of *Mangifera indica* Linn. I. Preliminary observations of the necrosis of the mango fruit with special reference to the external symptoms of the disease. Proceedings of Indian Academy of Sciences 39: 13-28.

Das Gupta, S.N. and Verma, G.S. 1939b. Studies in the disease of *Mangifera indica* Linn. II. Effect of infecting healthy mango fruits with extracts from naturally occurring necrotic mangoes. Proceedings of Indian Academy of Sciences B-12: 95-108.

Das Gupta, S.N. and Zachariah, A.T. 1945. Studies on the diseases of *Mangifera indica* Linn. Part V. on the die back disease of the mango tree. Journal of Indian Botanical Society, 24: 101-110.

Das Gupta, S.N., Verma, G.S. and Sinha, S. 1941. Studies in the disease of *Mangifera indica* Linn. III. Investigations into the effect of sulphur dioxide gas on the mango fruit. Proceedings of Indian Academy of Sciences B-13: 71-83.

Das Gupta, S.N., Iyer, S.N. and Verma, G.S. 1956. Studies in the disease of *Mangifera indica* Linn. XI. Isolation of brick kiln fumes constituent causing mango necrosis. Indian Journal of Agricultural Sciences 26: 259-266.

Das Gupta, S.N., Verma, G.S., Agarwala, S.C., Raj, J.N. and Iyer, S.N. 1950. Necrosis of the mango fruit. Current Science 9: 153.

Das, S.R. and Mohanty, N.N. 1972. A note on some fungi from Orissa. Current Science, 41: 430.

Datar, V.V. 1981. Chemical management of powdery mildew of mango. Third Int. Symp. on Pl. Patho. (Abs.) 14-18th Dec., New Delhi, p. 150-151.

Datar, V.V. 1983. Reaction of mango varieties to powdery mildew incited by *Oidium mangiferae.* Indian Journal Mycology & Plant Pathology, 13: 111-112.

Datar, V.V. 1985. Perpetuation of *Oidium mangiferae* Berth. causing powdery mildew of mango. Second Int. Symp. on Mango (Abs.), Bangalore, p. 61.

Datar, V.V. 1986. Management of powdery mildew of mango with fungicides. Indian Phytopathology, 39: 271-272.

Desai, M.C. 1966. Sponginess in mango fruits. *Junagarh Agriculture College Magazine* 5: 4-6.

Desai, M.V. and Patel, K.P. 1963. Relative susceptibility of several varieties of mango to blight disease. Indian Phytopatholgy, 16: 239-240.

Doidge, E.M. 1915. A bacterial disease of mango, *Bacillus mangiferae* n. sp. Ann. Biol. 2: 1-45.

Farr, D.F., Bills, G.F., Chamuris, G.P. and Rossman, A.Y. 1989. Fungi on plant and plant produces in United States. APS Press, The American Phytopathological Society, St. Paul, MN., p. 1252.

Fernando, M., Reddy, T.S., Singh, N.J., Sharma, O.P. and Sohi, H.S. 1960. Studies on blight disease of mango caused by *Macrophoma mangiferae.* Indian Phytopath. 13 : 137-143.

Gadre, U.A. 1979. Control of sclerotium rot of mango seedlings. Paper presented in Mango Workers Meeting held at Panaji (Goa), p. 202.

Garg, S.C. and Kasera, H.L. 1994. Antibacterial activity of essential oil of *Anacardium occidentale* Linn. Indian Perfumer 28: 95-97.

Ghosal, S., Biswas, K., Chakrabarti, D.K., Bhattacharya, S.K. and Chattopadhyay, B.K. 1977a. 2nd Conf. Common Wealth Pharm. Assoc., Bombay (Abstr.).

Ghosal, S., Biswas, K., Chakrabarti, D.K., Bhattacharya, S.K. and Chattopadhyay, B.K. 1977b. 12th Scientific Seminar in Indian Medicine, Varanasi.

Ginai, M.A. 1965. Malformation of mango inflorescence (West Pakistan). J. Agric. Res. 3 : 248-251.

Goldweber, S. 1975. The use of a chelated ion to restore normal vigour to Verticillium wilt infected mango trees. Proc. Fla. Sta. Hort. Soc., 88: 499-500.

Gunjate, R.T., Tare, S.J., Rangwala, A.D. and Limaye, V.P. 1979. Calcium content in Alphonso mango fruits in relation to occurrence of spongy tissue. Journal of Agricultural University, Maharashtra, 4: 159-161.

Gupta, J.H. 1976. Reaction of mango varieties to powdery mildew (*Oidium mangiferae* Berth.) in Uttar Pradesh. Progressive Horticulture, 8: 63-64.

Gupta, J.H. 1979. Influence of environmental facters on the development of powdery mildew of mango. Indian Journal of Horticulture, 36: 96-98.

Gupta, J.H. 1988. Dispersal of conidia of *Oidium mangiferae* Berthet, causing powdery mildew of mango. Progressive Horticulture, 20: 341-342.

Gupta, J.H. 1989a. Longevity of conidia of *Oidium mangiferae* causing powdery mildew of mango. Indian Journal of Mycology and Plant Pathology, 19 : 123-124.

Gupta, J.H. 1989b. Perpetuation and epidemiology of powdery mildew of mango. In: Proc. Second Int. Symp. on Mango, p. 528-533.

Gupta, P.L. and Dang, J.K. 1981. Occurrence and control of powdery mildew of mango in Haryana. Indian Phytopathology, (1980, publ. 1981) 33: 631-632.

Haq, C.A., Malik, M.T., Syed, S.A. and Khan, S.H. 1994. Evaluation of various fungicides against powdery mildew (*Oidium mangiferae*) in mango. Pakistan Journal of Phytopathology, 6: 17-20.

Hassan, A.S. 1944. Notes on *Eriophes mangiferae*. S.N. Bull. dela Soc. Fouad IER. Ent. 28 : 179.

Hernandez, J., Gallo, L., Lobet, L. and Jaizmevega, M.C. 1955. A preliminary study of the fungal species associated with pine apple, papaya and mango in Canary Islands. Anales del Instituto Nacional de Investigaciones Agrarias, Agricola, 28: 171-180.

Hingorani, M.K. and Sharma, O.P. 1956. Blight disease of mango. Indian Phytopathology, 9 : 195-196.

Hingorani, M.K., Reddy, T.S. and Singh, N.J. 1961a. Comparative studies of *Macrophoma mangiferae* and its ultraviolet light induced mutant. Indian Phytopathology, 12: 139-148.

Hingorani, M.K., Sharma, O.P. and Sohi, H.S. 1960. Studies on blight diseases of mango caused by *Macrophoma mangiferae*. Indian Phytopathology, 13: 137-143.

Hingorani, M.K., Sharma, O.P. and Singh, N.J. 1961b. Pycnidia formation in *Macrophoma mangiferae*, the causal organisms of blight disease of mango. Indian Phytopathology, 14: 48-52.

Hopkins, J.C.F. 1941. Diseases of fruits, flowers and vegetables in Southern Rhodesia. Rhod. Agric. J. , 38: 470-471.

Jadeja, K.B. and Vaishnav, M.U. 1984. Effective spray schedule for control of anthracnose and leaf blight of mango. Indian J. Pl. Prot., 12 : 93-96.

Jagirdar, S.A.P. and Shaik, M.R. 1968. Souvenir, Mango and Summer Fruit Show, Mirpur Khas, W. Pakistan.

Jawanda, J.S. 1963. Studies on mango malformation. Punjab Horticulture Journal, 3: 281.

Jha, K.K. and Siddiqui, M.A. 1965. Manganese toxicity, a cause of the health of mango trees in the Kosi flood affected areas of Bihar. J. Indian Soc. Soil Sci. 13: 233-236.

Johnson, G.I. 1994. Powdery mildew. In : Compendium of Tropical Fruit Diseases (eds. Ploetz, R.C., Zentmyer, G.A., Nishijima, W., Rohrbach, K. and Ohr, H.D.), , APS Press, St. Paul, pp. 38-39.

Joshi, G.D. 1975. Studies on spongy tissue of mango (*Mangifera indica* L.) fruits. M.Sc. (Agri.) Thesis, Konkan Krishi Vidyapeeth, Dapoli.

Joshi, H.U. and Chauhan, H.L. 1985. Effective control of powdery mildew of mango. (Abs.) Second Int. Symp. on Mango, Bangalore, India, p. 63.

Joshi, G.D. and Roy, S.K. 1989. Studies on spongy tissue in Alphonso mango. Acta Horticulture 231: 649-661.

Joubert, M.H. 1991. Implications of epidemiological studies on strategies for control of powdery mildew and anthracnose. Year Book-South African Mango Grower's Association, 11: 26-28.

Katrodia, J.S. 1979. The study into the cause of the development of spongy tissue in mango (*Mangifera indica* L.) fruit of cultivar Alphonso. Ph.D. Thesis, Marathawada Agricultural University, Parbhani.

Katrodia, J.S. and Rane, D.A. 1979. Study into the cause of the development of spongy tissue in mango (*Mangifera indica* L.) cv. Alphonso. Research Bulletin of Maharashtra Agricultural University, 3: 88-92.

Katrodia, J.S. and Rane, D.A. 1981. Effect of tree vigour in relation to spongy tissue development in Alphonso mango fruit. Haryana Journal of Horticultural Science, 10: 151-154.

Katrodia, J.S. and Rane, D.A. 1989. The pattern of distribution of spongy tissue in the affected Alphonso fruits at different locations. Acta Horticulture, 231: 873-877.

Katrodia, J.S. and Sheth, I.K. 1989. The spongy tissue development in Alphonso mango in relation to temperature and its control. Acta Horticulture, 231: 827-834.

Kausar, A.G. 1959. Malformation of inflorescence in mango. Punjab Fruit J., 22: 19-21.

Khader, S.E.S.A. 1988. Physiological changes in healthy and aborted fruits of mango at various stages of growth. Indian Journal of Plant Physiology, 31: 316-319.

Khader, S.E.S.A. 1989. A short note on clustering in mango- A new disorder. Haryana Journal of Horticultural Science, 18: 233-234

Khader, S.E.S.A., Rajput, M.S. and Biswas, P.P. 1988. Index to quantify black tip disorder of mango in orchards and its relationship with brick-kilns. Indian Journal of Agricultural Science, 58: 573-575.

Khan, M.D. 1943. Fruit and Differentiation in Mango. Thesis, Punjab University, Pakistan.

Khan, M.D. and Khan, A.H. 1960. Studies on mango inflorescence in W. Pakistan. Punjab Fruit J., 2: 247-258.

Kishun, R. 1981a. Loss in mango fruits due to bacterial canker *Xanthomonas campestris* pv. *mangiferaeinidicae*. Proc. 5th Int. Conf. Plant Path. Bacteria, Cali, pp. 181-184.

Kishun, R. 1981b. Preservation of viability and virulence of *Xanthomonas campestris* pv. *mangiferaeindicae* under different storages conditions. Indian Journal of Mycology & Plant Pathology, 16: 343-347.

Kishun, R. 1985. Stem injection of chemicals for control of bacterial canker of mango. Proc. 2nd Int. Symp. Mango, Bangalore, pp. 60 (abstr.).

Kishun, R. 1986. Role of insects in transmission and survival of *Xanthomonas campestris* pv. *mangiferaeindicae*. Indian Phytopathology, 39: 509-511.

Kishun, R. 1988a. Role of mango stones in survival of *Xanthomonas campestris* pv. *mangiferaeindicae*. In: Advances in Research on Plant Pathogenic Bacteria. Today and Tomorrow's Printers and Publishers, New Delhi, pp. 33-35.

Kishun, R. 1988b. Stem injection of chemicals for control of bacterial canker of mango. Acta Horticulture, 231: 518-552.

Kishun, R. 1993. Bacterial diseases of fruits. In: Advances in Horticulture-Fruit Crops Vol. III

(eds. Chadha, K.L. and Pareek, O.P.), Malhotra Publishing House, New Delhi, pp. 1189-1406.

Kishun, R. 1994a. Further studies in variability of *Xanthomonas campestris* pv. *mangiferaeindicae.* Indian Phytopathology, 47: 282.

Kishun, R. 1994b. Evaluation of phylloplane micro-organisms from mango against *Xanthomonas campestris* pv. *mangiferaeindicae.* Indian Phytopathology 47: 313.

Kishun, R. and Chand, R. 1988. Survival of *Xanthomonas campestris* pv. *mangiferaeindicae.* on weed hosts. Indian Phytopathology, 41: 269.

Kishun, R. and Chand, R. 1989. Mechanical transmission of *Xanthomonas campestris* pv. *mangiferaeindicae.* Indian Journal of Plant Pathology, 7(2) : 112-114.

Kishun, R. and Chand, R. 1994. Epiphytic survival of *Xanthomonas campestris* pv. *mangiferaeindicae* on weeds and its role in MBCD. Plant Disease Research, 9(1) : 35-40.

Kishun, R. and Joshi, S. 1986. Histopathology of mango infected with *Xanthomonas campestris* pv. *mangiferaeindicae.* Indian Journal of Plant Pathology, 4: 40-45.

Kishun, R. and Sohi, H.S. 1983. Bacterial canker in mangoes. Indian Farmer's Digest 14(1) : 21-23.

Kishun, R. and Sohi, H.S. 1984. Control of bacterial canker of mango by chemicals. Pesticides 18: 32-33.

Krishnamurthy, S. 1980. Internal breakdown during ripening of Alphonso (*Mangifera indica* L.) in relation to specific gravity of the fruit. Journal of Food Science and Technology, 17: 198-200.

Kueprakone, U., Saengkong, S., Pienpuck, K. and Choobumroong, W. 1986. *Phytophthora palmivora* (Butl.) Butl., the causal organism of black rot of mango seedling. Thai. Agric. Research Journal, 4: 67-73.

Kulkarni, D.K. and Kulkarni, U.K. 1978. Physiology of mango leaves infected by *Capnodium ramosum* Cooke II. *Mineral Contents.* Biovigyanam, 4: 173-174 (Heb.).

Kulkarni, G.S. 1924. Report of the work done in Plant Pathology Section during the year 1922-'23. Ann. Rept. Agri., Bombay Presidency for year, 1922-23, pp. 167-171.

Kumar, J. and Beniwal, S.P.S. 1987. Vegetative and floral malformation : Two symptoms of the same disease of mango. F.A.O. Plant Prot. Bull., 35: 21-23.

Kumar, J. and Beniwal, S.P.S. 1992. Mango malformation. In: Plant Diseases of International Importance (eds., Kumar, J., Chaube, H.S., Singh, U.S. and Mukhopadhyay, A.N.), Prenttice-Hall Inc., New Jersey, pp. 357-393.

Kumar, J., Singh, U.S. and Beniwal, S.P.S. 1993. Mango malformation : One hundred years of research. Ann. Rev. Phytopathol., 31: 217-222.

Kwee, L.T. and Chong, K.K. 1985. Diseases and disorder of mango in Malaysia. Tropical Press Sdn. Bhd., Kuala Lampur.

Lim, T.K. and Khoo, K.C. 1985. Diseases and disorders of mango in Malayesia. Tropical Press, Kuala Lumpur, Malayesia.

Limaye, V.P., Gunjate, R.T. and Joshi, G.D. 1975. Studies on occurrence of spongy tissue in some varieties of mango (*Mangifera indica* L.). Dapoli Agricultural Magazine, 10: 36-37.

Limaye, V.P., Joshi, G.D. and Gunjate, R.T. 1976. Occurrence of spongy tissue in relation to stage of harvest of Alphonso fruits. Journal of Agricultural University, Maharashtra, 1(Add.) : 292-293.

Lingaraj, D.S. 1969. Mango diseases and pests (*Mangifera indica* L.). Journal Mysore Horticulture Society, 14: 15-18.

Lonsdale, J.H. and Kotze, J.M. 1991. Increased mango yields through chemical control of blossom diseases. Year Book-South African Mango Grower's Association 11: 39-41.

Lourd, M. and Keuli, S.D. 1975. [Note Sur un chancre'a *Phtophthora* du manguier en cote d'Ivoire.] Fruit 30: 541-544.

Lynch, S.J. and Mustard, M.J. 1950. Mangoes in Florida. Fla. Dep. Agric. Bull., p. 135.

Lynch, S.J. and Ruehle, G.D. 1940. Little leaf of mangoes, a zinc deficiency. Proc. Fla. St. Hort. Soc., 53: 167-169.

Majumdar, P.K. and Sinha, G.C. 1972. Seasonal variation in the incidence of malformation in *Mangifera indica* L. Acta. Hort., 24: 221.

Malaguti, Gino and Reyes, De L.C. 1964. A gall disease of cacao and mango in Venezuela caused by *Calonectiria rigidiuscula*. Phytopath., 54: 499.

Mallik, P.C. 1959a. Mango malformation. Proc. ICAR Seminar on Diseases of Horticultural Plants held at Simla, 10th-15th June, 1959, pp. 107.

Mallik, P.C. 1959b. Studies on the malformation of mango inflrescence. Proc. Bihar Acad. Sci.: 8-9.

Mallik, P.C. 1963. Mango malformation symptoms, causes and cure. Punjab Hort. J., 3: 292-299.

Mallik, P.C. and Singh, D.L. 1959. Deficiency symptoms in mango due to the absence of trace elements. Indian J. Hort., 16: 228-231.

Malo, S.E. and Mc Millan, R.T. Jr. 1972. A disease of *Mangifera indica* L. in Florida similar to mango malformation. Fla. Sta. Hort. Soc. 85: 254-268.

Manicom, B.Q. 1986. Factors affecting bacterial black spot of mangoes caused by *Xanthomonas campestris* pv. *mangiferaeindicae*. Annals of Applied Biology, 109: 129-135.

Marlatt, R.B., Knight, R.J. and Goldweber, S. 1970. Verticillium wilt of mango (*Mangifera indica* L.) in Florida. Plant Disease Reporter, 54: 569-571.

Marloth, R.H. 1947. The mango in South Africa : Dieseases and Pests. Fmg. S. Afr., 22 : 615-619.

Matheron, M.E. and Matejka, J.C. 1988. Phytophthora crown and root rot of nursery grown mango trees delivered to Artizona. (Abs.) Phytopathology, 78: 1572.

McMillan Jr, R.T. 1973. Control of anthracnose , powdery mildew of mango with systemic and non-systemic fungicides. Trop. Agric. (Trinidad), 50: 245-248.

Mehta, N., Sandooja, J.K., Madan, R.L. and Yamdagni, R. 1986. Role of different chemicals in mango malformation and related physiological factors. Pesticides, 20 : 17-18.

Misra, A.K. and Om Prakash 1988. Favourable weather conditions for the rapid spread of powdery mildew (*Oidium mangiferae* Berthet) of mango. (Abs.) Fifth Int. Congress of Pl. Patho., Tokyo, Japan, p. 293.

Misra, A.K. and Prakash, Om 1992. Bacterial canker of mango incidence and control. Indian Phytopathology, 45 : 172-175.

Misra, A.K. and Prakash, Om 1993. Host range and efficacy of different chemicals for the control of sooty mould of mango. National Academy of Sci., 63(B) II : 233-235.

Misra, A.K. and Prakash, Om 1995. Epidemiological parameters of mango powdery mildew. Ann. Conf. and National Symposium on Recent Trends in Management of Biotic and Abiotic Stresses in Plants, Palampur (India), Oct., 20-31 : 14.

Mullar, H.R.A. 1940. Survey of the most important mango diseases in Dutch East Indies. Meded Alg. Proefstt. Linab., Bataivia 40 : 9.

Munshi, G.D., Jhooty, J.S. and Kaur Jasmit 1988. Perenation of powdery mildew of mango as leaf infections. Indian J. Mycol. Pl. Pathol., 18: 68-69.

Naik, K.C. 1934. Aam Ka Bagicha Me Eent Ka Bhatta. *Kishan* (Hindi Monthly), Bihar 4 : 240-242.

Nariani, T.K. and Seth, M.L. 1962. Role of eriophyid mites in causing malformation disease in mango. Indian Phytopathology, 15: 231.

Nauriyal, J.P., Chadha, K.L. and Rajput, M.S. 1972. Investigation on the control of black tip disorder in mango. Acta Horticulture, 24: 215-216.

Nirvan, R.S. 1953. Bunchy top of young mango seedlings. Sci. and Cult., 18 : 335-336.

Nuevo, P.A., Cua, A.U. and Lizada, M.C.C. 1984. Internal breakdown in "Carabao" mango

subjected to modified atmosphere III. Starch in the spongy tissue. Post Harvest Research Notes 1: 34-35.

Oppenheimer, C. and Gazit, S.H. 1961. Zinc deficiency in mango groves in Israel and its connection. Hort. Adv., 5: 1-2.

Padron Soroa, J. 1983. Factors responsible for low yield of mango. Boletin de Resenas Protection de Plantas, 10 : 37.

Pal, R.N. and Chadha, K.L. 1980. Black tip disorder of mango- A review. Punjab Horticultural Journal, 3 : 221-225.

Pal, R.N. and Chadha, K.L. 1993. Black tip and internal necrosis of mango. In : Advances in Horticulture Vol. 4 : Fruit Crops (eds. Chadha, K.L. and Prakash, O.P.), , Malhotra Publishing House, New Delhi. pp. 2081-2093.

Pal, N.L., Chatterjee, U.N. and Ranjan, S. 1937. Effect of gases from brick kilns on mango crop. Proc. 24th Indian Science Congress, pp. 270-271.

Pal, R.N. and Prakash, Om 1984. Package of recommended practices for mango. Leaflet, CIHNP, Lucknow, p. 6.

Palo, M.A. 1933. Sclerotium seed rot and seedling stem rot of mango. The Philippines J. Sci., 52(3) : 237-261.

Pandey, R.M., Sinha, G.C., Majumdar, P.K. and Singh, R.N. 1971. Mango decline caused by cation and anion imbalance. Current Science, 40: 356-357.

Pandey, R.M., Singh, R.N. and Sharma, V.K. 1974. Leaf scorch in mango, a new problem. Indian Horticulture, (April-June) 19: 7-8.

Pandey, S.C. and Mohammad, A. 1974-75. Studies on fruit rot of mango III. Brown spot caused by *Pestalotia magiferae* Butl. Proc. Bihar Acad. Sci., 22-23: 89-95.

Patel, M.K., Moniz, L. and Kulkarni, Y.S. 1948b. A new bacterial disease of *Mangifera indica*. Current Science, 17: 180-190.

Patel, M.K., Kulkarni, Y.S. and Moniz, L. 1948a. *Pseudomonas mangiferaeindicae* pathogenic on mango. Indian Phytopathology, 1: 147-152.

Peech, M. 1939. Chemical studies on soils from Florida citrus groves. Fla. Agric. Exp. Sta. Bull., p. 140.

Peethambharan, C.K. and Aravindakshan, M. 1975. Varietal susceptibility of mangoes to sooty mould. Agric. Res. J., Kerala, 16: 260-261.

Persley, D.M., Pegg, K.G. and Syme, J.R. eds. 1989. Fruit and nut crops- a disease management guide. Queensland Department of Primary Industries Information Series : 188018.

Peterson, R.A., Schipke, L.G. and Clarkson, P.C. 1991. Significance of two mango flower diseases in the dry tropics. In: Proc. 3rd Int. Mango Symp. held at Darwin, 24-29th Sept., 1989 : 338-343.

Ploetz, C.R.L. and Om, Prakash 1997 Foliar, floral and soil borne diseases. In: The Mango (eds. Litz, R.E). CAB, International, Wallingford, UK, pp. 281-325.

Pohronezney, K. and Marlatt, R.B. 1982. Some common disease of mango in Florida. Plant Patho. Fact Sheet, pp. 23, Univ. of Florida, Gainesville, 4 pp.

Prakash, Om 1975-76, 1978-79, 1980. Studies on black tip and internal necrosis disorders. Ann. Res. Reports of Plant Pathology, Central Mango Research Station, Lucknow.

Prakash, Om 1975-79. Internal Necrosis. Annual Report of Central Mango Research Station, Lucknow, India.

Prakash, Om 1977 & 1984. Annual Report, Central Mango Research Station, Lucknow, p. 28.

Prakash, Om 1978. A new mango disease and its crue. (Abst.) Symposium on Plant Disease Problems held at Jaipur, Oct. 1-3, p. 30-31.

Prakash, Om 1979. Chemical control of phoma blight in mango. Indian J. Mycology and Pl. Patho., 9(2) : 184-185.

Prakash, Om 1981. Annual Report of Plant Pathology Division (IIHR), Central Mango Research

Station, Lucknow : 133-134.

Prakash, Om 1982 & 1984. Annual Report, Central Mango Research Station, Lucknow.

Prakash, Om 1984. Annual Report of Pl. Patho., CIHNP, Lucknow, India, pp. 28.

Prakash, Om 1988. Report on mango in Malda region (W.B.). Submitted to ICAR, and Secretary, Govt. of West Bengal, p. 22.

Prakash, Om 1988. Sooty mould disease of mango and its control. Int. J. of Trop. Pl. Disease, 9 : 277-280.

Prakash, Om 1996. Principal diseases of mango causes and control. In: Advances in Diseases of Crops in India , Kalyani Publisher, Ludhiana, pp. 191-256.

Prakash, Om 1998. Diseases of Mango. In: Mango Cultivation, (ed. Prakash, Om), International Book Distributing Co., Lucknow,pp. 301-338 and 409-506 .

Prakash, Om and Eckert, J.W. 1992. Twig die back disease of mango (*Mangifera indica*). Abs. Proc. 4th Int. Mango Symposium at Florida (USA).

Prakash, Om and Eckert, J.W. 1998. Twig die back disease of mango (*Mangifera indica* L.) caused by *Botryosphaeria ribis* from California. Proc. Sixth Internatinal Mango Symposium, Pattaya, Thailand : pp. 139.

Prakash, Om and Misra, A.K. 1986. Evaluation of mango germplasm against powdery mildew under natural condition. Annual Report, CIHNP, Lucknow, pp. 62-63.

Prakash, Om and Misra, A.K. 1987. Annual Report of Plant Pathology Division, CIHNP, Lucknow, p. 180.

Prakash, Om and Misra, A.K. 1988. Growth of red rust *C. virescens* Kunz of mango during the unusual drought year 1987 in Kakori and Malihabad, mango belt of India. XXI Congress Brasileiro de Fitopatologia Bras., 13: 121.

Prakash, Om and Misra, A.K. 1989. Important diseases of mango and their effect on production. (Abs.) National Mango Seminar, Deptt. of Hort., U.P., Jan., 19-20 : 22.

Prakash, Om and Misra, A.K. 1992. Important diseases of mango and their effect on production. Biol. Mem., 18: 39-55.

Prakash, Om and Misra, A.K. 1993a. Fungal diseases of sub-tropical horticultural fruit crops. In: Advance in Horticulture, eds. Chadha, K.L., Malhotra Publishing House, New Delhi (India),pp. 1275-1372.

Prakash, Om and Misra, A.K. 1993b. Integrated approach in the management of mango diseases. (Abs.) National Conf. on Eco-friendly Approaches in the Management of Pests/Diseases and Industrial Effments, 20-22nd Dec. at CSAAU & T, Kanpur, pp. 70-71.

Prakash, Om and Raoof, M.A. 1979. Studies on die back disease of mango. Proc. Mango Worker's Meeting, Goa, 2-5th May, 1979): pp. 199-200.

Prakash, Om and Raoof, M.A. 1982. Effect of different fungicides, their concentration and spray intervals. Proc. All India Coordinated Fruit Improvement Project Workshop held at Nagpur, 9-14th Feb., p. 654-655.

Prakash, Om and Raoof, M.A. 1985. Bacterial canker in mango. (Abs.) IInd International Symposium on mango, Bangalore, p. 59.

Prakash, Om and Raoof, M.A. 1985a. Die back disease of mango (*Mangifera indica*), its distribution, incidence, cause and management. Fitopatol. Bras., 14 : 207-215.

Prakash, Om and Raoof, M.A. 1985b. New records of fungi on leaves and twigs of mango (*Mangifera indica* L.). Indian J. Pl. Patho., 3 : 243-244.

Prakash, Om and Raoof, M.A. 1985c. Perpetuation of powdery mildew of mango. Indian J. Pl. Patho., 3: 273-274.

Prakash, Om and Raoof, M.A. 1985d. Some new maladies of mango (*Mangifera indica*) of unknown etiology. Indian J. Pl. Pathology, 3: 245-251.

Prakash, Om and Raoof, M.A. 1985e. Spray schedule for the control of powdery mildew disease of mango. Indian J. Pl. Patho., 3: 51-52.Prakash, Om and Singh, U.N. 1976a. Basal rot of

mango seedling caused by *Sclerotium rolfsii.* Indian J. Myco. and Pl. Patho., 6(1) : 75.

Prakash, Om and Raoof, M.A. 1994. Studies on powdery mildew (*Oidium mangiferae*) disease of mango : Distribution, perpetuation, losses and chemical control. Bio. Memoirs, 207: 31-45.

Prakash, Om and Singh, U.N. 1976b. New Disease of mango. *Proc.* Fruit Res. Workshop, Hyderabad, May, 24-28th, p. 300-302.

Prakash, Om and Singh, U.N. 1977. Phoma blight, a new disease of mango (*Mangifera indica* L.). Plant Dis. Reptr,. 61 : 419-421.

Prakash, Om and Singh, U.N. 1979. Fungicidal control of red rust of mango. Indian Journal of Mycology and Plant Pathology, 9 : 175-176.

Prakash, Om and Singh, U.N. 1980. Root rot and damping off of mango seedling caused by *Rhizoctonia solani.* Indian Journal of Mycology and Plant Pathology, 10(1) : 69.

Prakash, Om and Singh, U.N. 1982. Evaluation of various fungicides for the control of powdery mildew of mango caused by *Oidium mangiferae.* Pesticides, 16(2) : 17-18.

Prakash, Om and Srivastava, K.C. 1987a. Mango Diseases and Their Management. Today and Tomorrow's Printer and Publisher, New Delhi : pp. 175.

Prakash, Om and Srivastava, K.C. 1987b. Mango diseases and their management. A World Review, Today and Tomorrow, Printer and Publisher, Karol Bagh, New Delhi, 180 p.

Prakash, Om, Kalra, S.K., Tandon, D.K. and Raoof, M.A. 1989. Incidence of powdery mildew in mango panicles and biochemical changes.Indian Phytopathology, 42 : 185-186.

Prakash, Om, Misra, A.K. and Raoof, M.A. 1994. Studies on mango bacterial canker disease. Bio. Memoirs, 20: 95-107.

Prakash, Om Misra, A. K. and Ram Kishun 1996. Some threatening diseases of mango and their management. In : Management of Threatenining Plant Diseases of National Importance. Malhotra Publishing House, New Delhi : 179-205.

Prakash, Om, Raoof, M.A., Singh, B.P. and Srivastava, K.M. 1985. Some new maladies of mango (*Mangifera indica*) of unknown etiology. Indian Journal of Mycology and Plant Pathology, 3 : 245-251.

Prasad, A. and Saran, M.D. 1968. Black tip disease in mango. Bagwani (Hindi) : pp. 20-23.

Prasad, A., Saran, M.D. and Singh, K. 1969-71. Black tip disease of mango and its control. Horticulture Advance, 8 : 4-10.

Prasad, A. and Singh, M.P. 1965. A short note on the control of mango necrosis. Science and Culture, **31** : 251.

Prasad, A., Nirvan, R.S. and Singh, S. 1972. Mango malformation - A review of the work done at Hort. Res. Institute, Saharanpur, India. Acta Hort., 24 : 227-229.

Prasad, A., Singh, H. and Shukla, T.N. 1965. Present status of mango malformation disease. Indian J. Hort., 22: 254-265.

Pruvost, O. and Luisetti, J. 1991. Effect of time of inoculation with *Xanthomonas campestris* pv. *mangiferaeindicae* on mango fruits susceptibility, epiphytic survival of *X. c.* pv. *mangiferaeindicae* on mango fruits in relation to disease development. Journal of Phytopathology**,**133: 139-151.

Pruvost, O., Couteau, A. and Luisetti, J. 1990. Development of bacterial black spot of mangoes and epiphytic population of the pathogen (*Xanthomonas campestris* pv. *mangiferaeindicae*) under natural conditions in Reunion Island. Fruits, 45: 125-140.

Puttarudiah, M. and Channabasavana, G.P. 1961. Mango bunchy top and the eriophyid mites. Curr. Sci., 30: 114-115.

Ragab, M.M., Sabet, K.A. and Dawood, N.A. 1971. *Botryodiplodia theobromae* Pat. The cause of fruit rot and die back of mango in A.R.E. Agricultural Research Review, Cairo 49: 81-97.

Rai, J.N. 1958. Studies in the diseases of *Mangifera indica* - girdle necrosis, A variation from typical mango necrosis. Indian Journal of Agricultural Science, 28: 243-247.

Raisinghani, G.S. 1945. Spring rains and mango in Sind. Indian Farming, 6 : 341.

Rajput, M.S., Kanwar, J.S. and Bajwa, M.S. 1971. Control black tip of mango with caustic soda. Punjab Horticulture Journal, 11: 49-51.

Ram, S. 1976. Internal necrosis in fruit. Indian Farmers Digest, 9: 37-40.

Ram, S. 1988. Factors associated with black tip and internal necrosis in mango and their control. Acta Horticulture, 231: 197-204.

Ram, S. 1989. Factors associated with black tip and internal necrosis in mango and their control. Acta Horticulture, 231 : 197-204.

Ram, S., Bist, L.D. and Dwivedi, T.S. 1978. Internal fruit necrosis. A new physiological disorder in mango. Pantnagar Journal of Research, 3: 196-203.

Ram, S., Bist, L.D. and Sirohi, S.C. 1989. Internal fruit necrosis of mango and its control. Acta Horticulture, 231: 805-813.

Ramos Leandro, J., Lara, S.P., McMillan, R.T. Jr. and Narayanan, K.R. 1991. Tip die back of mango (*Mangifera indica*) caused by *Botryosphaeria ribis.* Plant Dis., 75 : 315-318.

Rane, D.A., Katrodia, J.S. and Kulkarni, D.N. 1976. Problem of spongy tissue development in mango (*Mangifera indica* L.) cv. Alphonso - A Review. Journal of Agricultural University, 1 : 89-94.

Rangwala, A.D. 1975. Changes in chemical composition of Alphonso fruits during ripening with particular reference to spongy tissue. M.Sc. (Ag.) Thesis, Konkan Krishi Vidyapeeth, Dapoli.

Ranjan, S. and Jha, V.R. 1940. The effect of ethylene and sulphurdioxide on the fruits of *Mangifera indica* L. Proceeding of Indian Academy of Sciences B,12 : 267-288.

Rao, A.P., Rao, V.V.R. and Pandith, S.V. 1975. A note on mango canker in Andhra Pradesh. Indian Journal of Mycology and Plant Pathology, 7(1) : 71.

Rath, G.C., Swain, N.C. and Mohanan, M.K. 1978. A note on die back of mango in Orissa. Indian Phytopathology, 31 : 384-386.

Raut, B.T. and Anahosur, K.H. 1994. Pathogenic variability in *Xanthomonas campestris* pv. *mangiferaeindicae*. Proc. National Symposium on Ultrastructure, Cytology, Sexuality and Variability in Plant Pathogens. 46th Annual Meeting of Indian Phytological Society, Coimbatore, pp. 14 (Abstr.).

Rawal, R.D. and Ullasa, B.A. 1989. Control of powdery mildew (*Oidium mangiferae*) Berth. of mango by fungucides. In Proc. Second Int. Symp. on mango held at Bangalre (India) in 1985, pp. 534-536.

Reckhaus, P. 1987. Hendersonula die back of mango in Niger. Plant Dis., 71 : 1045.

Reddy, D.B. and Kapoor, S.P. 1966. Controlling mango black tip. Indian Horticulture, 10 : 5-6.

Ribeiro, I.J.A. 1980. [Seca da mangueira. Agentes causais eestudio da molestia.] Paginas, 123-130 in : Anais do I Simposio Brasileiro Sobre a cultura da mangueira, 24-28, Nov. 1980. L.C. Donadio, Ed. Facultad de Ciencias Agrarias e Veterinarias. Campus Jaboticobal, UNESP. Sociedade Brasieeira de Fruticultura, Jaboticobal, Brasil, pp. 213.

Rios-Castano, D. and Reuther, W. 1967-1968. Bark cracking of mango trunk. Proc. Trop. Res. Amer. Soc. Hort. Sci., 11: 16-22.

Robbs, C.F., Da Pante, J.J. and Da Gloria, S. M. 1978. Note on *Xanthomonas mangiferaeindicae* in North East Brazil. Fitopatologia Brasileira, 3: 215-218.

Rodriguez Landaeta, A. and Figueroa, R.M. 1963. The appearance of *Oidium* or ash on mangoes in Venezuela. Rev. Fac. Agron., Maracay, 3: 40-47.

Ruehle, G.D. 1956. A note on powdery mildew of mango. Proc. Fla. Hort. Soc., 68 : 277-278.

Ruehle, G.D. and Wolfenbarger, D.O. 1948. Diseases of pests of mango in Florida. Mimm. Rep. Fla. Subtropical Expt. Sta., 13: 6.

Ruehle, G.D. and Ledin, R.B. 1955. Mango growing in Florida. Fla. Agric. Exp. Sta. Bull., pp. 574.

Sanders, G.M., Verschoor, J.A., Van Wyngaard, S., Korston, L. and Kotze, J.M. 1994. Produc-

tion of monoclonal antibodies against *Xanthomonas campestris* pv. *mangiferaeindicae* and their use to investigate differences in virulence. J. Appl. Bacteriology, 77 : 509-518.

Sarkar, A. 1960. Leaf spot disease of Mangifera indica L. caused by *Pestalotia mangiferae* Butl. Loydia, 23 : 1-7.

Sattar, A. 1946. Diseases of mango in the Punjab. Punjab Farmer J., 10: 56-58.

Saxena, A.K. and Rawal, R.D. 1989. Wilt disease of mango, a new disease. Plant Disease Research, 4: 89.

Schoeman, M.H., Manicom, B.Q. and Wingfield, M.J. 1995. Epidemiology of powdery mildew on mango blossoms. Plant Disease, 79: 524-528.

Schwartz, A. 1968. A new mango pest. Farming S. Africa, 9: 7.

Sekhawat, G.S. and Patel, P.N. 1975. Studies on bacterial canker of mango. Z. Pflakrankh, Pflacchrtz, 82: 129-138.

Sen, P.K. 1941. Black tip of the mango. Science and Culture, 7: 56.

Sen, P.K. 1942. Further studies on black tip of the mango. Science and Culture, 8: 91-92.

Sen, P.K. 1943. Studies on black tip damage of the mango. Science and Culture, 9: 343-345.

Sen, P.K., Mallik, P.C. and Roy, P.K. 1943. Toxic effects of gases on plants. Science and Culture, 9: 87-88.

Sen, P.K., Roy, P.K. and De, B.N. 1947. Hunger signs on mango. Indian J. Hort., 5 : 35.

Sharma, B.B. 1953. Studies on malformation of inflorescence in mango. Proc. 40th Ind. Sci. Cong. : pp. 170-171 (Abstr.).

Sharma, I.M., Badiyala, S.D.and Sharma, N.K. 1993. Effect of fungicidal drenching agaisnt wilt of mango seedlings caused by *Fusarium solani* (Mart.) Sacc. Indian Journal of Mycology and Plant Pathology, 23: 326-327.

Sharma, O.P. and Tiwari, Anamika 1975. Studies on mango malformation. Pesticides, 9: 44-45.

Sheth, I.K. 1981. The study of temperature in relation to spongy tissue development in mango fruit of cultivar Alphonso and its control. M.Sc. (Ag.) Thesis, Gujarat Agricultural University, Navsari.

Siddiqui, S., Samdooja, J.K., Mehta, N. and Yamadagni, R. 1987. Biochemical changes during malformation in mango cultivars as influenced by various chemicals. Pesticides, 21: 17-19.

Simao, S. and Pimentel-Gomes, F. 1995. Sensitivity of mango to *Oidium* (*Oidium mangiferae*). Revista de Agricultura (Piracicaba), 70: 241-247.

Singh, B.H. and Chakravarty, S.C. 1935. Observations on a disease of mango at Bararas. Science and Culture, 1: 1-3.

Singh, D. 1967. The Mango : A Handbook, ICAR, New Delhi : pp. 17-18.

Singh, K.K. and Jawanda, J.S. 1961. Malformation in mangoes. Punjab Hort. J., 1 : 18-22.

Singh, L.B. (1960). The Mango. Leonard Hill (Book) Ltd., London : pp. 270-272.

Singh, L.B., Singh, S.M. and Nirvan, R.S. 1961. Studies on mango malformation - I, Review, Symptoms, Extent, Intensity and Cause. Hort. Advances, 5: 197-217.

Singh, N.N. 1961-62. Studies in the black tip disease of mango (*Mangifera indica* L.) I.III. Extent and intensity of damage and effect of certain micro-nutrient sprays. Annual Report, Horticulture Research Institute, Saharanpur : 102-106.

Singh, R.N. 1954. Studies on floral biology and subsequent development of fruit in the mango varieties Dashehari and Langra. Indian Journal of Horticulture, 11: 69-88.

Singh, S.P. and Singh, R.K. 1972. Studies on sooty mould of mango (*Mangifera indica* L.). Bihar (Abs.) Proc. 3rd Int. Symp. Subtropical and Tropical Horticulture, Bangalore, p. 121.

Sinha, P.P. and Hode, M.N. 1988. Performance of mango varieties against bacterial canker. Indian Phytopathology, 41: 466-467.

Smith, J.H.E. 1973. Bark cracking in mango trees. Citrus and Subtropical Fruit Growers. No. 479. Citrus and Subtropical Fruit Research Institute, Nelspruit (S. Africa).

Smith, P.F. and Seudder, G.K. 1995. Some studies of mineral deficiency symptoms in mango.

Proc. Fla. Hort. Soc., 64: 243.

Spencer, J.L. and Kennard, W.C. 1955. Out break and new records in Puerto Rico. F.A.O. Pl. Prot. Bull., 4: 43.

Srivastava, M.P. 1969. Biochemical changes in certain tropical fruits during pathogenesis. Phytopath. Z., 64: 119-123.

Srivastava, M.P. and Tandon, R.N. 1970. Factor affecting growth sporulation and spore germination of three isolates of *B. theobromae*, effect of pH and temperature. Proc. Nat. Acad. Sci., (India) 40: 43-48.

Srivastava, R.P. 1963a. The black tip disease of mango causes and control. Punjab Horticultural Journal, 8: 226-228.

Srivastava, R.P. 1963b. Effect of smokes and industrial gases on the horticultural plants with special reference to black tip disease of mango. Gardening, pp. 24-36.

Srivastava, R.P. 1964. Aam Ko Kala Roga Se Bachayin. Kheti, June : 64.

Stevens, N.E. and Shear, C.L. 1929. *Botryosphaeria* and *Physalospora* in Hawaiian Islands. Mycologia, 21: 313-320.

Subramanyam, H., Krishnamurthy, S., Subhadra, N.V., Dalal, V.B., Randhawa, G.S. and Chacko, E.K. 1971. Studies on internal breakdown, a physiological ripening disorder in Alphonso mangoes (*Mangifera indica* L.). Tropical Science, 15: 203-210.

Summarwar, A.S. and Raychaudhuri, S.P. 1968. The role of eriophyid mite (*Aceria mangiferae*) in the causation of mango malformation. Indian Phytopathology, 21: 463.

Summarwar, A.S., Raychaudhuri, S.P. and Pathak, S.C. 1966. Association of the fungus *Fusarium moniliforme* Shel. with the malformation in mango (*Mangifera indica* L.). Indian Phytopathology, 19: 227.

Sundaraman, S., Krishnan Nayar C. and Rama Krishnan, T.S. 1928. The stem bleeding disease of arecanut caused by *T. paradoxa*. Imp. Agric. Res. Inst. Pusa Bull., 169 : 12.

Tare, S.J. 1977. Studies on calcium in relation to occurrence of spongy tissue in Alphonso mango fruits. M.Sc. (Ag.) Thesis, Konkan Krishi Vidyapeeth, Dapoli.

Thirumalachar, M.J. 1945. An ascomycetous parasite of *Cephaleuros*. Proc. Indian Acad. Sci., B22 : 374-377.

Thirumurthy, V.S., Kashyap, R., Singh, J., Garg, K., Bhattachargee, S. and Shastri, M.B. 1981. Studies on red rust of mango. (Abs.) Third International Symposium on Plant Pathology, Dec. 14-18, New Delhi, p. 41.

Tripathi, R.B. 1954. 'Bunchy Top' and malformation diseases of the mango. Indian J. Hort., 11: 122-124.

Tripathi, R.D. 1954. 'Bunchy top' and malformation diseases of mango. Indian J. Hort., 11 : 122-124.

Tsao, P.H., Luzaran, P.B., de los Santos, A.B., Portales, L.A., Gochango, A.M. and Gruber, L.C. 1994. Phytophthora crown and root of mango detected in Philippine nurseries. Plant Disease, 78: 100.

Ullasa, B.A. and Rawal, R.D. 1985. Occurrence of a new post harvest disease of mango due to *Pestalotia grandicola*. (Abs.) Second International Symposium on mango, Bangalore (India), p. 65.

Uppal, B.N. 1937. Appendix, K. Summary of work done under the plant pathologist to government, Bombay Presidency, Poona for 1935-36. Rep. Dep. Agric., Bombay : 203-207.

Uppal, B.N., Patel, M.K. and Kamat, M.N. 1935. The fungi of Bombay B, 1-56. *Bull. Dep. Ld. Rec. Agric*. Bombay, 1934, VIII : pp. 56.

Uppal, B.N., Patel, M.K. and Kamat, M.N. 1941. Powdery mildew of mango. J. Univ. Bombay Ser., 9: 12-16.

Vaheeduddin, S. 1953. The mango. Dept. of Agric, Hyderabad 121+11, p. 23.

Vala, D.G., Solanki, K.U., Desai, V.D. and Joshi, H.U. 1985. Diseases of mango occurring in

Gujrat State. (Abs.) Second International Symposium on mango, Bangalore (India), pp. 64-65.

Varma, A. 1983. Mango malformation. Exotic Plant Quarantine Pests and Procedures for Introduction of Plant Materials : pp. 173-188.

Varma, A., Lele, V.C., Majumdar, P.K., Asha Ram, Sachidananda, J., Shukla, U.S., Singh, G.C., Yadav, T.D. and Raychaudhuri, S.P. 1969. Mango malformation. ICAR Workshop. Fruit Research, Ludhiana, 28-30th April, 1969.

Varma, A., Lele, V.C. and Goswami, B.K. 1974a. Mango malformation. In : Curr. Trends in Plant Pathology. (eds. Raychaudhuri, S.P. and Verma, J.P.). Bot. Dept., Lucknow Univ., Lucknow : pp. 196-208.

Varma, A., Lele, V.C., Raychaudhuri, S.P., Asha Ram and Asha Sang 1974b. Mango malformation: A fungal disease. Phytopath. Z., 79: 253.

Varma, A., Raychaudhuri, S.P., Lele, V.C. and Asha Ram 1971. preliminary investigation of epidemiology and control of mango malformation. Proc. Indian Nat. Sci. Acad. B., 37 : 291-300.

Varma, A., Raychaudhuri, S.P., Lele, V.C. and Asha Ram 1972. Towards the understanding of the problem of mango malformation. Acta Hort., 24: 237.

Vasudeva, R.S. 1953-54. Report of the division of Mycology and Plant Pathology. Sci. Rept. Agric. Res. Institute, New Delhi, pp. 87-101.

Vasudeva, R.S. 1960. Report of the Division of Mycol. and Plant Pathol., Sci. Rept. Agric. Res. Inst., Delhi (1957-58) : pp. 111-130.

Venugopal, V., Kishun, R. and Anil Kumar, T.B. 1991. Cultural and biochemical variation in *Xanthomonas campestris* pv. *mangiferaeindicae*, the bacterial canker pathogen of mango. Indian J. Pl. Pathol. ,9(1+2) : 27-32.

Verma, G.S. 1950. Tip pulp of the mango fruit. Current Science, 19 : 246.

Verma, G.S. 1952. The formation of lesions by gases on mango fruits. Journal of Indian Botanical Society, 31: 316-341.

Verma, O.P. and Singh, R.D. 1970. Epidemiology of mango die back caused by *Botryodiplodia theobromae* Pat. Indian J. Agric. Sci., 40 : 813-818.

Verma, O.P. and Singh, R.D. 1973. A method of testing varietal reaction in mango against *B. theobromae*. J. Myco. and Pl. Patho., 3: 110-111.

Vidhyasekaran, P. and Parambaramani, C. 1971a. Nitrogen metabolism of alga infected plants. Indian Phytopath., 24: 500-504.

Vidhyasekaran, P. and Parambaramani, C. 1971b. Carbon metabolism of alga infected plants. Indian Phytopath., 24: 369-374.

Voorhess, R.K. 1942. Life history and taxonomy of the fungus, *Physalospora rhodina.* Tech. Bull No. 371, Exp. Stn., Gainesville, USA, p. 91.

Wager, V.H. 1937. Mango diseases on South Africa. Fmg., South Africa, 12 : 4.

Wagle, P.V. 1928. Studies in the shedding of mango flowers and fruits. Part I, Mem. Dept. Agric., India Bot. Sci., 8: 219-249.

Wainwright, H. and Burbage, M.B. 1989. Physiological disorder in mango (*Mangifera indica* L.) fruit. Journal of Horticultural Science, 64(2) : 125-135.

Watt, G. 1891. The Mango Tree. In: A Dictionary of the Economic Products of India, Govt. Printing Press, Calcutta, India, 5 : pp. 149.

Woodhouse, E.J. 1909. The mango of Bihar. O. J. Dept. Agri, Bengal (India) 2 : 283.

Yamdagni, R. and Chandra, A. 1985. Effect of borax and sodium carbonate on the incidence of black tip in mango. Second international Symposium on Mango, Bangalore : p. 94 (Abst.).

Young, T.W. 1942. Investigations on the unfruitfulness of the Haden mango in Florida. Proceedings of State Florida Horticultural Society, 55: 106-111.

Young, T.W. 1957. Soft nose, a physiological disorder in mango fruits. Proceedings of State

Florida Horticultural Society, 70: 280-283.

Young, T.W. 1958. Mango Research on Sandy Soil. Proc. 17th Ann. Meeting Fla. Mango Forum (1957) : 26-30.

Young, T.W., Koo, R.C.J. and Miner, J.T. 1962. Effect of nitrogen, potassium and calcium fertilization on Kent mangoes on deep, acid, sandy soil. Proceedings of State Florida Horticultural Society, 75 : 364-371.

Young, T.W., Koo, R.C.J. and Miner, J.T. 1965. Fertilizer trials with Kent mangoes. Proceedings of State Florida Horticultural Society, 78 : 369-375.

Young, T.W. and Miner, J.T. 1961. Relationship of nitrogen and calcium to soft nose disorder in mango fruits. Journal of American Horticultural Society, 78 : 201-208.

Zakii, Z., Ershad, D. and Safavi, M. 1993. Occurrence of powdery mildew on mango in Iran. Applied Entomology and Phytopathology, 60 : 1-3.

Zhang Cheng In, Huang Hul Bai and Kuang Yan Hua 1995. A study of the cause of the mango black tip disorder. Scientia Horticulture, 64: 49-54.

Zhang, D.Y. 1984. Practical Manual For Enviromental Progection Workers. Metallurgical Industry Publishing House, Bejing, China : pp. 251.

Zimmerman, P.W. and Crocker, W. 1934. Toxicity of air containing Sulphur dioxide gas. Contibs. Boyce Thomps. Inst. 6: 455-470.

Epidemiology of Powdery Mildew, Downy Mildew and Anthracnose Diseases of Grapevine

T.S. Thind, J.K. Arora, C. Mohan and Prem Raj

Department of Plant Pathology, Punjab Agricultural University
Ludhiana – 141 004,Punjab, India

Abstract: Grape cultivation throughout the world is affected by various disease problems among which three fungal diseases viz. powdery mildew, downy mildew and anthracnose pose serious constraints in getting desired yields of good quality. Study of disease epidemiology plays an important role in working out strategies for effective and timely management of these diseases and in reducing the number of unwanted fungicide applications. A lot of work has been done on these three diseases in different countries to study host-pathogen-environment interactions and suitable correlations have been drawn. Weather parameters play an important role in initiation and development of these diseases. Both downy mildew and anthracnose appear to be largely affected by humid conditions and free moisture in the form of dew or rain are necessary for infection and rainy conditions lead to their epidemic build up. On the contrary, powdery mildew requires relatively dry conditions and moderate temperature. Effects of different climatic factors on the pathogen and disease development and secondary spread as reported for these diseases by various workers has been analysed in this review. Apart from this, some weather based epidemiological models proposed by different workers for these diseases have also been discussed their role in timing first and subsequent fungicide applications highlighted.

1. Introduction

Grape (*Vitis vinifera* L.) is one of the ancient fruits known to mankind and makes adequate mention in biblical records for its delicious fruits and wines prepared from its juice. The world-wide area under this crop is approximately 7.33 million hectares with average productivity of 7.83 ton/ha (FAO, 2000). Among grape growing countries, United States of America ranks first in terms of production with grape yield of 19.52 ton/ha. Other major grape growing countries are Italy, France, Spain, Australia, England, Germany, New Zealand and South Africa. The grape is mainly cultivated for fresh consumption and for wine making apart from resin production. In India, grapevine is being cultivated on an area of about 40,000 ha with annual production of 3.45 MT of table grape.

However, the successful cultivation of the crop is hindered due to attack of various diseases and their timely management is of utmost importance in getting desired quantitative and qualitative yield. Study of disease epidemiology plays an important role in understanding the behaviour of the pathogen population so that weaker links in its life cycle could be identified for their timely and effective management. The relationships between pathogen and host are of dynamic in nature and change

with time. Thus, study of pathogen population dynamics during crop growth period is important for working out disease management strategy. Effective disease management requires a sound knowledge not only of the host and the pathogen interactions but also the influence of various environmental factors on pathogen population and their interaction with host for initiation and development of the disease. In this article, an attempt has been made to review the progress made in the understanding of epidemiology of three important fungal diseases of grapevine, viz, powdery mildew, downy mildew and anthracnose and its implications in disease management programmes.

2. Powdery mildew

Powdery mildew of grapevine (*Vitis vinifera*) incited by biotropic ascogenous fungus *Uncinula* necator (Schw.) Burn. is a disease of wide occurrence, and is believed to have originated from North America (Viala, 1885). The first out-break of powdery mildew in Europe occurred in England in 1845 and was related to increased trade with America. The disease spread rapidly in only 7 years and it caused heavy losses in vine yards throughout Europe and the Mediterranean region (Bulit and Lafon, 1978, Viala, 1885). French grape industry suffered huge losses due to epidemics by this disease during 1850-1855.

The symptoms of the disease appear on all the green parts of vine, leaves, inflorescence and berries. The symptoms develop as whitish powdery areas on both the surfaces of the leaves. These whitish powdery patches consisting of conidia and conidiophores of the fungus may enlarge, coalesce and cover the whole area of the leaf. Young leaves curl when severely infected and drop prematurely. Infected spurs, canes, tendrils, panicles and berries have a white powdery appearance. When this superficial powdery growth is wiped or washed down due to rains, black discoloured areas beneath the powdery growth can be seen. Fruit set would be poor when flowers are infected. Infection on inflorescence lead to premature fruit drop and diseased berry turn dark in colour, crack and finally rot.

In spite of regularly applied control measures the disease causes huge economic losses. The disease not only reduces the yield but also lowers the fruit quality . The wine prepared from infected fruit often develops off flavour. Rao (1992) reported 50 per cent incidence in vine yards around Hyderabad in South India resulting in huge losses. Pool *et al* (1984) recorded 40 % reduction in vine size and 65% reduction in yield of cv. Rossetee.

2.1 Over-wintering and survival of the fungus

Dormant season survival and spread of powdery mildew in vine yards essentially depend on the inoculum at a given time, environmental factors and susceptibility of host plants The pathogen survives or over-winters from one growing season to the next as mycelium and conidia in dormant buds and as ascospores in cleistothecia. The over-wintering mycelium present in buds or leaf primordia get activated at bud break or with the onset of vegetative growth and covers the emerging shoots with mycelium. The conidia are produced abundantly on these infected shoots and these covered shoots

are called as flag shoots (Yossifovich, 1923, Lafon *et al*, 1966, Built and Lafon, 1978, Sall, 1982, Pearson and Gartel, 1985, Delye, 1998).

The pathogen is a heterothallic fungus and produces cleistothecia (ascogenous stage) on infected leaves which are later washed down and retained on the cracky bark of grapevine stem (Gadoury *et al.*, 1988, Cortesi *et al.*, 1995). In this case, the primary source of infection consists of ascospores produced in cleistothecia. However, their existence is not always certain. For more than 40 years after the introduction of grape powdery mildew into Europe, no cleistothecia were reported in the vineyards until 1892 when these were first observed. (Couderc, 1893). The cleistothecia have now been reported to appear in large number in several countries during or at the end of growing season depending on region (Wicks and Magarey, 1985, Bernard and Mur, 1986, Diehl and Heing, 1987, Pearson and Gadoury, 1987, Gadoury and Pearson, 1990, Cortesi *et al.,* 1998, Delye *et al.,*1998, Gee *et al.,* 2000). Although it is not yet possible to accord general significance to the over-wintering of the disease in the form of cleistothecia except the recent reports from Pearson and Gadoury (1987) and Gadoury and Pearson (1988) where cleistothecia are shown to be as primary source of inoculum . Willocquet (1995) observed that in case of massive and early infection cleistothecia can be observed early on the vines otherwise these are detected later in the well protected vine orchards or where the powdery mildew appears at the end of growing season. Subse-

Table 1: Effect of weather factors on germination of ascospores of *Uncinula necator* according to ANOVA

Effect	df	MS	F
Storage period (S)	4	94.46	1.60ns[a]
Temperature (T)	1	3621.30	61.54*
Humidity (H)		13406.91	227.84*
S x T	4	349.94	5.94*
S x H	4	628.29	10.67*
T x H	1	22.97	0.39ns
S x T x H	4	127.00	2.15ns
Error	40	58.84	

ns = Non significant; *$P < 0.001$; Source : Jailloux *et al.* (1998)

quently Diehl and Heintz (1987) and Stapleten *et al.* (1988) in Germany and California, respectively, reported that the cleistothecia produced play important role in the conservation of the pathogen. Their formation is induced in conditions like drought, cold, heat which are unfavourable to the pathogen (Galet, 1977, Diehl and Heintz, 1987,). In France, Jailloux *et al.,* (1998) worked on the condition for maturation of cleistothecia. Periodic wetting treatments of cleistothecia at 5 °C during 110 days were necessary to induce both ascospore (80 ascospores/cm^2) and their germination ability (62 %). This reported that the mature ascospores were pathogenic on healthy leaves at 20 °C and formed normal powdery mildew colonies indicating their probable role as primary inoculum source (Table 1).

The date of first appearance and dispersal of cleistothecia are a function of incidence of severity of powdery mildew. In contrast, in India, there is no dormancy of grapevines in southern states. Receptive grape shoots thus are present are throughout the years and the fungus does not need to have an over-wintering stage. In northern India, however, grape plants shed their leaves from December to February. However dormancy is never completely established and some shoots continue to develop under shade during this period. So pathogen may over-winter either in buds or in shoots. Sexual reproduction seems to be rare in India. It was observed occasionally in northern parts of country (Puttoo *et al,*1987*,* Munshi *et al.* 1996) but their role in disease cycle has not been established.

There are two sources of primary inoculum of this powdery mildew i.e. the conidia produced on young flag shoots sprouted from the buds infected with fungus and ascospores produced in cleistothecia.The primary infection leads to production of conidia, which then serve as means of secondary infection.

2.2 Dispersal and germination of ascospores

The anamorph of U. necator is widely regarded as xerophyte and rain is deleterious for development of powdery mildew but for the teliomorph rainfall is the essential event in release or detachment of the cleistothecia from their mycelial substrate. It washes them away, disseminates them over the plant itself and to the soil.

The asci and cleistothecia swell up simultaneously at maturity, both are opened by large site. The asci liberate ascospores via an aperture of dehiscence at their apex (Yossifovitch 1923). Pearson and Gadoury (1987) observed that cleistothecia from in-fected tissues in mid to late summer are dispersed by rain to the bark of vine, where they over-winter. Free water has been reported to induce dehiscence of cleistothecia and ascospores are released from asci (Diehl and Heintz, 1987). Gadoury and Pearson (1990) described that the period of rainfall occurring before the spore dispersal may trigger the liberation of ascospores. Jailoux *et al.* (1999) monitored the ascospore release for a five year period under natural conditions in the Bordeaux region of France and stated that ascospore release always began after bud burst and generally ended before blossom-ing. The release period of ascospores was always associated with a rainfall higher than 2.0 mm, a wetting duration greater than 2.5 h, an average temperature generally above 11ºC and daily mean temperature sum from November 1 to the first ascospore release above 1100ºC.

Gadoury and Pearson (1988) observed that temperature, day length, humidity, leaf age and host resistance did not affect the initiation of cleistothecia, which required only the pairing of compatible mating types. However, the rate of growth and matura-tion of cleistothecia was affected by temperature and host resistance. No growth oc-curred at 4 ºC and 32 ºC and cleistothecia grew more rapidly on susceptible cultivar compared to resistant cultivar. Cleistothecia split when these become wet by rains and ascospores are expelled from the asci and carried off by wind in spring. Ascospore germination takes place only in the presence of free water. Ascospores burst in water because of high pressure potential within the spores. Ascospores germinated equally well after 24 h at temperatures of 10-25 ºC, but germination was reduced to less than 5

% at 31 °C. Germinated ascospores failed to form appressoria at 5 and 31 °C and no infection was observed at these temperatures.

2.3 Dispersal and germination of conidia

Wind is essential climatic agent for separation and dissemination of mature conidia to neighboring vines. Conidial germination, survival and colony development depends on the environmental factors such as temperature, humidity and light. Willocquet (1995) showed that the course of a day without rain, the simultaneous increase in temperature, in radiation's, in speed of wind or decrease in RH provide favourable conditions for release of spores, the optimum being between 10 am and 6 p.m. During night the less favourable conditions mean a significant decrease in sporulation. Willocquet, *et al.,* (1998) designed a wind tunnel to study the effect of wind, relative humidity and colony age on dispersal of conidia and observed that wind speed as low as 2.3 m/s instantaneously triggered the dispersal of conidia from fixed leaf disc of 18 days old infection. The fraction of conidia dispersal at given wind speed increased with colony age from 12 to 24 days and conidia of 27 days old dispersed and germinated less easily. No maturation gradient was observed which explained that even newly formed conidia were able to germinate after dispersal.

2.3.1 Temperature

Temperature around 25°C is optimal for infection and disease development. Germination of conidia of *U. necator* is initiated around 4 °C. Temperatures above 35°C inhibit the germination of conidia, though they remain viable, but at temperature above 40 °C their viability is lost. At 25°C conidia germinate approximately in 5 h. The time from infection to sporulation at 25-30°C can be 5 to 6 days (Delp,1954, Oku *et al.*,1975).

2.3.2 Humidity

Water in liquid form results in poor and abnormal germination of conidia as well as bursting of conidia. Rainfall can be detrimental to disease development by removing the conidia and by disrupting the mycelium. A small proportion (12 %) of the conidia are able to germinate in a dry atmosphere of 20 % RH (Oku *et al.*, 1975). Relative humidity above 40 % has no significant difference in conidial germination or hyphal growth (Toma, 1974). Humidity has greater effect on sporulation than on germination. (Delp, 1954).

2.3.3 Light

Low diffused light favours the disease development. Germination is checked in bright sunlight. As an example 47 % germination of conidia in diffused light has been observed in comparison to 16 % in bright light . Willocquet (1995) showed that influence of light on germination of conidia which enables the quasi-absence of sporulant spots on the upper leaves to be explained atleast in part, especially in beginning of growing

season when coverage in not dense.

2.4 Conditions for disease development

Powdery mildew is a dry weather disease but may occur in rainy season provided dry conditions prevail. In grape growing areas period of high humidity is often accompanied by moderate temperature which are favourable for powdery mildew development. Temperature is the main environmental factor determining the severity of this disease. For the development of disease temperature around 20 –25°C is most suitable but the fungus can develop from 6 to 32°C. Temperatures between 35 and 40 °C check its development. Powdery mildew can also appear in the spring when temperature is around 10°C but its development is slow. The incubation period is shorter (5-6 days) between temperatures of 23-30°C, whereas it can be as long as 32 days at 7°C. Humidity has marked effect on development of this disease. Free water in the form of rainfall is not necessary and may even be harmful as it can carry away the conidia and disrupt the mycelium. Toma (1974) reported that the average number of spores produced in 24 hrs by the conidial chains varied as 2.07, 2.95 and 4.67 respectively, when the atmospheric humidity roses from 30 – 40, 60 – 70 and 90 – 100 percent.

In French vineyards near Bordeaux region, it was observed that primary infection caused by ascospores does not play much role in increasing disease incidence. On the other hand, the weather conditions during April (rainfall and temperature) strongly influenced the disease severity on berries by enabling good growth of the pathogens on leaves (Jailloux *et al.,*1999).

2.5 Biotic factors

Use of nitrogenous fertilizers decrease the phenolic content in leaves and increase their susceptibility to powdery mildew. The fungicides such as dithiocarbamates used against downy mildew and other diseases like anthracnose indirectly favour the development of powdery mildew by making the vines more vigorous. Willocquet (1995) noted that cultural practices such as application of pesticides caused vibration in the leaves, which resulted, into release of conidia.

2.6 Powdery mildew forecasting models

A powdery mildew risk assessment model has been developed at University of California, Davis, USA. (Gubler *et al.*, 1999) A portion of the model forecast ascospore release based on leaf wetness and temperature. Predictions are based on average temperature during an extended leaf wetness event. The model utilizes the 'Conidial Mills Table' at 2/3 value for hours of leaf wetness required at various temperatures. In general at least 12-15 hours of continuous leaf wetness are required when average temperatures are between 10-15°C. Once infection has occurred, the model switches to the risk assessment phase and is based entirely on the effect of temperature on the temperature on the reproductive rate of the pathogen.

The risk index for conidial increase ranges from 0 - 100. To trigger the index it

requires 3 consecutive days with at least 6 hours between 21-30°C. If three consecutive days at this temperature are not met, the index reverts to zero. For each day that this requirement is met, 20 index points are assigned. After 3 days, an index of 60 would be achieved thus triggering the index. Once the 3 consecutive day requirement is met, it no longer is a function of the model. The model will fluctuate between 0 and 100. Losing 10 points on days when the 6 hour requirement for 21-30°C was not met or if at any time during the day, the temperature rose to 35°C for at least 15 minutes. An index of 60 – 100 indicates the pathogen is reproducing every 5 days while an index of 0 –30 indicates a reproductive rate of 15 days or less. An index of 40 –50 is considered normal and would imply a reproductive rate of 8-11 days, i.e. somewhere between 5 and 15 day reproductive rate.

Daily analysis of the model allows grape growers to visualize what the conidial population will be approximately one week later and what the potential disease severity will be two weeks later, allowing them to know well in advance what their fungicide programme should be in terms of product and application interval.

3. Downy mildew

Downy mildew caused by *Plasmopara viticola* (Berk. and Curt.) Bal. and de Toni. is one of the most destructive diseases of grapevine and occurs in most of the grape growing areas of the world where warm and wet weather conditions prevail during the vegetative growth of the vine. It is widely prevalent in Europe, South Africa, Argentine, Brazil, East-North America, Eastern Australia, New Zealand, China and India.

The disease is characterized by light greenish yellow oily spots on the upper surface of leaves, which later turn yellow and necrotic. On the corresponding lower surface whitish downy growth of the fungus is seen which is more prominent in humid weather. In severe infection the entire lower surface of the leaf is covered with the white downy growth of the fungus consisting of sporangia and sporangiophores. Severely infected leaves dry and are shed. Flower panicles and small berries when infected turn black, become soft and are shed. Bigger berries turn dark brown.

If not checked timely, the disease may destroy foliage and young berries resulting into drastic reduction in berry yield and poor quality wine. European wine industry suffered a huge loss in 1870's when downy mildew introduced along with plant material appeared in epidemic form. Losses from 50 –100 % can occur when conditions favour downy mildew on flower and young berries. Damage is greatest following early infection when vines may be defoliated preventing the maturation of fruit and canes and exposing canes to sun burn. Vine vigour and crop potential may be reduced in the next season because downy mildew infection in subsequent defoliation depletes carbohydrates reserve. The bud burst may be reduced and crop potential reduced due to lower number of viable buds

3.1 Survival of the pathogen

The normal form of fungal perennation is through oospores, which are formed as result of fusion of an antheridium and oogonium, derived on the terminal expansion of hy-

phae. The oospores are resistant to low temperature (-20 to –26ºC) and are dependent on moisture content for survival. Capus (1916) and Roussel and Bouardo (1971) in their study of dynamics of oospores reported that maturation period from March to May and date of germination essentially depend on the amount of rainfall from October to January. The conditions for the production of oospore are not well understood in India specially when downy mildew remain in form of mycelium on diseased leaves, pruned canes and bud scales (Tripathi and Singh, 1998).

3.2 Dissemination of inoculum

3.2.1 Oospores

The pathogen overwinters mainly as oospores in fallen leaves but can also survive as mycelium in buds and in persistent leaves. Initial infection from oospores is followed by formation and spread of sporangia. The oospore germinate on moist soil or in the presence of free water at temperature rises above 11 ºC. The speed of oospore germination depends on the temperature. (Ronzon and Clerjeau, 1988). The zoospores produced by sporangia swim with the aid of flagella and are projected on to vine leaves near the soil by rain splashes. Bower system is popular in Indian conditions, so ground rain splashes do not play much role to cause the infection on lower parts of the vines.

3.2.2 Sporangia

The disease spread during the vegetative growth period through sporangia. The sporangia are very light and can break off easily by rain splash and are distributed to great extent with moist air. The life span of sporangia is about 45 days in dry air and 3 to 8 weeks in cold weather (Corbaz, 1972)

3.3 Germination of zoospores (Primary source)

The zoospores produced in sporangia of egg cell are the primary source of infection. They swim for few minutes in water with whirling motion, loose their flagella, become round and are enclosed in membrane called encyst and under moisture conditions nonseptate, flexuous, germ tubes are produced. The germ tube reaches to stomatal aperture and settles into sub-stomatal chamber and produces haustoria. When the nutrient substrate is exhausted pathogen send conidiophores exterior to under side of leaves through ostioles of stomata (Galet, 1977). The time between infection (penetration) and appearance of lesion (production of conidiophores) is called latent period. The incubation period to some extent is affected by air temperature, humidity and host. It varies between 5 –18 days with greater chance 7-10 days (Muller, 1936).

3.4 Germination of sporangia   (Secondary source)

As the result of primary source of infection, sporangia are produced on sporangiophores which determine the secondary spread with in the crop by means of zoopsores.

Germination of sporaniga depends on their age and presence of water. The speed of germination varies with weather conditions. Sporangia germinate very rapidly with in 1-2 h in moist weather, whereas in dry weather it takes 6-8 h for sporangia to germinate (Dubos, 2000).

The vitality of sporania under Indian conditions with day temp. above 10°C, conditioned by sun light. The sporangia collected at night (8.0 pm – 6.0 am) germinate with in 2-6 h where as sporangia harvested during day time (8.0 am - 6.0 pm) do not germinate. (Srinivasan and Jayarajan, 1977). Liberation of zoospores from sporangia occurs between temperature range of 3 – 9 and 28 – 30 °C with an optimum at 22 – 25 °C (Galet, 1977).

3.5 Role of environmental factors

Downy mildew is polycyclic disease and the optimum temperature for its development is about 25 °C. The occurrence, development and spread of disease is mainly based on rainfall and temperature. Rainfall is the principal factor in promoting disease rather than temperature. Therefore, the disease is favoured by all factors that increase the moisture content of soil, air and host plants. In India, development of disease is coincided with monsoon months (low temperature and high humidity). The optimum temperature for development of pathogen is 25 °C. Sporangiophores and sporangia production takes place during night which requires 90-95 per cent relative humidity at least 4 h of darkness and can appear on leaves after 4 days of infection (Brook, 1973, Blasher and Wertzlen, 1978).

Epidemiological study by Grunzel (1963) revealed that for infection leave must remain wet for 3 h at 12-15 °C or 1.5 to 2 h at 18-20°C. Galet (1977) explained that the rhythm of rains plays a principal and decisive role in the development of downy mildew and concluded that excessive and heavy rainfall has an adverse effect resulting in substantial run off of water from leaves which effectively removed the inoculum of *P. viticola* from vines. Brook (1992) established that rainfall less than 1.0 mm could not provide sufficient moisture for infection. His study conducted in three seasons explains that severe epidemic developed over period of 50 days in summer and autumn with rainfall for the period ranging from 36 to 58 mm. In two seasons, downy mildew became established in summer but its epidemic failed to develop in 50 days despite rainfall in this period measuring 99 and 234 mm. In this period temperature are similar to those in epidemic seasons.

Epidemiological study by Rao and Thind (2001) revealed that in south Indian situation, the disease is favoured by continuous cloudy weather for 3-4 days with sufficient rainfall which keep the leaves wet with a temperature range of 17-32.5 °C. In the Punjab state of India, disease may start in early April, remains in low intensity up to July, shows upward trend in August and reaches the maximum level in mid November (Thind *et al.*, 1992).

In European vineyards, when wet winter is followed by wet spring and warm summer with intermittent rainfall for 7 – 15 days , the epidemic of downy mildew occurs frequently.

3.6 Role of biotic factors

Cultural practices in vineyards influence the primary inoculum load and development of the disease directly or indirectly. Cultural practices like close planting and training the vines too low make them more susceptible by creating a congenial microclimate for infection and subsequent multiplication of sporangial inoculum. Excessive use of nitrogenous fertilizers promotes succulent growth and increases the disease incidence. Soyar (1987) and Soyar and Delas (1988) showed that balance between nitrogen and phosphorus plays a vital role in the susceptibility of vines to downy mildew. Vines lacking in nitrogen or phosphorus are less susceptible to downy mildew. The passes between the row promote the dead leaves on the ground, which help in survival of oospore for causing primary infection. Irrigation frequency, depth, drainage etc. aid to the aggressiveness of the pathogen during congenial weather conditions for development of this disease.

3.7 Epidemiological models

A forecasting model called Prediction of Oospore Maturity (POM) has been developed in France which predicts the date when most of the oospores get matured (Sung, 1990). The oospores of *P. viticola* constitute primary source of inoculum for causing downy mildew in grapevine. They help in winter survival of the pathogen. The maturation of oospores depends on weather conditions especially rainfall. The disease can be successfully predicted using POM model. In France, the risk of downy mildew is considered highest when oospores mature before end of March (Table 2) which leads to permit better timing of the first fungicidal spray and economizing on the number of their applications.

Four electronic alert systems/devices viz. Biomat, HP – 100, KMS – P and Metos are being used for providing early warning of downy mildew to the growers. Each model/apparatus is programmed specifically for temperature, humidity, leaf moisture and data for prognoses of the disease and had facilitate for storing and printing out results (Seigfried *et al.*, 1993).

Generalized linear mixed model (GLMM) was found suitable for analysing sporangial dispersal and disease incidence in Germany and the method is exemplified using data from randomized block experiments on the incidence of downy mildew (Piepho, 1999).

An electronic warning system for grape downy mildew based on the incidence of disease on the leaves of *vitis labrusca*, production of sporangia by *Plasmopara viticola* was tested over 7 years in Ohio, USA. Grapevines were sprayed with metalaxyl+mancozeb (Ridomil MZ) when the warning system indicated that the environmental conditions are favourable for sporulation and subsequent infection. In 7 years study, the plants were sprayed one to four times according to warning system as compared to 4 to 10 sprays according to standard calendar-based schedule. The warning system resulted in yearly reduction of one to six fungicidal sprays with incidence similar to the standard schedule treatments. So effective control of downy mildew can be achieved with the use of the warning system with fewer number of sprays than with

standard schedule (Madden *et al.,* 2000).

Egger *et al.* (1996) investigated the influence of different methods for measuring leaf wetness on the forecasting of attack of *Plasmopara viticola*. Leaf wetting was estimated using a mechanical sensor, electronic sensor (ADLO 9T of EDG and ADZ of SILTMCT) and a mathematical model proposed by S. Strizyk. The results showed that the use of different sensors and methods of estimate can have notable influence on the forecasting of the establishment and development of this disease. As per the model proposed by Strizyk for estimating leaf wetting and temperature, sum threshold of 200 supplied the best forecast of *P. viticola* for the area of Italy. Finally it was concluded that choice and evaluation of each forecasting model and method for measuring leaf

Table 2: Monthly maturation index (Im), calculated using the 'Prediction of Oospore Maturity' model and observed disease severity at bloom during different years in France.

Im	1977	1980	1984	1986	1988
September	-4	-9	-26	-26	-3
October	84	-15	175	-96	113
November	133	6	-126	-98	161
December	152	76	-79	-93	102
January	175	128	33	-19	189
February	258	79	68	16	270
March	266	118	95	11	337
Obs.Severity	4	3	1	1	4

Source : Sung *et al.* (1990)

wetting are very important and should take into account the variety and form of culture, the age of vineyard and all appropriate cultural characteristics. Satrahidayat *et al.* (1993) studied the epidemic of downy mildew disease in Tranguwisia Bail (Indonesia) where this disease is serious and found that rainfall duration, leaf wetness and number of sporangia are positively correlated with increased disease severity but air temperature had a negative correlation.

The model T- Metstation-Prototype is a useful low cost weather station/disease predictor designed in Australia to assist growers make better decisions in controlling this disease. An in-built computer system stores weather data from the vine canopy and enables prediction of primary infection for downy mildew (Magarey and Western, 1997).

PLASMO (*Plasmopara* simulation model), is a software computer programme/ model for forecasting downy mildew on grape and is followed in Italy. The model simulates disease development on the basis of climatic conditions. The model parameters include temperature, leaf wetness for inoculation phase , temperature and RH for incubation phase and the type of treatment applied. Numeric and graphic outputs are available and the program provides useful information for identifying fungicides application timing on the basis of actual downy mildew development (Rosa *et al* 1993).

4. Anthracnose

Anthracnose or bird's eye spot is a disease of European origin and is caused by *Sphaceloma ampilina* de Bary, (syn. *Gloeosporium ampelophagum* (Pass.) Sacc. with perfect stage *Elsinoe ampelina* (de Bary) Shear. The disease occurs regularly in areas where conditions during the growing season are usually humid and warm with rains during spring months.The disease affects all parts of vine starting from young shoots to mature berries. Infection on leaves results in premature defoliation and loss of photosynthetic activity. It produces small brown, circular spots, 1-5 mm in diameter on leaf lamina which later turn grey in the centre with a border of dark brown tissue. The central necrotic tissue often falls out leaving a shot hole appearance. Young leaves are most susceptible to infection. Lesions may cover the entire lamina or are concentrated along the midribs and main veins. On petioles and tendrils, light brown spots appear which are first circular in outline but later elongating into elliptical, sunken necrotic cankers. Young succulent shoots are highly susceptible to anthracnose. Shoot lesions are small and round in the beginning which later become elliptical with raised violet-brown margin giving a necrotic canker like appearance. These lesions may collapse and completely girdle the shoot and give dead arm or die-back appearance. Infection on berries appears as circular light brown spots surrounded by a dark brown to black margin giving bird's eye spot appearance.

Anthracnose has become a potential threat to grape cultivation in north India. It appears every year in these areas and reduces the quality and quantity of the crop apart from making the vines weak (Thind *et al.,*1992). Several workers have reported varying degree of losses in different parts of the world (Brook, 1992). Yield losses have been estimated up to the extent of 10 – 15 % in Haryana and Punjab (Bedi *et al.*, 1969). During 1981 – 83, serious outbreak of anthracnose was reported from Andhra Pradesh in India on variety Anab -e- Shahi and Thompson Seedless thus resulting in huge monetary losses (Rao, 1992). Apart from direct losses in yield, the disease also alters biochemical constituents of berries and affects the fruit quality (Thind *et al*, 1998).

4.1 Survival of pathogen

The pathogen over –winters primarily on diseased plant debris left on vineyard floor. Under favourable weather conditions conidia are produced in acervuli which give rise to primary infection on different plant parts viz. leaves, shoots, petioles (Anderson, 1956, Brook, 1973, Suhag and Grover, 1979). The fungus also over-winters as perithecia but the importance of ascospores in disease development is not clearly understood. Mirica (1988) studied that ascospores germinate and infect the tissues and produce the *Sphaceloma* phase which shows the existence of perfect stage of *Elsinoe ampelina*. However, conidia and ascospores over-winter on ground and give rise to primary infection.

4.2 Transmission

Rain water plays a major role in spread and development of disease. The mycelium

survives in lesions and form conidia after sufficient wetting and cause primary infection. The secondary spread of the pathogen is through splashing rains, dew or through air borne conidia (Suhag and Grover, 1979, Malakhova, 1977 and Suhag and Daulta, 1981).

In spring, pathogen produces numerous spores (conidia) on the surface of infected canes when they are wet for 24 h or more and the temperature is above 36 °F. the infective spores are spread by splashing rain and as little as 92 mm of rain can disseminate the spores to susceptible tissue. The spores can germinate and infect at temperature from 36 to 90 °F. Ascospores are discharged into air and may disseminate over long distance by wind currents to susceptible tissues and cause infection (Rao, 1992, Ellis, 1999). Brook (1973) detected the conidia in wind borne splash drops of rain at distance up to 7 meters.

4.3 Germination of conidia and infection

The conidia germinate if free water is present for at least 12 h and temperature ranged from 2 to 32°C. The incubation period varies from 13 days at 2°C to 4 days at 32°C. Optimum temperature for disease development is between 24 to 26°C (Dubos, 2000). In another study, maximum conidial germination was observed at 25°C followed by 20 and 30 °C as studied on water agar. Likewise, incubation period of the disease, studied by detached leaf method on water agar, was observed to be minimum i.e. 3 days at 25°C, 4 days at 30 °C and 5 days at 20 °C (Thind, 1995). It increased with further decrease in temperature and was observed as 14 days at 10°C . No symptoms developed at 35°C . A humid period of 7 days (close to 100 % RH) is needed for conidial germination. The leaf wetness period has pronounced effect on infection. Threshold of temperature and wetness for primary and secondary infection are assumed as 7-10 h leaf wetness at 12 °C decreasing 3-4 hrs at 21°C. The incubation period of 7-12 days at 16.5 °C, 5-7 days at 16.5°C and 3-4 days at 21°C have been reported. (Brook, 1973). Malakhova (1977) observed maximum spore germination at 27-32°C and incubation period of 3-4 days at 24-30 °C during frequent rains. Spore germination on the surface of leaf after incubation at room temperature (26°C) reaches maximum (95 %) after 24 h.

4.4 Role of environmental factors

Rainfall , humidity and temperature are the most important climatic factors influencing infection. The disease is severe in years of prolonged rains during the spring and early summer months. (Anderson, 1956). Wetness and temperature are key factors for the development of this disease. as a film of free water for 12 h is necessary for conidial germination (Dubos, 2000).

Rao and Satyanaryan (1989) and Rao (1992) reported that a minimum rainfall of 50 mm distributed over 3 days in a week associated with cloudy weather are sufficient for causing severe infection on vine foliage. A positive correlation between weather parameters and disease incidence was observed ,if humidity ,rainfall are higher and temperature is moderate the incidence of disease will be higher (Rao and Dakshinamurty, 1964, Prasad and Nirvan, 1965, Thind *et al.,*1992). The periodical progression of grape

anthracnose demonstrated by Thind *et al.* (1992) in Punjab (India) indicated that the disease initially appeared in the month of March and thereafter it remained low in April after which it gradually declined in May-June. With the onset of Monsoon rains in July-August, the severity of the disease showed increasing trend from July to September. Two peaks of disease severity during March-April and August-September showed correlation with adequate rainfall and temperature (max. 28-30 °C and min. 15-25°C). The decline of the disease during May-June was mainly due to negligible or no rainfall and higher temperature (>40°C).

In Auckland, a wetness period of 7-10 h at °C in spring down to 3-4h at 21°C in summer were reported necessary for causing infection of grapevine by this pathogen. In a similar study under controlled conditions on potted plants incubated at different temperatures in Phytotron, Thind (1995) observed that a minimum of 3h leaf wetness period was required for infection at 25 °C which seems to be the optimum temperature for anthracnose development (Table 3). It was between 3-4h at 30°C and between 4-5 h at 20 °C. At lower temperatures, the duration of leaf wetness period increased relatively and a minimum of 6-8h of leaf wetness was required for infection at 15°C. No symptoms developed at 35°C even after 24 h of leaf wetness.

Table 3: Influence of leaf wetness period at different temperatures on development of symptoms caused by *Gloeosporium ampelophagum* on potted plants of cv. Cinsaut

Leaf wetness period (hr)	*Mean infection grade on three top leaves at different temp. (°C)				
	15	20	25	30	35
0	0	0	0	0	0
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	1.2	0	0
4	0	T	2.0	2.3	0
5	0	0.4	2.8	2.7	0
6	0	0.9	4.7	5.0	0
8	T	4.3	5.2	5.3	0
24	3.5	5.6	5.3	5.1	T

* After 9 days of incubation (12 days at 15 °C) ;T= Traces ; Disease scale 0 –9
Source : Thind (1995).

A temperature of 24-30°C was observed to be optimum for the development of *Sphaceloma ampelinum* in USSR by Bychenko (1968). Todorova (1980) recorded that development of the fungus depends more on moisture than on temperature.

4.5 Role of biotic factors

Excessive use of nitrogenous fertilizers promotes susceptibility of the vines and increases disease incidence. Ravaz (1972) showed that use of nitrate of sodium, lime or potash fertilizers used at about 3 weeks before bud burst promotes the susceptibility of vines to disease. Wild hosts provide an excellent place for the disease to thrive and

serve as excellent reservoir of inoculum near vine yards.

4.6 Disease forecasting

In Punjab (India) a multiple linear regression model for the prediction of disease severity has been devised by Thind *et al.* (2001). It is fitted with weather parameters to predict the disease severity, Y= - 51.0158 – 0.0989 (maximum temp.) + 8.466 (minimum temp.) – 6.452 (average temp.) + 1.88 (morning relative humidity) – 0.830 (evening relative humidity) – 0.0257 (average relative humidity) + 0.0465 (rainfall), $R^2 = 62.62\%$.

The R^2 value (coefficient of determination) indicated that 62.62% variation in grape anthracnose could be monitored by prevailing air temperature, relative humidity and rainfall.

5. References

Anderson, H.W. 1956. Diseases of fruit crops. McGraw Hill, New York, 501p.

Bedi, P.S., Singh, G. and Suryanarayana, D. 1969. Field evaluation of Aureofungin and other chemicals to control anthracnose disease of grapes in Punjab. Hindustan Antibiotic Bulletin, 11: 251-253.

Bernard, A.C. and Mur, G. 1986. Presence de peritheces de I oidium en 1995, dan le midi. Progres Agricole et Viticulture, 103:258-261.

Blasher, M. and Weltzien, H.C. 1978. Die –Bedeutung von sporangienbidium assbreitung und – Keinmug fur die epidemiology von *Plasmopara viticola*. Pflanzenkr Pflanzen Schutz, 85 : 155-161.

Brook, P.J. 1973. Epidemiology of grapevine anthracnose caused by *Elsinoe ampelina* .New Zealand Journal of Agriculture Research, 16 : 333-342.

Brook, P.J. 1992 Epidemiology of grapevine anthracnose and downy mildew in Auckland, New Zealand vineyards. New Zealand Journal of Horticulture Science,.20 : 37-49.

Bulit, J. and Lafon, R. 1978. Powdery mildew of vine. In: "The Powdery Mildew" (ed. Spencer, D.M.) Academic Press, London, pp. 525-548.

Bychenko, N. 1968. Anthracnose and control measures against it. Review of Applied Mycology. 47 : (Abstract No. 1634).

Capus, J. 1916. Sur les invasions de mildiou de la vigne dans le sud-quest en 1916. Rev. Vit, 45 : 193-200.

Corbaz, R. 1972. Studies of fungus spores trap in the air in a vine yard. Phytopathologische – Zeitschrift, 74 :318-328.

Cortesi, P., Gadoury, D. M., Seem, R. C. and Pearson, R. C. 1995. Distribution and retention of cleistothecia of *Uncinula necator* on the bark of grapevine. Plant Disease, 79: 15-19.

Cortesi, P., Marescotti, L., Nicolodi, A. Ricciolind, M. Bisachm and Blaize, P. 1998. Cleistothecia of *Uncinula necator* addition source of inoculum in Italian vineyards. Integrated control in viticulture. Proceedings of the meeting of Godollo, Hungry 4-6 March, 1997, Bulletin – OILB – SROP. 21: 25-26.

Couderc, G. 1893. Sur les peritheces de I *Uncinula spirallis* en France. I'identification de i.oidium americian et de I oidium european C.R. Acad. Sci. Paris, 116 : 210 –211.

Delp, C.J. 1954. Effect of temperature and humidity on the grape powdery mildew fungus. Phytopathology, 44: 615-626.

Delye, C., Steievenard, C., Douencl, L. and Coriocostal, M.F. 1998. Grape powdery mildew, a dual problem. Phytoma, 510 : 38-42.

Diehl, H.J. and Heintz, C. 1987. Studies on the generative reproductions of grapevine powdery mildew (*Uncinula necator* Burk.). Vitis, 26 : 114-122.

Dubos,B. 2000. *Cryptogramic* diseases of vine : Wood and green tissue diseases caused by fungi. Editons Feret, Bordeaux, 161 p.

Egger, E., Marinelli, E. and Arcanglo, M. 1996. Influence of different methods for estimating leaf wetness on the forecasting of attack of downy mildew and grey mould on grape. Information Fitopathologica, 46: 57-61.

Ellis, Mile.1999. Anthracnose of grape in Ohio. New item, Ohio news item. pp 1-7.

FAO, 2000. FAOSTAT- Data base results. Internet Edition of 2000.

Gadoury, D.M. and Pearson, R. C. 1988. Initiation, development and survival of cleistothecia of *Uncinula necator* in New York vineyards. Phytopathology, 78: 1413-142.

Gadoury, D.M. and Pearson, R.C. 1990. Ascocarp dehiscence and ascospore dischagre in *Uncinulla necator*. Phytopathology, 80 : 393-40.

Galet, P. 1977. Les Maladies et les parasites de la vigne. Champignos et les vines. Vol.1, Paysan la Midi, Montpellier, France, pp 871.

Gee, L. M., Stummer, B. L., Gadoury, D. M., Beggins, L. T. and Scatt, E. S. 2000. Maturation of cleistothecia of *U. necator* and release of ascospore in South Australia. Australian Journal of Grapevine Research, 6 : 13-20.

Grunzel, H. 1963. Investigation on the vialblity of sporangia and zoospores of *Plasmopara viticola.* Abst. No. 2789. Review of Applied Mycology, 43: 1969.

Gubler, W.D., Rademacher, M.R., Vasquez, S.J. and Thomas, C.S. 1999. Control of powdery mildew using the UC Davis powdery mildew risk index. APSnet Feature*,* Jan.,1999.

Jailloux, F., Thind, T. and Clerjeau. 1998. Release germination and pathogenicity of ascospores of *Uncinula necator.* under controlled conditions. Canadian Journal of Botany, 70: 777-787.

Jailloux, F., Willoquet, L., Chapius, L. and Froidefond, G. 1999. Effect of weather factors on the release of ascospores of *Uncinula necator*, the cause of grape powdery mildew in the Boardeaux region. Candian Journal of Botany, 71: 1044-1051.

Lafon, R., Gouillaud, P. and Hude, R. 1966. Meladies et parasites de la vigne. In: "J.B. et Files", Bailliere, Paris , Vol. I, (3rd edition).

Madden, L. V., Ellis, M. A., Lalancette, N., Hughes, G. and Wilson, L. L. 2000. Evaluation of a disease warning system for downy mildew of grape. Plant Disease, 84: 549-554.

Magarey, P. and Wettern, M. 1997. The model-T-met station prototype, a useful low cost weather station/disease predictor. Australian and NewZeland Wine Industry Journal, 12: 237-276.

Malakhova, V. 1977. Anthracnose a dangerous disease of grapevine. Kazakhstana, 20: 46-48.

Mirica, I.I. 1988. Anthracnose In: "Compendium of grape diseases". APS Press, pp. 18-19.

Muller, K. 1936. The biological foundation for *Peronospora* control by the incubation calender method. Z. Pflkrankhs. XIV: 104-108.

Munshi, G.D., Thind, T.S., Singh,T., Mohan, C. and Sokhi, S.S. 1996. Occurrence of cleistothecia of *Uncinula necator* on grapes. Plant Disease Research,11: 52-53.

Oku, H., Hatomoto, M., Ouchi, S. and Fujii, C. 1975. Sci. Rep. Fac. Agric., Okayaamma University 45 : 16-20.

Pearson, R. C. and Gartel, W. 1985. Occurrence of hyphae of *Uncinula necator* in buds of grapevine. Plant Disease, 69: 149-151.

Pearson, R.C. and Gadoury, D.M. 1987. Cleistothecia, the source of primary inoculum for grape powdery mildew in Newyork. Phytopathology, 77: 1509-1514.

Piepho, H. P. 1999. Analysing disease incidence data from designed experiment by generalized linear mixed models. Plant Pathology, 48: 668-674.

Pool, R. M., Pearson, R. C., Welser, M. J., Lasko, A. N. and Seem, R. C. 1984**.** Influence of powdery mildew on yield and growth of rosette grapevine. Plant Disease, 68: 590-593.

Prasad, A. and Nirvan, R.S. 1965. Grape anthracnose and its control. Punjab Fruit Journal, 5: 185-190.

Puttoo, B. L. and Razdan, V. K. 1987. Powdery mildew of grapes. Indian Phytopathology, 40: 437.

Rao, K.C, 1992. Epidemiology of some common diseases of grape around Hyderabad. In: "Proceedings of International Symposium on Recent Advances in Viticulture and Oenology, Hyderabad, India", pp 323-329.

Rao,K.C. and Satyanarayan, A. 1989. Epidemiology of anthracnose of grapes (*Vitis vinifera*) caused by *Elsinoe ampelina* around Hyderabad. Indian Journal of Agriculture Sciences, 59 : 655-657.

Rao, K.C. and Thind, T.S. 2001. Diseases of grapes and their management. In: "Diseases of fruits and vegetables and their management" (ed. Thind, T.S.). Kalyani Publishers, New Delhi, pp. 86-110.

Rao, S.P. and Dakshinamurty, V. 1964. A note on the influences of certain environmental factors on the incidence of the diseases of grapevine. Andhra- Agriculture Journal, 18: 272–175.

Ravaz, L**.** 1972. On Anthracnose. Progres Agricole et Viticole. 87:357-360.

Ronzon, C. and Clerjeau, M. 1988. Technique for formation, maturation and germination of oospores of *Plasmopara Viticola* under controlled conditions. Plant Disease, 72 : 938-941.

Rosa, M., Genesio. R, Gozzini. B., Maracchi, G. and Orlandini, S. 1993. PLASMO – a computer programme for grapevine downy mildew development and forecasting. Computer and Electronics in Agriculture 9 : 205-215.

Roussel, C. and Bourad, J. 1971. Le mildiou in trait 'd' ampelogie – Ribereau – Geayon et Peynaud 2: 209, 238.

Sall, M.A. 1982. Epidemiology of grape powdery mildew : A Model. Phytopathology, 70:338-342.

Satrahidayat, I. R., Hadisutnsno, B. and Sudarama, I. M. 1993. Epidemic of the downy mildew disease (*Plasmopara viticola*) on grape in Tangguwisia- Buleleng-Bail. Agrivita. 16: 65-69.

Seigfried, W., Meier, H. and Holliger, E. 1993. Warning devices for fruits and grapevines. Schweizerische-Zeitschritft-fur. Obst und Weinbau, 129 : 120-123.

Soyer, J.P. 1987. Influence de divers facteurs agronomiques sue la sensibilite de la vignu au mildiou (*Plasmopara viticola*) colloque CEC. " Influence of environment factors on the control of grape pest, disease and weed." Thessalonque.

Soyer, J.P. and Delas, J. 1988. Fertilization rationnelle qualite de la production et prevention contral les parasites. INRA – VITI. Actualities sur la protection raisonnel des vignoable de qualities : 19-23.

Srinivasan, N. and Jeyarajan, R. 1977. Variablity of *Plasmopara viticola* sporangia produced at different times in a diurnal cycle. Current Science, 45: 106-107.

Stapleton, J.J., Gubler, W.D., Fogle, D., Chellemi, D., Bettinga, L, Leaviti, G., Verdegall, P. Smith, R. and Kelley, K. 1988. Relationship among climate, primary inoculum source, dormant and post emergence control spray and grape powder mildew in California. Phytopathology, 78: 153.

Suhag, L.S. and Grover, R.K. 1979. Epidemiology of grape anthracnose caused by *Sphaceloma ampelinum* in North India. Indian Phytopathology*,* 30 :460-465.

Suhag, L.S. and Daulta, B.S. 1981. A note on the incidence and distribuation of grapevine anthracnose under different systems of training. Indian Journal of Mycology and Plant Pathology,11: 108-109.

Sung, CTM., Sterizyk, S. and Clerjeau, I.1990. Simulation of date of maturity of *Plasmopara viticola* to predict the severity of primary infection of grapevine. Plant Disease, 74 : 120-124.

Thind, T.S. 1995. Report of the work done under IFCPAR project, New Delhi.

Thind, T.S, Munshi, G.D. and Sokhi, S.S., Chander Mohan and Grewal, R.K. 1992. Seasonal

periodicity in grape diseases in Punjab and their management. In: "Proceedings of the International Symposium on Recent Advances in Viticulture and Oenology, Hyderabad, India", pp. 305-310.

Thind, S.K., Monga, P.K., Kaur, N. and Arora, J.K. 1998. Effect of anthacnose disease on fruit quality of grapes. Journal of Mycology and Plant Pathology, 31: 253-254.

Thind, S.K., Arora, J.K., Kaur, N., Monga, P.K. and Arora, P.K. 2001. Periodicity and prediction model of grape anthracnose in Punjab an agrometeorological approach. Plant Disease Research, 16 : 63-67.

Todorova, M. 1980. Anthracnose of grapevine and means for its control. Rastitelna Zashchita, 28 :25-26.

Toma, N. 1974. These (resume), Fac Agron.Inst. Agron. N. Bucuresti.

Tripathi, S. and Singh, S.J. 1998. Fungal, viral and mycoplasma diseases of grapevine in India. In: "Diseases of Fruit Crops" (ed. Singh, S.J.), pp. 113-145.

Viala, P. 1885. L' oidium. In : Les maladies de la vigne. (eds. Viala, P. and Coulet, B.) Montpellier, France, pp. 67 – 124.

Wicks, T.J. and Magarey, P. 1985. First report of *Uncinula necator* cleistothecia on grapevine in Australia. Plant Disease, 69 : 727.

Willocquet, L. 1995. Influence des facteurs climatiques sur le development epidemique de I 'oidium' de la vigne. These, Universite de Paris xi. Orsay, 126 p.

Willocquet, L., Beurd, F., Rauox, L. and Clerjeau, M. 1998. Effect of wind, relative humidity and colony age on dispersal of conidia of *Uncinula necator*, causal agent of grape powdery mildew. Plant Pathology, 82 : 234-242.

Yossifovitch, M. 1923. Contribution a I' etude de l' oidium de la vigne et son traitment. Ph.D thesis, Universite de Tulouse, , France.

640

of infected plants and did not imply that all cases were caused by a single agent or even that the condition was infectious. Later, investigators attributed wilt disease to many causes, both parasitic and non-parasitic. Johnston (1934) reviewed the literature on the subject. Serrano (1934) was the first to associate mealybugs with pineapple wilt, but he did not believe that these insects were the primary cause of the disease. Carter (1933) provided the first convincing evidence that mealybugs were in some way connected with wilt, and in his later publications (1935, 1942, 1945, 1951, 1960, 1963) he established many facts concerning the nature and cause of the disease.

Illingworth (1931) was the first to associate mealybugs with the wilt disease of pineapple. He observed that the disease developed following infestation of the plants by the pink mealybug, *Dysmicoccus brevipes* (Ckll.). The role of mealybugs in the development of this disease was subsequently studied in detail by Carter.

2.2 Geographical distribution and losses

The disease occurs in practically all countries where pineapple is grown, such as Australia, Brazil, Ceylon (Sri Lanka), Cuba, Formosa, Hawaii, India, Jamaica, Malaya, Mexico, the Philippines, Puerto Rico and South Africa. Mealybug wilt is the most serious disease of pineapple in many of the pineapple growing countries of the world. Heavy losses have been recorded in Hawaii and other countries.

2.3 Symptoms

The most characteristic symptom of the disease is a pronounced reddening of the leaves, which later changes to pink. This is followed by a downward curling or reflexing of the leaf margins. The leaves lose their turgidity and assume a reddish to pink colour. The tips of affected leaves wither and become brown and dry. The root system ceases to develop and existing roots collapse and die, so that the plant is loosely anchored in the soil and can be pulled out easily. The affected plants may recover, particularly if the mealybug colonies are destroyed, but such plants usually remain stunted and produce small fruits of no commercial value.

of the roots. As early as 1912, Higgens of the University of Hawaii noted that wilt was limited to only a few fields. By 1920, the entire fields were being devastated by wilt (Illingworth, 1931). In 1925, A. Horner, Jr. from Hawaiian canneries of Kauai, was the first to point out the association between wilt and both mealybugs and ants and the first to suggest a means of controlling the ants. Although Illingworth (1931) was the first person to suggest that mealybugs were directly implicated in pineapple wilt, he also suggested that ants were a beneficial factor. Walter Carter in 1930 supplied the definite evidence for the relationship between mealybug feeding on pineapple plants and wilt (Carter, 1932). Carter worked for about 20 years to determine the nature of mealybug wilt, and he terminated his work with a reappraisal (Carter, 1963).

2.2 Geographical distribution

This disease has been found to occur in many countries viz., Africa, Central and South America, Jamaica, The Carribbian areas, Ceylon, Malaya, Australia and Fiji (Carter, 1934,1942,1949,1956). Jepson and Wiehe (1939) recorded this disease in Mauritius; Corbett and Pagden in Malaya (1941); Plank and Smith (1940) in Puerto Rico; Westgate (1945) in South Florida; Malan (1954) in South Africa; Real (1959) and Py *et al.* (1951) in Guinea in West Africa; Takahashi (1939) in Formosa; Lew (1958) in Taiwan; Serrano (1934) in Philippines; Singh and Sastry (1974) in India; Wakman *et al.* (1995) in Australia and Eduviges *et al.* (1998) from Cuba.

2.3 Symptomatology

The characteristic symptoms of the disease are drying and wilting of the leaves commencing from tips downwards, accompanied by reddish yellow colour of the wilting plant which can be grouped in to following four different stages (Singh and Sastry, 1974).

Stage-I: Bronze to red colour appears from third to fourth whorl outwards, leaves show inner reflexing of margins; tips of the leaves not curled backward. Plant not evidently smaller than nearby plants.

Stage-II: Leaves exhibit bright pink and yellow colour; lose turgidity, slight browning of the tips, leaves occasionally show tip curling and necrotic areas on the apex .

Stage-III: Leaves of 4th and 5th whorls show downward reflexing, their edges turn yellow, otherwise pink, tips of the leaves curl tightly back at edges. Plants definitely smaller than healthy ones.

Stage-IV: Central whorl upright but without turgor; leaf tips curl tightly back, brown and withered; colour dull green, with pink colour sparse and principally in reflexed leaves. Complete root decay and wilting and death of the plants.

In severe case, the plant is apparently moribund. The wilt disease, however, clearly includes root collapse, and wilted plants invariably have poor roots. The relationship of root collapsed to wilt was determined by Carter (1948) by growing plants in mist chambers.

An interesting diagnostic procedure has been suggested from Malaya to separate drought wilt from mealybug wilt. If a thin strip of epidermis is removed from the

upper surface of a wilted leaf, sufficient to remove the layer of cells containing antho-cyanin, the underline tissue will appear white or yellowish if mealybugs are the cause of the wilt; but the colour will be green if the wilt is not due to mealybugs. This technique was used with the variety grown in Singapore; it does not seem to apply to smooth Cayenne.

2.4 Transmission

The pineapple wilt virus (PWV) is transmitted successfully by species of mealybugs i. e., *Dysmicoccus brevipes* Ckl., *D. neobrevipes* (Singh and Sastry, 1972,1974; Sether *et al.,* 1998). Even though a single specimen of the mealybug can transmit the virus, however, for cent percent transmission of the virus a minimum of 20 mealybugs per plant are required. The inoculated plants show wilt symptoms 50-60 days following inoculation. Bird (1954) observed that a single specimen of *B. brevipes* Ckl. could transmit PWV to healthy susceptible seedlings when the mealybugs were confined to test plants for 24 hours. Sether *et al.* (1998) reported that all stages of *D. neobrevipes* acquired PMWV, although vector efficiency decreased significantly in older adult fe-males. The probability of a single third- instar immature transmitting the virus was 0.04. Both species of mealybug acquired and transmitted PMWV from infected pineapple material that had been clonally propagated for decades.

2.5 The species of mealybugs concerned

For almost 30 years it was presumed that only one species, *Pseudococcus brevipes* (Ckl.). Ferris (1948) removed the pineapple mealybug from the genus *Pseudococcus* and placed it in the new genus *Dysmicoccus* as *D. brevipes.* Beardsley (1959,1965) demon-strated there were valid morphological differences between Ito's pink and gray forms and recognized the gray form as a distinct new species, *D. neobrevipes*. *D. brevipes* is non-green spotting strain and *D. neobrevipes* is green spotting type. In Hawaii, *D. neobrevipes* (gray species) occurs primarily on the crown of the pineapple plant, in-cluding the developing fruit. *D. brevipes* (pink species) is confined largely to the lower portions of the pineapple plant, near ground level or below. These two mealybug spe-cies are now widely distributed through out the pineapple growing areas of the world. It is now known to occur in Fiji, Micronesia, Philippines, Taiwan, Malaysia, Mexico and Jamaica (Rohrbach *et al.*, 1988).

Singh and Sastry (1974) reported the association of *D. brevipes* with mealybug wilt disease in India and they found that this species transmits the wilt virus under fields conditions.

In addition to *D. brevipes* and *D. neobrevipes*, one more species of mealybug occasionally infests pineapple in Hawaii and can cause wilt. This is the long-tailed mealybug (*Pseudococcus longispinus*) (Targioni – Tozzetti), which gets its name from the long waxy filaments extending, tail-like, from the posterior end of the insect. Unlike the pink and grey species, the long-tailed mealybug apparently can survive fairly well without being tended by ants, and because of this, spotty infestations occasionally develop even in fields where ants have been controlled. The long-tailed mealybug has

a number of natural enemies, however, and once these have located an infestation, they will generally eliminate the population within a few weeks.

2.6 Relationship between mealybugs and ants

The recognition of this relationship is basic to an understanding of the epidemiology of mealybug wilt. *D. neobrevipes* appear to be completed dependent on the activity of ants for its vigorous growth and reproduction, but not all ant species fill the mealybug's need in this respect equally. Many species of ants will attend the mealybugs casually to the benefit of the insect. Ants are a problem in pineapple fields only because of their association with mealybugs. It is the ants caretaking behavior that allows the mealybug species *D. brevipes* and *D. neobrevipes* to prosper (Carter, 1935; Nixon, 1951; Serrano, 1934). The rapidity of spread is pronounced when *Pheidole megacephala* (Fabr.) is an attendance. *Solenopsis germinata* Forel. and its varieties can, on occasion, build huge nests around colonies of mealybugs on scattered plants, but these generally are colonies of *D. brevipes*, which is normally subterranean species, and spread is limited. With *P. megacephala*, on the other hand, large colonies of the mealybug develop on the aerial portions of the plant, and spread can be rapid. Two more introductions of ants into Hawaii, the Argentine ant (*Iridonyrmex humilis*) (Mayr) and the long-legged ant (*Anplolepis longipes* Jerdon), have successfully displaced the bigheaded ant (*P. megacephala*) where environmental conditions are suitable.

2.7 Etiology

For more than 50 years, the wilt disease of pineapple has been associated with mealybug feeding and was thought to be caused by the secretion of toxins from mealybugs as they fed on the plants. The first evidence that the pineapple mealybug wilt disease is caused a virus came from the experiments on mealybug transmission (Singh and Sastry, 1974). Singh and Sastry (1974) reported that when these mealybugs reared on the healthy pineapple plants were transferred to another healthy plant, no disease symptoms appeared. When the healthy colonies of the mealybugs were allowed to feed on diseased plants for 24-48 hours and then transferred on to healthy pineapple plants, the initial disease symptoms appeared after 40-50 days. This result was confirmed by a series of transmission studies. These investigators ruled out the toxin theory and gave an indication that the pineapple mealybug wilt disease is caused by a virus which is transmitted by mealybug, *Dysmicoccus brevipes* (Singh and Sastry, 1974). Singh and Sastry (1974) proposed the name "pineapple wilt virus" instead of pineapple mealybug wilt.

Following of the data from Carter (1933), Ito (1938) and Singh and Sastry (1974) seems to support the concept that wilt disease is caused by a virus transmitted by mealybugs. i. Mealybugs must feed on a plant that is designated as a positive source, since mealybugs fed on artificial media for more than 4 hours can not transmit the disease when transferred to healthy plants. ii.The mealybugs must move to another plant, which is normally accomplished with the help of ants. iii. The amount of wilt within a field is very dependent on the average number of mealybugs per plant and the

average length of time these mealybugs feed on each plant. iv. The above ground symptoms appear faster after mealybugs feed on young plants (i.e. symptoms peaked at 75 days after feeding on 5 month old plants Vs. about 120 days on 9 month old plants). v. Recovered plants do not become symptomatic when re-infected with mealybugs from positive source plants (cross protection via a mild strain).

Rohrbach *et al.* (1988) produced further evidence that pineapple wilt is caused by a virus. The discovery of double stranded RNAs (ds RNAs) in infected pineapple led to the isolation and characterization of a virus proposed to be named pineapple wilt virus. This virus could be recovered constantly from plants with mealybug wilt symptoms. The extremely long, flexuous shape of the virus particle, the molecular wt. of the largest ds RNA (about 4.4×10^6 daltons), the presence of multiple smaller ds RNAs, and the molecular wt. of its coat protein (23×10^3 daltons) are consistent with the properties of subgroup II closterovirus (Francki *et al.,* 1985). The discovery of virus-like particles associated with mealybug wilt (Gunasinghe and German, 1986, 1987, 1988, 1989) seemed to point to a viral etiology for the disease. However, despite further work (Hu *et al.,* 1997; Thomson *et al.,* 1996; Ullman *et al.,* 1989; Ullman *et al.*, 1991; Wakman *et al.,* 1995; Eduviges *et al.,* 1998), this still remains to be established (Hu *et al.,* 1997; Sether *et al.,* 1998).

2.8 Particle morphology

The virus particles are long flexuous rods measuring 1200 – 1500 nm x 12 nm in size. (Rohrbach *et al.,* 1988; Ullman *et al.*, 1989). Wakman *et al.* (1995) reported closterovirus – like particles measuring c. 1700 – 1900 x 12 nm. Eduviges *et al.* (1998) reported long flexuous rod shaped virus like particles measuring 1200 – 1450 nm with a width of 12 nm.

2.9 Viral coat protein

Protein was analyzed by sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE). Partially purified virus preparations revealed the presence of one major protein and some minor lower molecular weight protein component. Molecular weight of 23,800 Da was calculated for PWV. A protein of this size is consistent with its being the coat protein of a closterovirus group (Gunasinghe and German, 1989).

2.10 Analysis of dsRNA

The double stranded RNA (dsRNA) was extracted from pineapple plants using the procedure of Dale *et al.* (1986). dsRNA was found in green leaf material from wilting pineapple plants (Gunasinghe and German, 1989). They observed that the molecules were resistant to RNAs treatment under high salt conditions but were degraded by RNAs in low salt buffer indicating thereby that they are dsRNA with molecular weight of 8.35 million daltons.

2.11 Serology and detection

Ullman *et al.* (1989) and Wakman *et al.* (1995) produced an antiserum to a closterovirus-like particles consistently associated with pineapple plants (*Ananas comosus* 'Smooth Cayenne') affected with mealybug wilt of pineapple. Purified immunoglobulins (IgG) specific to pineapple virus and goat anti-rabbit IgG were used to develop and indirect enzyme linked immunosorbent assay (ELISA) for detection of pineapple virus in crude pineapple leaf extract. Specific reaction between IgG specific to pineapple virus and virus from pineapple plants were shown by serologically specific electron microscopy, ouchterloney double diffusion tests and ELISA. The virus can also be detected by ISEM technique (Wakman *et al.,* 1995), tissue blotting immunoassay (TBIA) (Hu *et al.,* 1997) and cDNA probes (Gunasinghe and German, 1988).

2.12 Epidemiology

Factors affecting field incidence of this disease include mealybug population, climate, soil, agronomy and pineapple variety. In general temporary plantings in virgin soil are rarely affected. Corbett and Pagden (1941) noted the prevalence of wilt in pineapple grown in the lateric mineral soils as compared to peat soils. Carter (1942) noted that in pineapple growing on the fringe of the plants geographic economic range, low temperatures during the winter materially reduced the size of mealybug colonies. Seasonal rainfall and high temperature, favour mealybug multiplication which results in high wilt incidence. The incidence of heavy rains in reducing leaf populations of mealybugs and favouring establishment of subsurface colonies is also noted as a factor in minimizing wilt incidence in Malaya. Land rich inorganic matter in Zanzibar, where small plantings on virgin soil were observed, showed dubious cases of wilt. On the adjacent East African mainland, however, in soils deficient in organic matter and subject to seasonal rainfall and high temperatures, mealybug wilt was a limiting factor which has since caused the abandonment of the plantings. In 1938, plantings in Kenya showed only small and scattered colonies of mealybugs on small plantings on virgin land. With the development of pineapple growing, the establishment of canning industry in that area, and the use of old sisal land for pineapple planting, wilt had increased considerably by 1956. Jepson and Wiehe (1939) reported that in Mauritius mealybugs were known on pineapple plant long before but the wilt problem developed only when the area in pineapple cultivation increased. In the State of Pernambuco, where more Tropical conditions obtain, wilt is more common inspite of the fact that the variety grown there and known as white paulista is more resistant than the yellow paulista of the south. The virgin land used there is a cleared, low scrub forest.

In Taiwan, in August, the normal planting month is followed by a dry season during which mealybug populations multiply rapidly until the heavy rains in the following summer. With the advent of the next dry season, populations again rise rapidly and infestation becomes general. It is not until the rains begin in the third year, the beginning of the ratoon crop, that rapid collapse of the plants occurs. In Hawaii, Fullway (1924) observed that mealybug infestation appeared to start from the outer edges of pineapple fields and gradually work in toward the centre.

2.13 Assessment of loss

Singh and Sastry (1974) studied the effect of pineapple wilt virus infection on the growth and yield of pineapple plant and found that there was a considerable difference in all the observations taken in healthy and infected plants. It was also observed that the number of leaves emerged were very few when compared to those on healthy plants. In fourth stage of infection, there was no emergence of leaves.

2.14 Management

Pineapple wilt could be controlled considerably by controlling the mealybug vector and ants. Dipping of planting material is recommended by spraying the growing plant with an organo-phosphorus insecticide (Ting-Wei, 1958; Py *et al.,* 1957; Vilardebo, 1955). Clorinated hydrocarbons have been found effective for the control of ants. Mirex and heptachlor have been found effective against ants.

Singh and Sastry (1974) reported that pineapple wilt virus can be controlled by hot water or dry heat treatments. Virus infected planting material could be freed from virus by subjecting them to hot water treatment at 50°C for one hour or dry heat treatment at 55°C for one hour. Treated plants did not exhibit wilt symptoms. Ullman *et al.* (1991) reported that pineapple crown infected with pineapple wilt (Closterovirus) were made virus-free by hot water treatment at 40,50 and 60°C for 30,60 and 120 minutes at each temperature. Plant survival was 80-100% at 40 and 50°C and ELISA test demonstrated that 60-100% of these surviving plants were rendered free of closterovirus-like particles.

2.15 Future management strategies

The following points have been suggested by Rohrbach *et al.* (1988):

i. Screening of chemicals to control ants and mealybugs should be continued. Bait formulation such as Logic, Affirm, Amdro, and Prodrone are more desirable than spray because of their low hazard to the applicator, minimal impact on non-target organisms and the environment, and selectivity to ants. Any formulation showing promise should be tested.

ii. Detailed studies on ant ecology and behaviour should be conducted. Little is known about the in-migration rate of big-headed ants in to fields, survival after tillage, food source in fallow fields, foraging behaviour and foraging distance, recruitment to food, seasonability and population trends with plant phenology, among others. Information on these areas may reveal a point of vulnerability in the biology of the ants that can be manipulated for better control.

iii. Studies should continue on the role of a virus in disease development, to identify alternate hosts of the virus and explore the removal of these from areas adjacent to pineapple as a means of control. Virus-free vegetative "seed" material, should be identified and studied for use as disease free propagative material for planting.

iv. Biological control of mealybugs should be reconsidered. It probably never was

given a fair trial because of the introduction of diesel oil spray in 1935 that not only destroyed the mealybugs but also reduced mealybug predator populations. Biological control of mealybug will not be effective without control or suppression of ant populations. A programme integrating the management of ant populations with biological control of mealybugs should be initiated.

v. Ant predators and parasites should be studied. Research have discovered a number of   natural enemies of fire ants (Jouvenaz, 1986; Wojcik, 1986a,b). It appears that little has been done in this area with bigheaded ants.

vi. Physical barriers such as fences or rows of beds running parallel to the field periphery have been partially successful in the past and should be considered. Whether these particular barriers are used or not, they do illustrate that ant behavior can be modified by changing their physical environment.

vi. Reconsideration of the use of mirex could serve as a stopgap control until more suitable chemicals and/or biological control methods are developed.

3. Pineapple bacilliform virus

Wakman *et al.* (1995) reported the association of a bacilliform virus in asymptomatic or mealybug wilt infected pineapple plants, from Australia. The virus appears to belong to the badnavirus genus of plant viruses. This is supported by I) bacilliform shape and size of particles, II) serological cross reactivity with antiserum to sugarcane bacilliform virus (SCMV), III) PCR amplification of PBV DNA using degenerate badnavirus primers, and IV) nucleotide sequence similarity with badnaviruses in the RNAs H and RT genes.

The virus particles measure c. 133 x 33 nm in size. The virus is efficiently transmitted through seeds. The expression of viral promoter may indicate the possibility of seed transmission of badnaviruses.

The presence of the virus can be detected serologically using ELISA or ISEM (Wakman *et al.,* 1995) and also by PCR (Thomson *et al.,* 1996). Pineapple bacilliform virus (PBV) was detected in tissues of leaves and roots. PBV has also been detected in plantlets passed through meristem tip culture. The widespread distribution of PBV can be attributed to the vegetative propagation of pineapples. PBV is distributed throughout the plant. Leaf soak extracts (Thomson and Dietzgen, 1995) is easy to prepare and appear to provide sufficient template for detecting PBV in small quantities of tissue. PBV was detected in mealybugs (*Dysmicoccus brevipes*) (vector of pineapple mealybug wilt) (Thomson *et al.,* 1996). The relationship of the PMWV and PBV and possible iteraction are still undetermined. Thomson *et al.* (1996) used the sequence of 448 bp fragment to design the specific primers PBV 1 and PBV 2. Using either partially purified PBV, TNAE or leaf soak extracts, the specific primers amplified a 403 bp fragment from smooth leaf pineapples including cv. Smooth Cayenne and rough leaf pineapple including cv. Queen collected from the field. There was 100% correlation between PCR and IEM results of the same plants.

PBV DNA contains conserved badnavirus sequences in the RNAs H and RT genes. PBV had 42-45% nucleotide homology to the other badnaviruses when the sequences were aligned between the primer regions.

4. Pineapple yellow spot

4.1 History and geographical distribution

This disease occurs in all pineapple growing places. This disease was thoroughly defined and described by Illingworth in 1931, but the cause of the disease was not known definitely at that time. The viral nature of the disease and its method of spread were determined definitely by Linford in 1932. The disease was first observed in a small area in Hawaii in 1926. In subsequent years, it spread rapidly throughout the islands. Yellow spot was reported in the Philippines in 1935 (Serrano, 1935).

4.2 Symptomatology

The disease takes its name from the appearance of the early symptoms on pineapple leaves, namely, a small, round, yellowish spot, of the upper surface of the leaves. These spots are also called "initial spot" which ranges from 1/8 –1/2 inch in size. These later elongate downwards towards the leaf base, increasing in to an irregular streak. This may also take the form of a succession of elongated spots, connected or not connected, become wider and longer as it approaches the leaf base. It has a dark centre surrounded by a halo of yellow. The spots are usually found 3-8 inches from the base of the leaf, its centre often marking the oviposition puncture of the vector (*Thrips tabaci* Lind.). A yellow streak eventually appears, extending from the spot to the white tissue at the base of the leaf. After this initial yellow streak reaches the leaf base, other yellowish streaks are soon seen ascending the next leaf above the originally infected one. Then in succession other still young leaves become involved, brown or blackish necrosis develops and the plant bend over towards the side where the initial yellow spot appeared. The tissues of the stem on the affected side cease to elongate so that axis becomes curled towards the affected side. Finally the entire plant drops and dies. The distorted one-sided nature of the affected stems gave rise to the earlier name "side rot". The infected leaves become stunted, chlorotic and somewhat brittle and tend to tighten together while the lower leaves remain apparently normal. When cut lengthwise, diseased plants reveal patches of browned tissues in the stem around the attachment of the leaf which had the initial spot (Serrano, 1935).

The disease may also, however, appear in crowns and pass down in to the fruits, causing rotting of the fruit, or it may start in the fruit directly, during the blossom or bud stage growth, and cause large necrotic areas of cavities.

4.3 Transmission

The virus is mechanically sap transmitted and in nature it is transmitted by the onion thrips, *Thrips tabaci* L. from diseased weed hosts on which the thrips breed (Linford, 1932). This virus is also transmitted by other species of thrips i.e. *Frankliniella schultzii*, *F. fusca* and *F. occidentalis*. Adult thrips are unable to pickup the virus *de novo*; it must be acquired by the larval form first, the subsequent adult can then transmit the virus. An incubation period of 10 days in the insect vector is required. The minimum acquisi-

tion feed is 15 minutes. The latent (incubation) period varies from 4-8 days according to the species of thrips involved. The incubation of the virus in pineapple and *Emilia sagittata* varies from 7-15 days.

4.4 Etiology and Physical properties

Pineapple yellow spot disease is caused by a strain of tomato spotted wilt virus. The thermal inactivation point of the virus is 42°C, the dilution end point lies between $1:10^{-4}$ to $1:10^{-5}$, longevity *in vitro* is very short, about 5 hours at room temperature. The virus is spherical and measure 85-102 nm in diameter.

4.5 Host range

Host range of spotted wilt virus is very wide (Cho *et al.,* 1987). *Petunia hybrida* and *Nicotiana glutinosa* are local lesion hosts.

4.6 Detection

The virus can be readily detected by serological techniques like ELISA, ISEM and also by cDNA probes.

4.7 Management

Biological control through imported parasites (*Thripoctenus ruselli* Crawford and *T. brui* Vuillet.) have been attempted. Application of systemic insecticides may prove helpful in controlling thrips.

5. Pineapple chlorotic leaf streak

This disease has been reported only from Brazil by Kitajima *et al.* (1975).

5.1 Symptomatology

Plants of a giant variety of pineapple (*Ananas comosus* (L.) Merill) were introduced by the S. Frutas de Clima Tropical of the Instituto Agronomico de Campinas, from the region of Tarauaca, Acre. Infected plants showed conspicuous chlorotic streaks in their leaves.

5.2 Etiology and particle morphology

The etiologic agent of this disease is a virus belonging to rhabdovirus group. Attempts to transmit the chlorotic streak agent to seedling of commercial pineapple varieties and a large number of other test plants by mechanically inoculation have been unsuccessful but this mite have been due to inhibitors present in the pineapple leaf juice.

Negatively stained leaf dip preparations from affected plant contained bullet or bacilliform particle 60-70 nm wide and 200-250 nm long (Fig. 16). Sometimes a regular hexagonal lattice as well as small surface projections are noticed in these particles. In few cases, the stain penetrate in to the particle, revealing an inner striated, tubular core. Groups of bacilliform particles are consistently found, mostly in epidermal and parenchymatous cells. In sections, the particle diameter is about 60 nm and the length somewhat variable, being the maximum around 250 nm.

6. References

Beardsley, J. W. 1959. On the taxonomy of pineapple mealybugs in Hawaii, with a description of a previously unnamed species (Homoptera: Pseudococcidae). Proc. Hawaii Entomol. Soc., 17: 29-37.

Beardsley, J. W. 1965. Notes on the pineapple mealybug complex, with descriptions of two new species (Homoptera: Pseudococcidae). Proc. Hawaii Entomol. Soc., 19: 55-68.

Bird, J. 1954. Ann. Rep. Dept. Plant Path. Agric. Stat. Univ., Puerto Rico 1953-54.

Carter, W. 1932. Studies on populations of *Pseudococcus brevipes* (Ckl.) occurring on pineapple plants. Ecology, 13: 296-304.

Carter, W. 1933. The pineapple mealybug, *Pseudococcus brevipes* (Ckl.) and wilt of pineapples. Phytopathology, 23: 207-242

Carter, W. 1934. Mealybug wilt and green spot in Jamaica and Central America. Phytopathology, 24: 424-426.

Carter, W. 1935. The symbionts of *Pseudococcus brevipes* (Ckll.). Ann. Entomol. Soc. Am., 28: 60-61.

Carter, W. 1942. Geographical distribution of mealybug wilt with notes on some other insect pests of pineapple. J. Econ. Entomol., 35: 10-15.

Carter, W. 1948. The effects of mealybugs feedings on pineapple plants grown in finely atomized nutrient solutions. Phytopathology, 38: 645-657.

Carter, W. 1949. "Insect notes from South America with special reference to *Pseudococcus brevipes* and mealybug wilt". J. Econ. Entomol., 42: 761-766.

Carter, W. 1956. Notes on some mealybugs (Coccidae) of economic importance in Ceylon. FAO, U. N. Plant Prot. Bull., 4: 49-52.

Carter, W. 1963. Mealybug wilt of pineapple – a reappraisal. Annls of the New York Acad. Sci., 105 : 741-764.

Cho, J. J., Mau, R. F. L., Mitchell, W. C., Gonsalves, D. and Yudin, L. S. 1987. Host list of plant susceptible to tomato spotted wilt virus (TSWV). Res. Ext. Series 078, College of Trop. Agric. and Human Resources, Univ. of Hawaii.

Corbett, G. H. and Pagden, H. T. 1941. A review of some recent entomological investigations and observations. Malayan Agric. J., 29: 347-379.

Dale, J. L., Phillips, D. A. and Parry, J. N. 1986. Double stranded RNA in banana plants with bunchy top disease. J. Gen. Virol., 67: 371-375.

Eduviges, G., Borroto, Cintre, Mayra, Gonsalvez, J. and Borroto, C. 1998. First report of closterovirus-like particles associated with pineapple plants (*Ananas comosus* cv. Smooth Cayenne) affected with pineapple mealybug wilt in Cuba. Plant Disease, 82: 263.

Ferris, G. F. 1948. *Atlas of scale insects of North America, Series 5, Pseudococcidae*. Stanford University Press, Stanford, CA. 278 pp.

Francki, R. I. B., Milne, R. G. and Hatta, T. 1985. Atlas of plant viruses vol. 2. CRC Press, Boca Raton, Fl., 284 pp.

Fullway, D. T. 1924. Insects affecting pineapple production. 2nd Ann. Short Course Pineapple

production, Univ. Hawaii, 1923, 52-61.

Gunasinghe, U. B. and German, T. L. 1986. Association of virus particles with mealybug - wilt of pineapple. (Abstr.) Phytopathology, 76: 1073.

Gunasinghe, U. B. and German, T. L. 1987. Further characterization of virus associated with mealybug- wilt of pineapple (Abstr.). Phytopathology, 77: 1176.

Gunasinghe, U. B. and German, T. L. 1988. Detection of viral RNA in mealybugs associated with mealybug-wilt of pineapple. Phytopathology, 78: 1584.

Gunasinghe, U. B. and German, T. L. 1989. Purification and partial characterization of a virus from pineapple. Phytopathology, 79: 1337-1341.

Hu, J. S., Sether, D. M., Liu, X. and Wang, M. 1997. Use of a tissue blotting immunoassay to examine the distribution of pineapple closterovirus in Hawaii. Plant Disease, 8: 1150-1154.

Illingworth, J. F. 1931. Preliminary report on evidence that mealybugs are an important factor in mealybug wilt. J. Econ. Entomol., 24: 877-889.

Ito, K. 1938. Studies on the life history of the pineapple mealybug. *Pseudococcus brevipes* (Ckll.). J. Econ. Entomol., 31: 291-298.

Jepson, W. F. and Wiehe, P. O. 1939. Pineapple wilt in Mauritius. Mauritius Dept. Agric. Gen. Ser. Bull. No., 47.

Jouvenaz, D. P. 1986. Diseases of fire ants. Problems and opportunities. In: Fire Ants and Leaf Cutting Ants: A Synthesis of the Current Knowledge (eds. Lofgren, C.S. and Vander Meer, R.K.) Westview Press, Boulder, CO., pp. 327-338.

Kitajima, E. W., Giacomelli, E. J., Costa, A. S., Costa, C. L. and Cupertino, F. P. 1975. Bacilliform particles associated with chlorotic leaf streak of Giant pineapple (*Ananas comosus* L.) Merrill. Phytopath. Z., 82: 83-86.

Larsen, L. D. 1910. Diseases of Pineapple. Hawaii Sugar Planters Assoc. Pathol. Physiol. Ser. Exp. Stn. Bull. No., 10: 1-72.

Lew, W. T. 1958. Pineapple mealybug control in Taiwan. Hofchen Briefe, 11: 114-120.

Linford, M. B. 1932. Transmission of the pineapple yellow-spot virus by *Thrips tabaci*. Phytopathology, 22: 301- 324.

Malan, E. F. 1954. Pineapple production in South Africa (with special reference to the Eastern Transvaal). Union S. Africa, Dept. Agric. Bull., No. 339.

Nixon, G. E. J. 1951. The association of ants with aphids and coccids. Commonwealth Institute of Entomology, London, 36 pp.

Plank, H. K. and Smith, M. R. 1940. A survey of the pineapple mealybug in Puerto Rico and preliminary studies of its control. J. Agric. Univ. Puerto Rico 24: 49-76.

Py, C., Tisseau, M. A., Oury, B. and Ahmada, F. 1951. The culture of pineapples in Guinea. Institut Francairs recherches fruitieres d'outre Institut des fruits e agrumes coloniaux (I.F.A.C.), Paris.

Real, P. 1959. Le Cycle annual de la cochenille *Dysmicoccus brevipes* (Ckll.), Vectrice d'une 'Wilt' de l'ananas en basse Cote d' Ivoire; son determinesme. Rev. Pathol. Vegetale et Entomol. Agr. France, 38: 1-111.

Rohrbach, K. G., Beardsley, J. W., German, T. L., Reimer, N. and Sanford, W. G. 1988. Mealybug wilt, mealybugs and ants on pineapple. Plant Disease, 72: 558-565.

Serrano, F. B. 1934. Pineapple mealybug wilt in the Philippines. Philipp. J. Sci., 55: 363-377.

Serrano, F. B. 1935. Pineapple yellow spot in the Philippines. Philipp. J. Sci., 58: 481-493.

Sether, D. M., Ullman, D. E. and Hu, J. S. 1998. Transmission of pineapple mealybug wilt-associated virus by two species of mealybug (*Dysmicoccus* spp.). Phytopathology, 88: 1224-1230.

Singh, S. J. and Sastry, K. S. M. 1972. Pineapple mealybug wilt- a new virus disease in India (Abstr.). Proc. III Inter. Symp. on Trop. and Subtrop. Hort. , Bangalore, p. 125, 1972.

Singh, S. J. and Sastry, K. S. M. 1974. Wilt of pineapple: a new virus disease in India. Indian

Phytopath., 27: 298-303.

Singh, S. J. 1996. Pineapple diseases. In : Advances in Diseases of Fruit Crops in India. (ed. Singh S. J.) Kalyani Publishers., pp. 317-330.

Takahashi, R. 1939. Insect pests of pineapple, especially *Pseudococcus brevipes* (Ckll.). Bull. Agric. Res. Inst. Formosa, 161: 1-17.

Thomson, K. G. and Dietzgen, R. G. 1995. Detection of DNA and RNA plant viruses by PCR and RT-PCR using a rapid virus release protocol without tissue homogenization. Jour. Virol. Methods, 54: 85-95.

Thomson, K. G., Dietzgen, R. G., Thomas, J. E. and Teakle, D. S. 1996. Detection of pineapple bacilliform virus using the polymerase chain reaction. Ann. Appl. Biol., 129: 57-69.

Ting-Wei Lew, G. 1958. Pineapple mealybug control in Taiwan. Hofchen Briefe, 11: 114-120.

Ullman, D. E., German, T. L., Gunasinghe, U. B. and Ebesu, R. H. 1989. Etiology of closterovirus-like particle associated with mealybug wilt of pineapple. Phytopathology, 79: 1341-1345.

Ullman, D. E., German, T. L., Mc Intosh, C. E. and Williams, D. D. F. 1991. Effect of heat treatment on a closterovirus-like particle associated with mealybug wilt of pineapple. Plant Disease, 75: 859-861.

Vilardebo, A. 1955. La Cochenlle de l' ananas et le wilt quelle provoque. Fruits (Paris), 10: 59-66.

Wakman, W., Teakle, D. S., Thomas, J. E. and Dietzgen, R. G. 1995. Presence of a clostero-like virus and a bacilliform virus in pineapple in Australia. Australian Jour. Agric. Res., 46: 947-958.

Westgate, P. J. 1945. Mealybug wilt of pineapple in South Florida. Proc. Florida State Hort. Soc., 58: 194-196.

Wojcik, D. P. 1986a. Status of the search for natural control agents in South America. Pages 29-37 In: Proc. Fire Ant. Conf.

Wojcik, D. P. 1986b. Observations on the biology and ecology of fire ants in Brazil. In: "Fire Ants and Leaf Cutting Ants. A Synthesis of the Current Knowledge" (eds. Lofgren, C.S. and Vander Meer, R.K.) Westview Press, Boulder, CO. pp. 88-103.

Author Index

Subject Index