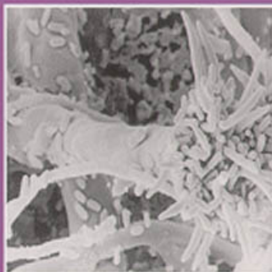
Tropical Mycology:

VOLUME 2

MICROMYCETES



Edited by R. Watling, J.C. Frankland, A.M. Ainsworth, S. Isaac and C.H. Robinson

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Tropical Mycology: Volume 2, Micromycetes

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Edited by

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Dedication

It is with great pleasure that this volume is dedicated to Dr Reginald W.G. Dennis, who has made many important contributions to our understanding of tropical fungi, especially in unravelling the complexities of members of the large genus *Xylaria* and allies. His mycological interests range from the hyaloscyphaceous and helotiaceous ascomycetes to pyrenomycetous taxa, agarics, rusts and gasteromycetes. Visits to Trinidad and Venezuela, in 1949 and 1958, resulted in several papers, which were to be only a foretaste of his *Fungus Flora of Venezuela and Adjacent Countries*, published in 1970 and illustrated with his own watercolours. It has become an indispensable companion to any mycologist working in South America and is a useful introductory guide for all tropical fungus researchers. He began his career as a plant pathologist working in Scotland at the West of Scotland College of Agriculture and Dept of Agriculture of Scotland, East Craigs, as they were then, with a sandwich period between in the School of Agriculture, Cambridge. He moved to taxonomy from his earlier work on plant pathogens, including virus diseases of cereal and root crops, when he joined the staff of the Royal Botanic Gardens, Kew in 1944, where he still works even though retired. He is an Honorary Member of the British Mycological Society, The Mycological Society of America and the Swiss Mycological Society, and is a Corresponding Member of the Botanical Society of Argentina.

A special Festschrift published in Vol. 31 of the *Kew Bulletin* was dedicated to Dr Dennis on his retirement from service and includes therein an account of his works and interests. He was 90 in July 2000.

Roy Watling, June 2001

(See Spooner, B.J. (2000) *Mycological Research* 104, 1410.)

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Preface

Half of the membership of the British Mycological Society resides abroad and many of these members are from countries which are generally considered to be situated in 'The Tropics'. Since the Sixth General Meeting of the Society held in Birmingham in 1988, the Society has recognized the increasing importance of tropical mycology and Council felt that it would be appropriate to bring both these factors of membership and research interests together to celebrate the millennium. A symposium therefore was organized for April 2000 to cover as many aspects as possible of the tropical mycobiota. This volume is one of two which result from this Millennium Symposium held at the Liverpool John Moores University. In order to broaden the scope and the depth of the subjects covered, other chapters not formally presented at the symposium are included. This present volume deals with the microfungi; Volume 1 deals with the macrofungi.

The British Mycological Society has now organized two meetings specifically on Tropical Mycology and both were held in Liverpool, coincidentally a city with a long history of contacts with the areas of the world which lie between 25° North and South of the equator and known to us all as 'The Tropics'. The present pair of books will therefore act as a companion to the Proceedings of the meeting held in 1992, which were published 1 year later under the title *Aspects of Tropical Mycology.* The chapters included here have expanded the ideas first discussed therein and each is a summary of the state of knowledge in the respective field. Some of the studies have indirect connections to the two expeditions the Society has organized to tropical areas, the first to Ecuador in 1993 and the second to Thailand in 1997.

In the selection of subjects, attempts have been made to bring together

papers which offer a wide spectrum of information, linking results from studies in the New World to data from the Old World tropics. Contributions by 26 researchers provide 11 chapters covering the major area of the tropics. Although our knowledge of the tropical mycotas is far from adequate, the surge of interest in the fungi of these areas has expanded our knowledge several-fold since 1992, as documented in the first chapter, the contents of which could equally apply to both the macro- and the microfungi. However, in this first chapter, the micro-forms have been emphasized by the author and so it acts as a convenient opening statement to the book.

The additional chapters undoubtedly interlink, but they have been arranged in such a way as to bring together like interests for easy reference. It is refreshing to see that several of the chapters cover various aspects of mycogeography placed in a geological timescale. Chapters 2–4 cover broad issues of systematics whilst Chapters 5–7 explore the interrelationships between fungi and plants, a theme which is continued in Chapters 8–10, but in these the interacting organisms are either algae or from the animal kingdom. The concluding chapter explains recent discoveries of pharmacologically active metabolites isolated from tropical fungi and what the future holds in this area of exploration.

It is essential in all fields of mycology to have a firm taxonomic base-line from which to work and a good key by which researchers can identify the organisms they are studying. This has been made possible for the first time for the nectriaceous fungi by the authors of Chapter 2. Theirs has been a wide-reaching study, which will place many unfamiliar and obscure names into context and make it possible to address the relationships of some of our widespread nectriaceous plant pathogens both in the tropics and in the temperate world. For instance, the 'Panama disease' of bananas, which is currently showing a resurgence, is caused by a race of the nectriaceous *Fusarium oxysporum* and is a devastating pathogen on a crop on which many tropical economies rely.

Chapter 3 deals with the finer details of the systematics of sooty moulds, a group of fungi widespread in the tropics but also a familiar sight in glasshouse conditions in the temperate world. The authors extend the patient work commenced by a former vice-president of the British Mycological Society, Stan Hughes. It was he who first unravelled the relationships for this group of fungi, the various sexual and asexual stages, the latter often multiple, and made sense of the biology. More importantly, many of the herbarium collections languishing in national herbaria under the broad name of *Hormiscium* etc. were finally determined. Reynolds expanded this work and with his colleagues in Chapter 3 continues to tease apart the complex typification of some of these obscure fungi.

Terence Ingold and John Webster, two former presidents of the British Mycological Society, have explored the relationships of aeroaquatic hyphomycetes. Their studies were based mainly, but not exclusively, on

temperate taxa, and it was they who encouraged the search for the sexual stages of these fungi which produce such beautiful branched water-borne conidia. Several links are now known, but the authors of Chapter 4 extend these observations to the anamorphic and teleomorphic stages of lignicolous fungi in the tropics of South-East Asia.

In 1995, the author of the first chapter of this volume expressed the view that there were of the order of 1,500,000 fungi worldwide. The authors of Chapter 5 examine this estimate in connection with the fungi found on members of the *Pandanaceae*, a tropical family of flowering plants resembling the palms in many ways in form and structure. They found that there is a multitude of fungi on members of this family, many of which are restricted in their host range. This theme is continued in Chapter 6 where various aspects of downy mildews of grasses are considered. With again so much specificity, it was only logical for the authors to examine the coevolution of the host, the grasses, and the downy mildews. This allowed them to extrapolate and place these fungi in the same frame as the flowering plants when enquiring into the geographical area of origin of these fungi and pattern of migration in geological time. Equally, such evolutionary discussions provoke further questions as to the uniformity of the mildew taxa, and the opportunity is taken to address this issue.

Chapter 7 deals with invasive neotropical pathogens of two major crops, the export of products of which many developing countries depend on for foreign exchange. The crops are cacao and rubber, the fungi ranging from ascomycetes to basidiomycetes. The chapter also unravels some of the taxonomic problems encountered in naming the causal organisms.

Lichens have only recently been incorporated into general discussions about fungi, but they should be considered even though they are only lichenized forms. 'Only' really does not reflect their unique role in plant assemblages or their long history of being recognized as organisms in their own right. It is fitting therefore to include a chapter on lichens within the framework of this publication. Chapter 8 tackles various aspects of tropical lichens, based on extensive fieldwork, especially in South-East Asia. Their diversity, distribution within and outwith the rainforest canopy, and the possible environmental and anthropogenic factors which affect their continual growth in specific sites are all discussed.

Animals have long been associated with fungi but generally the interactions which they establish, except for perhaps the medical fungal pathogens, are much less publicized than those for plant diseases caused by fungi. However, these fungi do play an important role in naturally occurring invertebrate and vertebrate populations. In addition, as described in Chapter 9, the estimate of world fungi by Hawksworth will certainly have to be reconsidered when a full study of the fungi associated with invertebrates is taken into account, for there appears to be great specificity expressed in these fungi. There are estimated to be about three times, if not more, insects than fungi! The author of Chapter 9 has taken into account, in the title of his contribution, that there are many species

pathogenic on spiders and mites and that entomogenous or even entomopathogenic does not cover the real world. This chapter also focuses heavily, although broadly, on results of work in South-East Asia, introducing the reader to these fungi as potential biocontrol organisms and as sources of useful secondary metabolites, a fact developed further in the last chapter.

Humans, although parasitized by fungi, as demonstrated in the penultimate chapter, are organisms scarcely mentioned in fungal texts, and especially tropical taxa. Thus, Chapter 10 is doubly important as it places another type of fungal/animal interrelationship into context with the rest of general mycology and demonstrates the increase in these diseases as a result of freer world travel between tropical and temperate destinations. This chapter summarizes the state of knowledge in this potentially threatening scenario where many practising mycologists and doctors, although well aware of the symptoms of ringworm and athlete's foot, are unaccustomed to the conditions caused by some of these tropical agents.

For a long time it has been argued that we must conserve the biodiversity of the world because it may hold hidden secondary metabolites which will cure disease. Although this is a commendable reason, it is folly and unsubstantiated to base the conservation of the world's fauna and flora purely on this single selling point, although it is true that some of the most effective pharmacological compounds have been isolated from nature. Indeed, the first three top-selling pharmaceutical compounds are derived from fungi. The last chapter looks at some aspects of novel compounds isolated from fungi and the possible future of such activities in industrial mycology. The development of new technologies based on what we already know of active fungal compunds is discussed.

Scientists, including many botanists, are blissfully unaware of the role of fungi in the world's ecosystems, and it is hoped that this collection of chapters along with the sister volume will emphasize their importance, bringing mycologists up to date in areas with which they are less familiar. It is also hoped that these two books will stimulate students of mycology and be helpful to a whole range of biologists interested in tropical biodiversity. It would be outstanding if they could act as a pump primer to support research programmes and even necessitate a demand to launch a third British Mycological Society tropical expedition.

It is my great pleasure to thank an industrious team of editors: Martyn Ainsworth, Susan Isaac, Clare Robinson, who have all given their all unselfishly since April 2000 in reviewing and editing the chapters for this publication and its sister volume, and especially Juliet Frankland, who has helped me finalize the drafts ready for technical adjustment and presentation, so they could be brought together as soon after the meeting as possible in order to act as a base-line for future studies.

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Why Study Tropical Fungi? 1

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Introduction

The question 'Why study tropical fungi?' can be addressed in different ways, and at a variety of levels, depending on both the perspective of the person being asked and also its relevance to the interests and concerns of the questioner. The reasons why tropical fungi should be studied are numerous. Here in the author's view are the ten most important. Tropical fungi are a major component of biodiversity, essential to the survival of other organisms, crucial in global ecological processes, a source of novel bioactive compounds, a source of biocontrol agents, a source of plant pathogens, a threat to human health, able to contribute to sustainable development, a part of human culture, 'and are there...'. Each reason will be justified and a prognosis of tropical mycology presented.

The subject has attracted fresh interest, and is now more active than at any time in the last 50 years. However, support for basic mycological work in developed countries has fallen at a time when the demand from a wide range of ecologists, conservationists, and those interested in sustainable development is greater than it has ever been. Encouragingly, the focus of work on tropical fungi is moving to centres in the tropics where active research schools, workshops, symposia, and mycological organizations and publications are being established. Mycologists are now encouraged to commit to actively promoting the study of tropical fungi, in order to achieve a steady improvement in both knowledge and utilization.

The rekindling of interest in tropical mycology, particularly in fungal diversity and utilization, has mainly occurred in the last 10 years. The subject has

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risen to a level not paralleled since the colonial period came to an end in the 1940s and 1950s. This revival is exemplified by several books and symposia focusing on tropical mycology, particularly in the wake of the British Mycological Society's pioneering meeting on *Aspects of Tropical Mycology* held in Liverpool in April 1992 (Isaac *et al.*, 1993), and including *Tropical Mycology* (Janardhanan *et al.*, 1997) and *Biodiversity of Tropical Microfungi* (Hyde, 1997).

A new journal devoted entirely to the topic, *Fungal Diversity*, started publication in 1998. Other books have focused on particular areas in the tropics (e.g. Hennebert, 1994; Rossman *et al.*, 1998) or on exploitation (Palm and Chapela, 1997; Pointing and Hyde, 2001). In addition, fungi have received a heightened profile in general assessments of diversity in tropical and other areas (Heywood, 1995).

At the 1992 meeting, I surveyed the extent of the tropical fungal biota, examined the resource base available for its study, touched on its pertinence, suggested what might be done, and cautioned that, if mycologists did not become involved in the biodiversity arena, mycology would be increasingly marginalized (Hawksworth, 1993). It is gratifying to have witnessed the progress made over the last 8 years.

Fungi, while still 'orphans' and trailing in the biodiversity stakes (Hawksworth, 1997), are now being noticed by a wider range of international and national bodies, government departments, conservation agencies, ecologists, biodiversity scientists, and naturalists than ever before. See, for example, Kaul (2002). This is not an inconsiderable achievement, but needs to be followed through. There is no room for complacency if we are to capitalize on our investments to date. The next task is to enthuse the array of potential sponsors, and newly found supporters, with impelling reasons why work on tropical fungi should be incorporated into their portfolios and programmes.

I should stress that the following points are necessarily selective and what other cogent reasons can be found elsewhere in this and the companion volume (Watling *et al.*, 2002) as to why tropical fungi should be studied.

Because they are . . .

(1) . . . a major component of biodiversity

Fungi are now generally accepted as the largest group of organisms on Earth after the insects; as working figures, the Global Biodiversity Assessment (Heywood, 1995) accepted estimates of 1.5 million fungi and 8 million insects, respectively. Dissensions from this view are primarily from non-mycologists who do not appreciate the long-term studies necessary to determine how many fungi are present in a site or the extent of host specificity and geographical differences (e.g. May, 2000). An analysis of estimates of species

numbers made since the 1.5 million figure was first proposed (Hawksworth, 1991) suggests that, if anything, the figure is an underestimate (Hawksworth, 2001).

A remarkable 179 governments are committed to the conservation and sustainable use of biodiversity, through their ratification of the Convention on Biological Diversity (UNEP, 1992). This was drawn up in 1992, after the Society's *Aspects of Tropical Mycology* meeting, and entered into force on 29 December, 1993. Article 7 requires signatory countries (and the European Union) to identify the components of biodiversity important to the Convention's objectives. This therefore embraces fungi, as will be stressed later, and many developed countries in particular are now taking fungal conservation more seriously than ever before. Governments of some tropical countries are also starting to take a broader view, including fungal components in projects supported through agencies such as the World Bank's Global Environment Facility.

(2) . . . essential to the survival of other organisms

Fungi can be essential to the survival of other organisms at the individual, species and ecosystem levels. At the individual level, the role of fungi in mutualisms is well known, especially in the case of mycorrhizas formed with perhaps 90% of the world's plants, and the extent of involvement with insects is becoming clearer. In the tropics, a surprising proportion of beetles, for example, are fungus-feeders, over 40% of 1.2 million collected in Sulawasi, Indonesia (Hammond, 1990). This is not just an issue for plants and smaller organisms. Herbivorous mammals need anaerobic fungi in their stomachs to aid the breakdown of cellulosic materials on which they feed.

Termites culturing *Termitomyces* mushrooms, and the gardens of leaf-cutter ants (Fisher and Stradling, 2002) are increasingly featured in museum displays and even introductory textbooks. Images of moths and spiders camouflaged against lichens are also becoming increasingly familiar (e.g. Gilbert, 2000).

However, it is the role of fungi in ecosystem functioning overall that is the most significant for the survival of other organisms in tropical forests (Lodge *et al.*, 1996). They are basal in complex food webs, not least in soil where they are the key food of many nematodes and collembolans, for instance, which in turn are devoured by larger invertebrates. In their absence, crucial soil processes, including decomposition and nutrient cycling, would fail and above-ground ecosystems be endangered. Further, there are indications that elevated carbon dioxide levels lead to some changes in populations of soil fungi, which in turn affect the numbers of fungus-feeding invertebrates (Jones *et al.*, 1998).

Our understanding of interactions between fungi and other organisms is in its infancy. The significance of endophytic fungi is still only starting to be

appreciated (Bacon and White, 2000), connections and nutrient transfers between different plant species via mycorrhizas have only recently been recognized (Linderman, 1997), and the role of pathogenic fungi in both limiting species and creating fungal habitats and microenvironments in natural ecosystems is scarcely studied. The complexities to be found can only be guessed at. Who would have thought that soil *Pythium* species could influence tree patterns in mixed forests (Packer and Clay, 2000)? The significance of such phenomena in tropical forests awaits exploration.

(3) . . . crucial in global ecological processes

Fungi have a key role in the global carbon cycle in particular. They are the only organisms able to break down materials composed of lignin, releasing methylated gases during the decay process. Quantative data on litter and wood decay in tropical forests remain scant (Lodge *et al.*, 1996), but the rates achieved must be enormous as humic layers remain shallow in relation to the volume of material to be processed.

In addition fungi are often ignored as a carbon sink, tying up what must be gigatonnes of carbon in their mycelia, and further by converting rock and soil minerals to oxalates. It is also not always remembered that, in lichen associations, fungi are involved in fixing substantial amounts of carbon dioxide photosynthetically through the included algae or cyanobacteria. Cyanobacteria in lichens have another role too, fixing atmospheric nitrogen, a nutrient often in short supply in rainforests.

(4) . . . a source of novel bioactive compounds

Ever since penicillin hit the headlines in the press in 1942, fungi have been recognized as a source of novel compounds important for human health. The probabilities of finding a drug that will become a world leader are small but the rewards are immense. Leading pharmaceutical companies have been well aware of the potential benefits from screening tropical fungi, and encouraged by successes achieved. Many companies probably hold isolates of undescribed species in their collections as description and identification are not necessary prior to screening. As so many of the fungi yet to be discovered are tropical, it follows that exploitable compounds are likely to be present in tropical species as well as in those from other regions. See Bills *et al.* in this volume.

In addition to providing pharmacologically active compounds, tropical fungi may also be sources of new antifungal agents and enzymes for industrial use, including bioconversions. The full array of uses to which fungi can be put is now immense, and overviews of many aspects are provided in Pointing and Hyde (2001).

Most tropical fungi have yet to be collected and grown in culture, some may never be, and others grow only very slowly. However, this is now less of a constraint to exploitation of useful products since the pertinent genes can be cloned and expressed through bacterial and fungal (especially yeast) species that are easily grown.

The Convention on Biological Diversity is concerned with the equitable sharing of benefits from biodiversity and requires 'prior informed consent' to bioprospecting and screening. Partnerships between tropical countries and pharmaceutical companies are the way forward, and individual countries can do much to facilitate the search and discovery process.

(5) . . . a source of biocontrol agents

The biocontrol of insects and weeds is high on the agendas of developed and developing countries. The prospects of environmentally friendly specific controls that do not require repeated applications are attractive. Tropical insects have long been considered a source of fungal strains for testing against agricultural pests, but more recently attention has focused on weed and even crop pathogens (see Evans, this volume).

Plants originating in particular places where they are a benign normal component of the vegetation can become aggressive weeds if they become established in areas where their usual pest and disease organisms are absent, or rather have not been introduced with them. The endemic sites of weeds are those that have the greatest diversity of associated organisms, including fungi, and where species that have potential as biocontrol agents need to be sought. In the case of weeds originating in particular tropical countries, they may have the key to their control elsewhere. For example, fungi new to science meriting evaluation for the control of *Lantana camara* L. have been discovered in Brazil (Barreto *et al.*, 1995).

(6) . . . a source of plant pathogens

Reasons for studying tropical fungi are not because species are always beneficial. Fungi that can cause diseases in agricultural crops, garden plants, and native species in one country may be present in others. This can be at the species or strain levels.

A recent example pertinent in the UK is the rust of *Bellis perennis* L., which originated in Australia, became established in continental Europe, and has now spread first to cultivated and then to native daisies (Preece *et al.*, 2000). More complex threats can arise when previously isolated fungal species are brought together by human interference and hybridize, posing threats to hosts previously immune from their effects (Newcombe *et al.*, 2000).

Potentially serious for tropical countries are strains of species that could threaten economically important crops, for example the special form of *Fusarium oxysporum* Schlecht that could devastate the oil-palm industry in Malaysia, f.sp. *elaeidis*, should it get into South-East Asia from West Africa (Holliday, 1980).

The risks of pathogens arriving in a new country are accentuated with increased travel and movement of plants and seeds; see Evans, this volume. Tropical and other countries need to take steps to increase their vigilance over material entering the country that could affect wild or cultivated plants. Regrettably, insufficient awareness of the risks invariably results in token controls at national boundaries and other points of entry.

(7) . . . a threat to human health

There is always a need to know the poisonous fungi in an area, to document these, and to ensure public awareness. However, they are only a very small proportion of the species present. Of more widespread concern is the recognition and limitation of mycotoxins in stored foodstuffs in the tropics. The expertise to recognize the species involved needs to be developed; this a prerequisite for action. The extent of concern over the threat of mycotoxins to human health is reflected in the large number of pertinent papers appearing in journals such as the *African Journal of Mycology and Biotechnology*.

The spectrum of human pathogens is greater in tropical than in temperate regions, whether of dermatophytes or species causing serious deep-seated mycoses; see Evans and Ashbee, this volume. There is a need for heightened awareness of species that are endemic in certain tropical regions, such as *Neotestudina rosatii* Segretain & Destombe, which can be detected at early stages when their effects may not be so severe. With a warming climate and increased travel, some fungal problems now mainly associated with tropical countries may become of increasing concern in temperate regions. Medical pathology laboratories need to be aware of what species may be present in particular countries in order to facilitate rapid diagnoses. However, the training of medical mycologists and identification guides for medical laboratories tend to focus on the well-known disease-causing fungi.

(8) . . . able to contribute to sustainable development

Mushrooms for human consumption are perhaps one of the greatest hopes for feeding a spiralling world population (see Härkönen, 2002). Their nutritional values rival those of most vegetables apart from legumes, and, in addition to being low in calories and saturated fatty acids, they are rich in amino acids and vitamins, including some otherwise obtainable only from

animal products. It has been eloquently argued that the devloping world now needs a mushroom-based Non-Green Revolution (Chang, 1999).

Mushroom production can be a household or a factory enterprise, and use lignocellulosic wastes, such as paddy straw and wood chips, which otherwise themselves cause pollution problems.

The use of fungi in biocontrol (see above) also means reduced inputs of pesticides, which can be costly, uncertain in availability, and need repeated applications. A successful biocontrol programme can operate in perpetuity following a successful introduction.

Using fungi for food, biocontrol, and the degradation of wastes are 'planks' of what is currently the drive for 'White Agriculture' in China. Indeed, it is because of these multifarious contributions fungi can make to sustainability that a book on the role of fungi in sustainable development concludes with a cartoon captioned 'No fungi. No future' (Palm and Chapela, 1997).

(9) . . . a part of human culture

Certain mushrooms have long histories as a part of human cultures: in traditional medicine, religion, and art. In China, for example, an amazing array of fungi is used in traditional medicine, some of which are clearly effective even if the pharmacological basis of the effects has not always been clearly elucidated, as is the case for *Ganoderma*-based preparations (Buchanan *et al.*, 1995; Härkönen, 2002).

The involvement of mushrooms with hallucinogenic effects in the development of religions is increasingly being recognized and is deeply rooted, even in western countries, although rarely acknowledged or its extent fully appreciated (Wasson *et al.*, 1986). The religious interest spills over into art and artefacts, in which it is the hallucinogenic species that invariably feature the most strongly. The array of artefacts utilizing the mushroom form is staggering (Taylor-Hawksworth, 2000), although the deep-rooted historical reasons for this empathy are not always recognized.

(10) . . . and there

Scientific curiosity and explorative instincts are a part of human nature. The need to search for new stars, send uncrewed spacecraft to planets and to pass close to occasional comets, characterize ever smaller parts of atoms, map areas where no people live and the floors of oceans, and climb hitherto unconquered peaks is something ingrained in the human pysche and attracts considerable media interest and major funding. So few of the world's fungi are known, even in temperate regions, let alone tropical forests, that there is always the excitement of discovery. When mycologists make a microscope slide

of that small black speck on a decaying stem, or look in detail at the features of a freshly collected mushroom, they can have the joy of seeing an organism no human has ever observed or documented before. It can have novel and even aesthetically beautiful features, or unsuspected combinations of known features, that can affect our current views and understanding of the range of, and relationships between, the organisms with which we share planet Earth.

Now that the larger animals and plants are relatively well-known, the excitement of finding an unknown organism is most likely to be found in the fungi and other microbial groups. The fascination of the new and the unknown is such that the leading biodiversity scientist, sociobiologist and entomologist Edward Wilson, in his autobiography, indicated that, if he had his time again, he would spend it as a microbiologist (Wilson, 1995).

A Personal Prognosis

In 1992 I forecast that, for tropical mycology to progress, the prerequisite organization and planning needed to be in place by 1995 (Hawksworth, 1993). While some progress was made, a sufficiently concerted campaign for particular activities did not materialize, some doors are now closed, and, sadly, opportunities have been missed.

However, the subject has not become as marginalized as feared at that time. The heightened publicity that has been achieved and the general increase in awareness of fungi already referred to have been gratifying. The estimate that there might be as many as 1.5 million fungi on Earth reappears even in the most semi-popular articles and also in radio and television items worldwide. Fungi scarcely even entered the arena of the ecologist and conservation scientist in the late 1980s. In addition, the role of fungi recognized by work undertaken as part of the International Biological Programme (e.g. Kjøller and Struwe, 1982) in the previous decade seemed to be passed over. Further, information being promulgated in the 1980s was often erroneous and not contributed by mycologists. The major change that has occurred since that time is exemplified by the book from the symposium that launched the word ‘biodiversity’ in 1986 (Wilson, 1988). That publication had a mere six index entries on fungi (Wilson, 1988). In contrast, the proceedings of its successor symposial meeting 10 years had not only a whole chapter devoted entirely to fungi, but 28 other references to them, not including mentions of particular taxa (Raven and Williams, 2000). This is a major change mycologists have effected.

Nevertheless, I remain pessimistic over the situation of mycology in developed countries. The number of positions and the standing of organismal biology generally, including mycology, continue to be eroded against a tide of recognition of increased importance. There are currently fewer professional

mycologists now active in the UK than at any time this century; indeed, there is now only one involved in identification and systematic work in the entire UK university system and he will retire this decade.

This situation arises because of historical patterns of funding and differing responsibilities and perspectives of the responsible agencies, departments and other official institutions. Further, there is a preoccupation with reductionist, and thus simplistic, science generally in developed countries, and a lack of appreciation of the value of work on complex systems that cannot be reduced to short-term experiments with few variables. Fortunately, the situation in developed countries is not mirrored in all parts of the world. Several individuals have had major roles in developing schools of mycology in areas where there was previously no or little activity in the field. Amongst these are Drs K.D. Hyde in Hong Kong and N. Hywel-Jones in Thailand, and Profs R.K. Mibey in Nairobi and G. Guzmán in Mexico.

I have been impressed by the number of new mycologists in tropical countries, and by both the quantity and quality of fungal data now being generated from, and projects that are running in, such countries. Encouraged by the International Mycological Association, African, Asian and Latin American associations have all been formed in the last decade, and they now organize their own series of congresses, workshops, directories, and even journals. These groups now need to share their experiences, knowledge and ideas; forging south–south as well as north–south partnerships.

As mycologists we need to face the challenges of asserting our identity, having a clear mission, and taking individual as well as corporate action; this is something we all need to contribute to on a regular basis – 2 h a week spent by each active mycologist equates to numerous full-time campaigners (Hawksworth, 1995). This is a matter for each of us and not only for corporate society-organized action; we should not expect outside help but appreciate any that comes as a bonus, and work both separately and together to realize the potential mycology holds for so many areas of human concern.

If we do have this shared commitment, my prognosis is of one of steady improvement in the tropical situation, but more slowly than ideal and at a lower rate than might have been possible – the rate sadly being hindered by a decreased ability to help from declining numbers of mycologists and mycological institutions in developed countries.

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Key to Tropical Species of *Nectria*-like Fungi

2

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Introduction

Fungi historically identified as *Nectria* are a conspicuous element of the tropical biota. The sexual fruit bodies of these hypocrealean ascomycetes are primarily light- to bright-coloured, uniloculate perithecia that can occur singly on decaying leaves or in masses of bright red or orange aggregations bursting through the bark of recently dead trees. The fungi may colonize the plant substrata in various ways, e.g. via the soil or plant roots, possibly long before perithecia are formed. As invaders, endophytes, plant pathogens, or saprotrophs, they play important ecological roles. In addition to decaying woody and herbaceous debris, *Nectria*-like fungi occur on insects, pyrenomycetous stromata and other ascomycetes, lichens, basidiomycetous fungi and living leaves. These fungi are often encountered as isolations from soil and diseased plant tissue and are manifested *in vitro* in their asexual forms. This chapter consists of a key to the *Nectria*-like fungi frequently encountered in tropical regions.

Until recently, the genus *Nectria* included over 1000 species described over a period of 100 or more years, most of which have not been characterized in the modern sense. These taxa are known to be linked to a diverse array of anamorphs. To understand this large genus, groups of related species were recognized in an informal sense initially by Weese (1916, 1919), followed by Booth (1959), Samuels (1976), and Rossman (1983). These groups were correlated with characteristics of the ascomatal wall morphology and anamorph as well as biological features, particularly of the host. Rossman (1989) circumscribed the genus *Nectria* in a narrow sense to include only one group of

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related species based on the type species, *Nectria cinnabarina* (Tode : Fr.) Fr. and its anamorph, *Tubercularia vulgaris* Tode : Fr. This left many hundreds of orphan species that did not belong in *Nectria* but had not been placed elsewhere.

In a comprehensive account of three major families of the *Hypocreales*, many of these species described in *Nectria* were placed in segregate genera in the two families *Bionectriaceae* and *Nectriaceae* (Rossman *et al.*, 1999). Many of the tropical *Nectria*-like species were placed in segregate genera and are included in this key. Additional *Nectria*-like species are included here that do not belong to the narrowly circumscribed genus *Nectria* and have not been placed in segregate genera. For these species, the generic name is placed in quotes and the affinity with a segregate genus is indicated if known. No new combinations are proposed in this key.

This key to tropical *Nectria*-like fungi is limited to those species belonging to the *Bionectriaceae* and *Nectriaceae* that are relatively common in tropical regions. In general, species that are known only from the type specimen are not included in the key, although there are exceptions especially for distinctive or well-characterized species. The authors have collected primarily in the Neotropics, with brief forays in the Asian and African tropics. Although distribution data are limited at present, it is suspected that many of these species are pantropical. Reference is given to a full description of the species, although often under another generic name as indicated. Users are urged to delve into the literature for similar taxa if a specimen of a *Nectria*-like fungus from the tropics cannot be identified using this key.

Key to Common Tropical Species of *Nectria,* Genera Segregated from *Nectria,* and *Nectria*-like Fungi

Species with yellow-orange, orange, red to purple perithecia, becoming darker in 3% KOH, primarily belonging to the *Nectriaceae*

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Species with white, yellow, orange or brown perithecia, not turning dark in 3% KOH, primarily belonging to the *Bionectriaceae*

1. Ascospores averaging 15 µm or less in length **2**
1. Ascospores averaging more than 15 µm in length **37**
    2. Ascospores averaging 3.5 µm or less in width **3**
    2. Ascospores averaging more than 3.5 µm in width **22**
3. On myxomycetes **4**
3. On diverse substrata but not myxomycetes **5**
    4. Perithecial hairs tapering from base to tip; ascospores 4.5–5.5 × 2–3 µm .... *Nectriopsis exigua* (Pat.) W.Gams (Samuels, 1988a)

4. Perithecial hairs cylindrical, hyphal; ascospores 5.5–7 × 2.5–3.5 µm ....*Nectriopsis oropensoides* (Rehm) Samuels (Samuels, 1988a)

5. On living leaves, sometimes on *Meliolaceae* **6**

5. Lignicolous or fungicolous but not on leaves...**11**

6. Perithecia not on fungi; stiff, thick-walled setae arising from perithecial surface; ascospores 13.5–16.5 × 3–3.5 µm, smooth.......................... *Trichonectria erythroxylifoliae* Samuels (Samuels, 1988a)

6. Perithecia on *Meliolaceae* **7**

7. Ascospores striate, 12.5–16.5 × 3–4 µm ....*Dimerosporiella leucorrhodina* (Mont.) Rossman & Samuels (Samuels, 1988a as *Nectriopsis leucorrhodina*)

7. Ascospores smooth or spinulose **8**

8. Ascospores spinulose, 8–10 × 2.5–3 µm...........*Dimerosporiella guarapiensis* (Speg.) Rossman & Samuels (Samuels, 1988a as *N. guarapiensis*)

8. Ascospores smooth **9**

9. Perithecia glabrous; ascospores 14–16 × 2–3.5 µm....*Dimerosporiella sensitiva* (Rehm) Rossman & Samuels (Samuels, 1988a as *N. sensitiva*)

9. Perithecia with saccate or hyphal hairs arising from the ostiolar region **10**

10. Perithecia with saccate hairs arising from around the ostiolar region; ascospores 9–11 × 3–4 µm...........*Dimerosporiella pipericola* (Henn.) Rossman & Samuels (Samuels, 1988a as *N. pipericola*)

10. Perithecia with thick-walled hyphae arising from around the opening; ascospores 11.5–15.5 × 2.5–4 µm................... *Dimerosporiella cephalosporii* (Hansf.) Rossman & Samuels (Samuels, 1988a as *N. cephalosporii*)

11 (5). On bark or decaying herbaceous debris **12**

11. On *Lachnum* or on other fungi including superficial or immersed pyrenomycetes, *Stereum*, polypores or lichens **16**

12. Perithecia smooth or slightly roughened **13**

12. Perithecia with solitary hyphae or triangular fascicles of hairs arising from perithecial surface **14**

13. Perithecia solitary or in small groups; ascospores 10–12.5 × 2.5–3.5 µm, verruculose, rarely smooth; anamorph *Sesquicillium* sp. ....*Bionectria sesquicillii* (Samuels) Schroers & Samuels (Schroers, 2001)

13. Perithecia on a well-developed, erumpent stroma; ascospores 9.5–11 × 3–3.5 µm, spinulose; conidiophores dimorphic, either verticillium-like or penicillate, *Clonostachys rosea* ....*Bionectria ochroleuca* (Schwein.) Schroers & Samuels (Schroers *et al*., 1999; Schroers, 2001)

14. Perithecia yellow to orange-yellow, covered with white to

pale golden hyphae; ascospores 7.5–10 × 1.5–2.5 µm, smooth........................*Nectriopsis squamulosa* (Ellis) Samuels (Samuels, 1988a)

14. Perithecia orange to orange-brown with solitary to fasciculate hairs **15**

15. Perithecia orange with orange, solitary to fasciculate hairs; ascospores 11–15 × 3–4 µm, smooth ....*Lasionectria sylvana* (Mouton) Rossman & Samuels (Rossman *et al.*, 1999)

15. Perithecia orange to brown-orange with white to brown, fasciculate hairs; ascospores 12.5–17 × 3–4 µm, spinulose .... *Hydropisphaera rufofusca* (Penz. & Sacc.) Rossman (Samuels *et al.*, 1990 as *Nectria brasiliensis*)

16 (11). Ascospores averaging more than 7 µm in length.....**17**

16. Ascospores averaging less than 7 µm in length **20**

17. On *Lachnum*; ascospores 11–20 × 2–3 µm ....*Nectriopsis discophila* (Rogerson & Samuels) Samuels (Samuels, 1988a)

17. On other fungi **18**

18. Perithecia pale orange; ascospores 13–15 × 2–3 µm............ *Nectriopsis ostiolorum* (Berk. & Cooke) Samuels (Samuels, 1988a)

18. Perithecia yellow; ascospores averaging less than 14 µm in length..**19**

19. Ascospores 10.5–13 × 2.5–3 µm ....*Nectriopsis epimyces* Samuels (Samuels, 1988a)

19. Perithecia yellow, with chains of thin-walled, globose cells arising from the perithecial surface; ascospores 6.5–11 × 2–3 µm, smooth to spinulose; on ascomata, lichens or non-fungal substrata................*Nectriopsis mindoensis* (Petr.) Samuels (Samuels, 1988a)

20 (16). Perithecia yellow, smooth; ascospores 5.5–6.5 × 2–2.5 µm, smooth; on perithecia and stromata of *Nectria, Bertia* and *Xylariaceae*..........................*Nectriopsis epimycota* Samuels (Samuels, 1988a)

20. Perithecia white, with hyphal hairs arising from perithecial apex or surface; ascospores smooth to finely spinulose.....**21**

21. Perithecia white, with white, unbranched, septate hairs forming a fringe around the apex; ascospores 5.5–7.5 × 2–2.5 µm, smooth to finely spinulose; on perithecia of *Hypoxylon*, *Nectria* and *Xylaria* ....*Nectriopsis perpusilla* (Mont.) Samuels (Samuels, 1988a)

21. Perithecia white, with spinulose hyphae arising from the perithecial surface; ascospores 5.5–7 × 2.5–3.5 µm, spinulose; on diverse substrata including *Stereum*, polypores and myxomycetes .... *Nectriopsis oropensoides* (Rehm) Samuels (Samuels, 1988a)

22 (2). Ascospores averaging more than 3.5 and less than 5 µm in width..**23**

22. Ascospores averaging 5–6 µm in width **33**

23. On stromata of *Balansia* or immersed ascomycetous structures on grass; ascospores 9.5–11.5 × 3.5–4 µm, smooth to slightly spinulose.............. *Bionectria epichloë* (Speg.) Schroers (Schroers, 2001)

23. On herbaceous or lignified, monocotyledonous or dicotyledonous material including bamboo, lichens and fungi..**24**

24. Ascospores smooth or spinulose **25**

24. Ascospores striate **28**

25. On perithecia of *Neonectria*; perithecia pale yellow; ascospores 9.5–13 × 3.5–4 µm, smooth; anamorph gliocladium-like...................*Nectriopsis byssotecta* (Rehm) Samuels (Samuels, 1988a)

25. On non-fungal substrata **26**

26. Perithecia orange, with large white to off-white warts; warts made of cells that are unevenly thickened on one side; ascospores 11–13 × 3.5–4.5 µm, smooth or spinulose..................... *Bionectria byssicola* (Berk. & Broome) Schroers and Samuels (Schroers *et al.*, 1999)

26. Perithecia smooth, granulose or slightly scaly **27**

27. Perithecia smooth to granulose; ascospores 9.5–17 × 3.5–6 µm, spinulose; anamorph synnematous; synnemata grey to black, forming orange drops of conidia.....................*Stilbocrea gracilipes* (Tul. & C. Tul.) Samuels & Seifert (Rossman *et al.*, 1999)

27. Perithecia smooth to scaly; ascospores 12–16 × 4–5 µm, spinulose; conidiophores sporodochial, dendrodochium-like ....*Bionectria* Samuelsii Schroers (Schroers, 2001) (previously referred to as *Nectria aureofulva* Cooke & Ellis, see Samuels *et al.*, 1990)

28 (24). Perithecia caespitose, sometimes appearing as if immersed in a superficial, hyphal stroma (*Hypocrea*-like); ascospores 13–17 × 4–5 µm, with few striations that extend the full length of the spore ....*Protocreopsis pertusa* (Pat.) Samuels & Rossman (Rossman *et al.*, 1999)

28. Perithecia solitary or caespitose but not immersed within a stroma **29**

29. Ascospores 8.5–10.5 × 3.5–4 µm, striate; perithecia yellow, globose, thin-walled, collapsing by lateral pinching when dry, with spinulose hairs or modified cells around apex of immature fruit bodies................. *Nectriopsis albofulta* (Petch) Samuels (Samuels, 1988a)

29. Ascospores averaging more than 10 µm in length; perithecia not collapsing by lateral pinching..**30**

30. Perithecia pale yellow with conspicuous warts; ascospores 11–13.5 × 4.5–5 µm, striate; anamorph *Clonostachys* sp. (*Bionectria grammicospora* (Ferd. & Winge) Schroers & Samuels (Samuels *et al.*, 1990)

30. Perithecia yellow to orange or brown, smooth to slightly roughened or with fasciculate hairs **31**

31. Perithecia with triangular, fasciculate hairs arising from surface; ascospores 12–17 × 4–5 µm, finely striate ....*Hydropisphaera suffulta* (Berk. & M.A. Curtis) Rossman & Samuels (Samuels *et al*., 1990 as *N. suffulta*)

31. Perithecia glabrous to slightly roughened **32**

32. Perithecia solitary, yellow, on decaying wood or herbaceous debris; ascospores 14.5–17.5 × 4–5 µm, initially smooth but becoming finely to coarsely striate; anamorph synnematous, forming dark greenish conidial masses, myrothecium-like.......................‘*Nectria*’ *chlorogloea* Samuels (Samuels, 1988b)

32. Perithecia solitary or in caespitose clusters, orange to brown, on decaying monocotyledonous leaves; ascospores 14–16 × 3.5–4 µm, smooth or striate; anamorph acremonium-like ....*Hydropisphaera arenula* (Berk. & Broome) Rossman & Samuels (Samuels, 1978 as *N. arenula*)

33 (22). Perithecia completely immersed within an effuse white stroma; ostiolum orange; ascospores 10–14 × 4–6 µm, verrucose; anamorph synnematous, black or hyaline...........*Stilbocrea macrostoma* (Berk. & M.A. Curtis) Höhn. (Rossman *et al*., 1999)

33. Perithecia superficial **34**

34. Ascospores pale green, 13–17 × 5–7 µm; perithecia tan; anamorph *Penicillifer macrosporus*..........................*Viridispora penicilliferi* Samuels & Rossman, *Nectriaceae* (Samuels, 1989b; Rossman *et al*., 1999)

34. Ascospores hyaline; perithecia yellow or orange; anamorphs not *Penicillifer* **35**

35. On non-fungal substrata; perithecia glabrous, globose, collapsing cupulate; ascospores 11–14 × 5–7 µm, striate; anamorph acremonium-like.......................................*Hydropisphaera peziza* (Tode : Fr.) Dumort. (Rossman *et al*., 1999)

35. On ascomycetous fruiting structures or stromata................................**36**

36. On *Xylariaceae*; ascospores coarsely striate, 11.5–14 × 4.5–6 µm ....*Nectriopsis puiggarii* (Speg.) Samuels (Samuels, 1988a)

36. On perithecia of various pyrenomycetes but not *Xylariaceae*; ascospores smooth, 9.5–13.5 × 4–6 µm................*Nectriopsis lasioderma* (Ellis) Samuels (Samuels, 1988a)

37 (1). Ascospores averaging more than 25 µm in length........................**38**

37. Ascospores averaging 15–25 µm in length **59**

38. Ascospores dictyosporous, 48–97 × 10–16 µm; perithecia white to orange, with long, triangular, fasciculate hairs

around the apex; on herbaceous and woody debris...............
Ijuhya dictyospora (Rossman) Rossman & Samuels
(Rossman, 1983 as *N. dictyospora*)

38. Ascospores 1- or more septate, not muriform **39**

39. Ascospores 1-septate **40**

39. Ascospores 2- or more septate **47**

40. On lichens; asci each with four large ascospores (34–50 × 12–18 µm) and four small ascospores (8–17 × 3.5–7 µm)....................................'*Nectria*' *parmeliae* (Berk. & M.A. Curtis) D. Hawksw. (Hawksworth and Booth, 1976 as *N. heterospora*)

40. Ascospores of homogeneous size **41**

41. Ascospores 50–76 × 6.5–9 µm, striate; perithecia immersed in a compact white, hyphal stroma.....*Protocreopsis fusigera* (Berk. & Broome) Doi (Rossman *et al.*, 1999)

41. Ascospores averaging 25–35 µm in length **42**

42. On lichens; perithecia brown-orange, yellow in 3% KOH; ascospores 22–25 × 7–8 µm, smooth ....
'*Nectria*' *lichenophila* Speg.

42. On *Xylaria* or on bark of recently dead trees.....................**43**

43. On stromata of *Xylaria* spp.; perithecia immersed in a minute, yellow, pulvinate stroma; ascospores 28–31 × 4.5–6 µm, smooth......................
'*Hypocreopsis*' *xylariicola* Samuels (Samuels, 1988a)

43. On bark of recently dead trees...**44**

44. Perithecia brown..**45**

44. Perithecia yellow to orange **46**

45. Perithecia brown, red in 3% KOH; ascospores 26–35 × 10–13 µm, striate ....'*Nectria*' (*Neonectria*) *balansae, Nectriaceae* (Samuels and Brayford, 1994)

45. Perithecia brown, associated with a chrome-yellow *Cylindrocarpon* anamorph; ascospores 25.5–31.5 × 8–10 µm, smooth..................
'*Nectria'* (*Neonectria*) *cinnamomea* Brayford & Samuels, *Nectriaceae* (Brayford and Samuels, 1993)

46. Ascospores striate, 26–32.5 × 8.5–10.5 µm; perithecia orange, with orange to white warts; anamorph clonostachys-like .... *Bionectria lucifer* (Samuels) Schroers & Samuels (Samuels 1988b; Schroers, 2001)

46. Ascospores smooth, 24–29 × 8–10 µm; anamorph myrothecium-like ....*Bionectria* pityrodes (Mont.) Schroers (Samuels *et al*, 1990; Schroers, 2001) Mont. (Samuels *et al.*, 1990)

47 (39). Ascospores three septate...**48**

47. Ascospores more than three septate **54**

48. Ascospores conspicuously striate **49**

48. Ascospores smooth or only faintly striate **50**

49. Ascospores 38–55 × 11–13 µm; perithecia yellow to dark yellow, smooth; anamorph *Didymostilbe echinofibrosa* .... *Peethambara spirostriata* (Rossman) Rossman (Rossman *et al*., 1999)

49. Ascospores 24–35 × 7–10 µm; perithecia white, grossly warted, cells of warts unevenly thickened; anamorph *Fusarium decemcellulare*; on diverse substrata including cacao pods .... *Albonectria rigidiuscula* (Berk. & Broome) Rossman & Samuels, *Nectriaceae* (Rossman *et al*., 1999)

50. Ascospores 48–55 × 6–7 µm; perithecia dark orange with orange hairs .... *Hydropisphaera gigantea* (Speg.) Rossman & Samuels (Samuels, 1976 as *N. gigantea*)

50. Ascospores averaging less than 50 µm in length **51**

51. Perithecia white **52**

51. Perithecia orange to brown **53**

52. Ascospores 40–48 × 10–12.5 µm; perithecia white, grossly warted, cells of warts unevenly thickened .... *Albonectria albosuccinea* (Pat.) Rossman & Samuels, *Nectriaceae* Rossman *et al*., 1999)

52. Ascospores 38–41 × 8–11 µm; perithecia smooth................. '*Nectria*' *albida* Rossman (Rossman, 1983)

53. Ascospores 18–29 × 4–6 µm; perithecia orange to brown, smooth to slightly roughened .... *Hydropisphaera erubescens* (Desm.) Rossman & Samuels (Rossman, 1983 as *N. erubescens*)

53. Ascospores 25–38 × 5–7 µm; perithecia brown with brown hairs .... *Hydropisphaera dolichospora* (Penz. & Sacc.) Rossman & Samuels (Samuels *et al*., 1990 as *N. dolichospora*)

54 (47). Perithecia pale yellow to yellow with a bright red papilla; ascospores 56–95 × 6–9 µm, 7–11-septate; on dead culms of bamboo, often at the nodes.......................... '*Nectria*' *rubrostoma* Rossman (Rossman, 1983)

54. Perithecia concolorous **55**

55. Ascospores 11–19-septate, 50–70 × 6–7 µm; perithecia smooth to slightly roughened, orange to umber............ *Hydropisphaera multiloculata* (Samuels) Rossman & Samuels (Rossman, 1983 as *N. multiloculata*)

55. Ascospores 5–9-septate, averaging less than 60 µm in length **56**

56. Perithecia white to orange, with long, triangular, fasciculate hairs around the apex; ascospores 5–7-septate, 30–60 × 4–7 µm; on decaying herbaceous debris .... *Ijuhya peristomialis* (Berk. & Broome) Rossman & Samuels (Rossman *et al*., 1999)

56. Perithecia smooth or with conspicuous warts.................. **57**

57. Ascospores 24–38 × 4–5.5 µm, 7–9-septate; perithecia smooth, with abundant, orange oily droplets in middle wall region .... *Ochronectria*

calami (Henn. & E. Nyman) Rossman & Samuels (Rossman *et al.*, 1999)

57. Ascospores longer than 40 µm; perithecia smooth or warty but without oily droplets **58**

58. Ascospores 40–60 × 5–7 µm, 5–7-septate; perithecia smooth........................'*Nectria*' *pseudopeziza* Rossman (1983)

58. Ascospores 42–62 × 7–9 µm, 5–9-septate; perithecia grossly warted, cells of warts unevenly thickened .... *Albonectria verrucosa* (Pat.) Rossman & Samuels, *Nectriaceae* (Rossman *et al.*, 1999)

59 (37). Ascospores averaging less than 5 µm in width............................**60**

59. Ascospores averaging 6–8.5 µm in width **65**

60. Perithecia densely caespitose, often immersed in a hyphal stroma (*Hypocrea*-like); ascospores striate; typically on monocotyledonous leaves **61**

60. Perithecia solitary or in groups of a few but neither densely caespitose nor forming *Hypocrea*-like stromata..................**62**

61. Perithecia clothed in tan to brown hyphae; ascospores 21–27 × 4–5 µm ....*Protocreopsis foliicola* (Berk. & M.A. Curtis) Samuels (Rossman *et al.*, 1999)

61. Perithecia clothed in white to tan hyphae; ascospores 15–18 × 4–5 µm ... *Protocreopsis javanica* (Höhn.) Rossman & Samuels (Rossman *et al.*, 1999)

62. On meliolaceous fungi on living leaves; ascospores 17–22 × 3–4 µm, striate......................*Dimerosporiella oidioides* (Speg.) Rossman & Samuels (Samuels, 1988a as *Nectriopsis oidioides*)

62. On decaying wood or herbaceous debris **63**

63. Perithecia orange, globose, wall pseudoparenchymatous; ascospores 14.5–17.5 × 3.3–5 µm, striate; anamorph *Septomyrothecium*................. '*Nectria*' *septomyrotheciae* Samuels (Samuels, 1988b)

63. Perithecia white to pale yellow, globose or flattened above, wall hyphal in structure **64**

64. Ascospores 14.5–20 × 3–5 µm, spinulose..........*Ijuhya parilis* (Berk. & Broome) Rossman & Samuels (Samuels, 1988a as *Peristomialis parilis*)

64. Ascospores 21.5–24.5 × 4–5 µm, coarsely striate .... *Ijuhya paraparilis* (Samuels) Rossman & Samuels (Samuels, 1988a as *Peristomialis paraparilis*)

65 (59). Ascospores striate, 16.5–19 × 5.5–7 µm ....'*Nectria*' (*Bionectria*) *subquaternata* Berk. & Broome (Samuels *et al.*, 1990)

65. Ascospores smooth to spinulose or warted..**66**

66. On foliicolous ascomycetes; ascospores 19.5–24.5 × 5.5–6.5 µm, smooth to spinulose..............*Bionectria tonduzii* Speg. (Rossman *et al.*, 1999)

66. On wood or bark **67**

67. Perithecia completely immersed in stroma, stroma appearing *Hypocrea*-like; ascospores 15–22 × 7–10 µm, spinulose............*Stilbocrea impressa* (Mont.) Samuels (Rossman *et al.*, 1999)
67. Perithecia superficial; ascospores 16–33 × 4.5–9.5 µm, verrucose.......... *Bionectria apocyni* (Peck) Schroers & Samuels (Samuels, 1976 as *Nectria apocyni*)

Species with yellow-orange, orange, red to purple perithecia, becoming darker in 3% KOH, primarily belonging to the *Nectriaceae*

1. Perithecia with spinulose, golden, rarely whitish, hairs arising from the surface; ascospores striate..**2**
1. Perithecia glabrous or hairs not spinulose; ascospores smooth, striate, spinulose or tuberculate...**4**
2. Ascospores 24–30 × 7–9 µm; anamorph synnematous........... *Lanatonectria mammiformis* (Chardon) Samuels & Rossman (Rossman *et al.*, 1999)
2. Ascospores less than 20 µm in length; anamorph synnematous or sporodochial...**3**
3. Ascospores 13.5–18 × 4–6 µm; anamorph sporodochial.......*Lanatonectria flavolanata* (Berk. & Broome) Samuels & Rossman (Rossman *et al.*, 1999)
3. Ascospores 10–13 × 3–4.5 µm; anamorph synnematous......*Lanatonectria flocculenta* (Henn. & E. Nyman) Samuels & Rossman (Rossman *et al.*, 1999)
4. Associated with *Chaetopsina* anamorphs **5**
4. Associated with other anamorphs, not *Chaetopsina*...............**9**
5. Ascospores more than 20 µm in length...**6**
5. Ascospores less than 20 µm in length..**7**
6. Ascospores 36–41.5 × 6–7 µm, smooth; anamorph *Chaetopsina* sp. ....*Cosmospora macrochaetopsinae* (Samuels) Rossman & Samuels (Samuels *et al.*, 1990 as *Nectria macrochaetopsinae*)
6. Ascospores 25–42 × 6–10 µm, striate; anamorph *Chaetopsina penicillata*...........*Cosmospora chaetopsinae-penicillatae* (Samuels) Rossman & Samuels (Samuels and Brayford, 1994 as *N. chaetopsinae-penicillatae*)
7. Ascospores 8–9.5 × 2–3 µm, smooth; anamorph *Chaetopsina* cf. *fulva*....... *Cosmospora chaetopsinae* (Samuels) Rossman & Samuels (Samuels, 1985 as *N. chaetopsinae*)
7. Ascospores more than 10 µm in length...**8**
8. Ascospores 11–15 × 3–3.5 µm; anamorph *Chaetopsina polyblastia*, conidiogenous cells proliferating percurrently; conidia 11–15 × 3–3.5 µm, borne in slimy heads ....*Cosmospora*

chaetopsinae-polyblastiae (Samuels) Rossman & Samuels (Samuels, 1985 as *N. chaetopsinae-polyblastiae*)

8. Ascospores 11–13 × 3.5–4.5 µm; anamorph *Chaetopsina catenulata*, conidiogenous cells determinate; conidia 10–19.5 × 1.9–3.5 µm, borne in chains ....*Cosmospora chaetopsinae-catenulatae* (Samuels) Rossman & Samuels (Samuels, 1985 as *N. chaetopsinae-catenulatae*)

9 (4). Ascospores non-septate **10**

9. Ascospores with one septum or more **12**

10. Ascospores 5.5–7 × 1.5–2 µm, smooth; on decaying plant parts of *Agavaceae*.....................*Nectria miltina* (Mont.) Mont. (Rossman *et al*., 1999)

10. Ascospores more than 7 µm in length, smooth or rugose **11**

11. Associated with decaying leaves and fruits of *Clusia*; ascospores 9–11 × 3–4 µm, ellipsoid, smooth; anamorph *Gliocephalotrichum bulbillium*........ *Leuconectria clusiae* (Samuels & Rogerson) Rossman *et al*. (Rossman *et al*., 1993, 1999)

11. Primarily isolated from soil, causing fruit- and root-rots and seedling damping-off; ascospores 10–15.5 × 7.5–12 µm, subglobose, rugose; anamorph acremonium-like .... *Neocosmospora vasinfecta* E.F. Sm. (Rossman *et al*., 1999)

12 (9). Ascospores with more than 1 septum.........................**13**

12. Ascospores with 1 septum...**30**

13. Ascospores dictyosporous, 17–31 × 6–15 µm; anamorph synnematous; perithecia on recently dead or decaying woody substrata............*Nectria pseudotrichia* Berk. & M.A. Curtis (Samuels *et al*., 1990)

13. Ascospores phragmosporous..**14**

14. Ascospores 13–25-septate, 150–270 × 8–12 µm, faintly striate; perithecia solitary, warty; on decaying woody substrata ....*Ophionectria trichospora* (Berk. & Broome) Sacc. (Rossman *et al*., 1999)

14. Ascospores 3–5-septate **15**

15. Ascospores mainly 3-septate..**16**

15. Ascospores mainly 5-septate..**29**

16. Perithecia dark brick-red to dark blue or purple, appearing black *en masse*...**17**

16. Perithecia yellow-orange to red or dark-red **21**

17. On stromata of *Phyllachora* on living leaves; perithecia dark brick-red; ascospores 26–35 × 8–10 µm, smooth..................*Allonectella guaranitica* (Speg.) Rossman (Rossman *et al*., 1999)

17. On decaying herbaceous and woody substrata **18**

18. Ascospores averaging less than 20 µm in length **19**

18. Ascospores averaging more than 20 µm in length **20**

19. Ascospores smooth, 13–18 × 5–8 µm; anamorph *Fusarium lateritium*..........................*Gibberella baccata* (Wallr.) Sacc. (Booth, 1971)

19. Ascospores roughened unequally over upper cell, 15–20 × 5–6.5 µm; anamorph *Fusarium xylarioides*..............*Gibberella xylarioides* R. Heim & Saccas (Booth, 1971)

20. Ascospores 22–27 × 5.5–7 µm, 3-septate; anamorph *Fusarium sambucinum*................*Gibberella pulicaris* (Fr.) Sacc. (Rossman *et al*., 1999)

20. Ascospores 19–24 × 3–4 µm, 0–1-, eventually 3-septate; anamorph *Fusarium graminearum*; primarily on grasses........ *Gibberella zeae* (Schwein.) Petch (Booth, 1971)

21 (16). On scale insects; ascospores 25–35 × 6–8 µm, smooth.................. *Cosmospora diploa* (Berk. & M.A.Curtis) Rossman (Rossman, 1983 as *N. diploa*)

21. On decaying woody or herbaceous substrata or on ascomycetous stromata or rust cankers...**22**

22. On ascomycetous stromata or rust cankers of *Gymnosporangium*...**23**

22. On decaying woody or herbaceous substrata....................**24**

23. On cankers of *Gymnosporangium* on *Cupressaceae*; ascospores 23–32 × 7–10 µm, finely spinulose................‘*Nectria*’ (*Neonectria*) *gymnosporangii* (Jaap) Rossman (Rossman, 1983)

23. On stromata of *Botryosphaeria* and *Valsa* on decaying wood; ascospores 25–39 × 8.5–14 µm, striate..........*Cosmospora diminuta* (Berk.) Rossman & Samuels (Rossman *et al*., 1999)

24. Perithecia with a dark apical disc, smooth; ascospores 50–67 × 10–14 µm, smooth.................‘*Nectria*’ (*Neonectria*) *phaeodisca* Rossman (Samuels and Brayford, 1993)

24. Perithecia smooth, scaly or warted; ostiolar region concolorous **25**

25. Perithecia smooth, apex acute, lacking a disc; ascospores 24–40 × 8–12 µm, smooth.....................*Cosmospora glabra* (Rossman) Rossman & Samuels (Rossman, 1983 as *N. glabra*)

25. Perithecia roughened to warty or scaly with or without a flattened apical disc...**26**

26. Perithecia scaly, with a flattened apical disc; ascospores 42–65 × 7–11 µm, smooth to slightly roughened; anamorph *Cylindrocarpon*......................‘*Nectria*’ (*Neonectria*) *microdisca* Rossman (Rossman, 1983)

26. Perithecia warty, without an apical disc; anamorph synnematous or *Cylindrocladium*...**27**

27. Ascospores 14–20 × 4–6 µm, striate; anamorph synnematous; on recently dead wood...........*Nectria lateritia* (P. Karst.) Rossman (Samuels and Brayford, 1994)

27. Ascospores smooth; anamorph *Cylindrocladium*; on decaying leaves, stems and other herbaceous debris..**28**

28. Perithecia orange-yellow with blood-red base; ascospores 30–70 × 4.5–8 µm, 3-septate; anamorph *Cylindrocladium colhounii*..................*Calonectria colhounii* Peerally (Crous and Wingfield, 1994)

28. Perithecia red to dark red; ascospores 40–70 × 4–7 µm, smooth, 1–3-septate; anamorph *Cylindrocladium ilicicola*....... *Calonectria pyrochroa* (Desm.) Sacc. (Rossman *et al.*, 1999)

29 (15). Perithecia scaly, with a well-developed apical disc, on a well-developed stroma with erect hyphae protruding from the surface; ascospores 65–98 × 8–15 µm; lignicolous.....................'*Nectria*' (*Neonectria*) *fusispora* Rossman (Samuels and Brayford, 1993)

29. Perithecia smooth, lacking an apical disc, not obviously stromatic; ascospores 54–65 × 7–10 µm; on bamboo.........'*Nectria*' (*Neonectria*) *lae tidisca* Rossman (Samuels and Brayford, 1993)

30 (12). Ascospores averaging less than 20 µm in length **31**

30. Ascospores averaging 20 µm or more in length................**59**

31. Ascospores green, 13–17 × 5–7 µm, smooth; anamorph *Penicillifer bipapillatus*.......................*Viridispora alata* (Samuels) Samuels & Rossman (Rossman *et al.*, 1999)

31. Ascospores hyaline or yellow-brown..**32**

32. Perithecia forming on insects, old termite nests or ascomycetous stromata **33**

32. Not obviously associated with insects or ascomycetous stromata...**40**

33. On scale insects or adelgids or old termite nests **34**

33. On stromal surface of ascomycetes or their anamorphs......................**36**

34. On old termite nests; ascospores disarticulating into broadly ellipsoid part-spores, 3–4 × 3–3.5 µm, yellow-brown, densely spinulose.............................*Haematonectria termitum* (Höhn.) Samuels & Rossman (Rossman *et al.*, 1999)

34. On scale insects or adelgids; ascospores not disarticulating...**35**

35. Ascospores broadly fusiform, 12–15 × 5.5–6.5 µm................*Cosmospora aurantiicola* (Berk. & Broome) Rossman & Samuels (Booth, 1981a as *N. aurantiicola*)

35. Ascospores ovoid to ellipsoid, 16–20 × 7.5–10 µm ....*Cosmospora flammea* (Tul. & C. Tul.) Rossman & Samuels (Booth, 1981b as *N. flammea*)

36. On *Parodiella* sp. on living leaves; ascospores 14–19 × 5.5–7 µm, smooth....................*Cosmospora papilionacearum* (Seaver) Rossman & Samuels (Samuels *et al.*, 1991 as *N. papilionacearum*)

36. On diverse ascomycetes **37**

37. Red hyphal hairs arising from the perithecial surface; ascospores 12–13 × 5–7 µm, spinulose to verrucose; on *Cucurbitaria* .... *Cosmospora rubrosetosa* (Samuels) Rossman & Samuels (Samuels *et al.*, 1990 as *Nectria rubrosetosa*)

37. Perithecia glabrous **38**

38. Ascospores 16–17 × 7–9 µm, spinulose; anamorph acremonium-like; on *Pseudovalsa berkeleyi*...........*Cosmospora wegeliana* (Rehm) Rossman & Samuels (Samuels *et al.*, 1991 as *N. wegeliana*)

38. Ascospores less than 16 µm in length **39**

39. Ascospores 8–11 × 4–5.5 µm, tuberculate; anamorph *Acremonium butyri* with green conidia, common ....*Cosmospora vilior* (Starbäck) Rossman & Samuels (Samuels *et al.*, 1990 as *N. vilior*)

39. Ascospores 11–13 × 6–7.5 µm, smooth to finely tuberculate; anamorph acremonium-like.........*Cosmospora pseudepisphaeria* (Samuels) Rossman & Samuels (Samuels *et al.*, 1991 as *N. pseudepisphaeria*)

40 (32). Perithecia completely immersed in a compact stroma; ascospores 15.5–19 × 5–6.5 µm, smooth or striate................ '*Nectria*' *paraguyensis* Speg. (Samuels and Brayford, 1994)

40. Perithecia not completely immersed in a stroma; ascospores striate, smooth or verrucose... **41**

41. Perithecia smooth, often reflecting light, apex darker than the body, sometimes with a flattened disc; cells at the surface of the perithecial wall difficult to discern; anamorph *Cylindrocarpon* **42**

41. Perithecia warted, hirsute or smooth but not glistening; cells at perithecial surface easily discerned; anamorphs *Cylindrocarpon*, *Fusarium* or others.. **43**

42. Ascospores 11.5–17 × 5–7.5 µm, spinulose; cultures purple ....*Neonectria discophora* (Mont.) Mantiri & Samuels (Samuels *et al.*, 1990 as *N. discophora*)

42. Ascospores 12.5–15 × 5.5–6.5 µm, finely spinulose; cultures white to tan..............'*Nectria*' (*Neonectria*) *lucida* Höhn. (Samuels *et al.*, 1990)

43. Perithecia with red hyphal hairs arising from surface; ascospores 7–8.5 × 2–3.5 µm, smooth to spinulose ....*Cosmospora geastroides* (Samuels) Rossman & Samuels (Samuels *et al.*, 1991 as *N. geastroides*)

43. Perithecia glabrous, scaly or warted, without red hyphal hairs; ascospores averaging greater than 8 µm in length **44**

44. Ascospores striate **45**

44. Ascospores smooth, spinulose, verrucose, warted or tuberculate... **49**

45. Ascospores golden-brown, coarsely striate, 10–17 × 5–8 µm; perithecia with greenish warts ....*Rubrinectria olivacea* (Seaver) Rossman & Samuels (Rossman *et al.*, 1999)

45. Ascospores at most pale yellow-brown, striations fine, sometimes difficult to see; perithecia with concolorous reddish warts **46**

46. Cells at the surface of the perithecial wall typically less than 15 µm diam. and their walls typically less than 2 µm thick; perithecia cupulate when dry **47**

46. Cells at the surface of the perithecial wall typically more than 20 µm diam. and their walls typically more than 2 µm thick; perithecia cupulate when dry or not **48**

47. Ascospores 8.5–13.5 × 4–5 µm, smooth, becoming striate; perithecia typically dark red.........*Nectria pseudocinnabarina* Rossman (Samuels and Brayford, 1994)

47. Ascospores 14.5–16.5 × 6.5–8 µm; perithecia typically red-orange........ *Nectria guarapiensis* Speg. (Samuels and Brayford, 1994)

48. Perithecia warted, collapsing by lateral pinching when dry; cells at the perithecial surface angular, 20–25 µm diam.; ascospores yellow-brown, finely striate, 13–16 × 6–8 µm; associated with *Fusarium* anamorph ....*Haematonectria haematococca* (Berk. & Broome) Samuels & Nirenberg (Rossman *et al.*, 1999)

48. Perithecia at most scaly, cupulate when dry; cells at the perithecial surface circular, 15–25 µm diam.; ascospores hyaline, finely but conspicuously striate, 13.5–18 × 4.5–6.5 µm; anamorph *Cylindrocarpon rugulosum*..............*Neonectria rugulosa* (Pat. & Gaillard) Mantiri & Samuels (Samuels and Brayford, 1994 as *Nectria rugulosa*)

49 (44). Ascospores tuberculate, rarely smooth **50**

49. Ascospores smooth, spinulose or striate **55**

50. Perithecia obpyriform with conical papilla; ascospores 8–12 × 4–8 µm ....*Cosmospora obscura* Lowen (Rossman *et al.*, 1999)

50. Perithecia globose to broadly pyriform or obpyriform with flattened apex; ascospores averaging more than 10 µm in length **51**

51. Perithecia obpyriform, sometimes flattened apically, but without an apical disc; solitary or caespitose, often forming laterally and terminally on orange mycelial cords; ascospores 14–21 × 5–9 µm, yellow-brown, tuberculate ....*Corallomycetella repens* (Berk. & M.A. Curtis) Rossman & Samuels (Rossman *et al.*, 1999)

51. Perithecia globose to broadly pyriform, smooth to scaly with a conspicuous apical disc or with concolorous or paler warts; solitary to densely gregarious; ascospores hyaline, smooth, roughened or warted...........**52**

52. Perithecia smooth to scaly with a conspicuous apical disc **53**

52. Perithecia warted with concolorous or paler warts...........**54**

53. Ascospores 15–19.5 × 6.5–8 μm, roughened, rarely smooth; anamorph *Cylindrocarpon candidulum*......................‘*Nectria*’ (*Neonectria*) *veuillotiana* Sacc. & Roum. (Brayford and Samuels, 1993 as *Nectria veuillotiana*)

53. Ascospores 9.5–11 × 3.5–4.5 μm, smooth or warted; anamorph *Cylindrocarpon permirum*......................‘*Nectria*’ (*Neonectria*) *platycephala* Brayford & Samuels (Brayford and Samuels, 1993)

54. Ascospores 11.5–13.5 × 4.5–5.5 μm, warted; anamorph *Cylindrocarpon arcuatum*........‘*Nectria*’ (*Neonectria*) *rubrococca* Brayford & Samuels (Brayford and Samuels, 1993)

54. Ascospores 16.5–19 × 6–7 μm, warted; anamorph *Cylindrocarpon torpidum*.........................‘*Nectria*’ (*Neonectria*) *verrucospora* Brayford & Samuels (Brayford and Samuels, 1993)

55 (49). Perithecia scaly to warted, surface cells distinctive, circular, thin-walled; perithecial wall more than 20 μm in width....................**56**

55. Perithecia smooth, surface cells lacking a distinctive outline; perithecial wall less than 20 μm in width **57**

56. Perithecia with an apical disc; ascospores 15–19.5 × 6.5–8 μm, smooth or warted; anamorph *Cylindrocarpon candidulum*..............‘*Nectria*’ (*Neonectria*) *veuillotiana* Sacc. & Roum. (Brayford and Samuels, 1993 as *Nectria veuillotiana*)

56. Perithecia without an apical disc; ascospores 10–13 × 3–5 μm, smooth; anamorph *Cylindrocarpon destructans*............... *Neonectria radicicola* (Gerlach & Nilsson) Mantiri & Samuels (Samuels and Brayford, 1990 as *Nectria radicicola*)

57. Perithecia densely gregarious on immersed, orange stroma; ascospores 12–14.5 × 5.5–7.5 μm, smooth to minutely spinulose; anamorph acremonium-like ....*Cosmospora meliopsicola* (Henn.) Rossman & Samuels (Samuels *et al.*, 1991 as *Nectria meliopsicola*)

57. Perithecia solitary to aggregated in groups of a few; ascospores 10–12 × 2.5–4 μm, smooth; anamorph *Stilbella* or *Volutella*..........................**58**

58. Anamorph *Volutella*; ascospores 10–11 × 3–4 μm................. *Cosmospora consors* (Ellis & Everh.) Rossman & Samuels (Samuels, 1977 as *N. consors*)

58. Anamorph *Stilbella*; ascospores 10–12 × 2.5–3 μm.............. *Cosmospora stilbellae* (Samuels & Seifert) Rossman & Samuels (Samuels *et al.*, 1991 as *N. stilbellae*)

59 (30). Ascospores less than 25 μm in length **60**

59. Ascospores more than 25 μm in length **65**

60. Ascospores 19–26 × 3.5–4.5 μm; on stromata of *Phyllachora*...................‘*Nectria*’ (*Cosmospora*) *prodigiosa* Syd.

60. Ascospores more than 6 μm in width, not obviously fungicolous **61**

61. Ascospores yellow-brown, smooth, 18–21 × 6.5–7.5 µm; perithecia forming on a rust gall .... '*Nectria*' (*Cosmospora*) *uredinaecola* Pat.

61. Ascospores hyaline, lignicolous **62**

62. Perithecia minute, with conspicuous saccate cells around the apex; ascospores 16–24 × 5.5–8.5 µm, striate, sometimes appearing spinulose or smooth ....*Neonectria coronata* (Penz. & Sacc.) Mantiri & Samuels (Samuels and Brayford, 1994 as *Nectria coronata*)

62. Saccate cells not formed **63**

63. Ascospores smooth to finely punctate, 19.5–24.5 × 7–8.5 µm; perithecia caespitose, red-orange, deeply cupulate when dry ....'*Nectria*' *noackiana* Syd.

63. Ascospores striate; perithecia collapsing laterally pinched or not at all when dry **64**

64. Ascospores 17.5–22 × 6.5–8 µm, finely striate; perithecia smooth, not collapsed when dry; anamorph not known .... '*Nectria*' *seriata* Rehm (Samuels and Brayford, 1994)

64. Ascospores 19–27 × 8–10.5 µm, striate; perithecia conspicuously warted, collapsing laterally pinched or not collapsed when dry; anamorph *Fusarium*......'*Nectria*' (*Haematonectria*) *borneensis* Petr. (Samuels and Brayford, 1994)

65 (59). Perithecia on *Dothideales* on bamboo leaves; ascospores 28–37 × 8–13.5 µm, smooth, pale brown ....*Cosmospora tungurahuana* (Petr.) Rossman & Samuels (Samuels *et al*., 1991 as *Nectria tunguarhuana*)

65. Not on bamboo leaves and not obviously fungicolous **66**

66. Ascospores striate **67**

66. Ascospores smooth or warted **71**

67. Ascospores 35–52 × 11–14 µm, coarsely striate; perithecia with a felty white matrix covering the surface ....*Calostilbe striispora* (Ellis & Everh.) Seaver (Rossman *et al*., 1999)

67. Ascospores averaging less than 35 µm in length **68**

68. Ascospores 26–35 × 10–13 µm, finely striate; perithecia caespitose, immersed in *Hypocrea*-like stroma ....'*Nectria*' *balansae* Speg. (Samuels and Brayford, 1994)

68. Ascospores averaging less than 30 µm in length **69**

69. Perithecia pyriform, dark red, with a knobby, often dark red apex, smooth, often glistening; ascospores 22–29 × 9–10 µm, coarsely striate .... *Neonectria jungneri* (Samuels and Brayford, 1994 as *Nectria jungneri*)

69. Perithecia subglobose, orange-red to red, warted or roughened **70**

70. Ascospores 25–30 × *c*. 10 µm, smooth to finely striate .... '*Nectria*' (*Haematonectria*) *subsequens* Rehm

70. Ascospores 22.5–32 × 9.5–13 µm, smooth to coarsely striate......................'*Nectria*' (*Neonectria*) *pulcherrima* Berk. & Broome (Samuels and Brayford, 1994)

71 (66). Perithecia typically formed at the base of red synnemata, superficial on an erumpent stroma, caespitose, with white to pale yellow furfuraceous coating on the lower third; ascospores 29–35 × 9–11 µm, yellow-brown, smooth .... *Corallomycetella jatrophae* (A. Möller) Rossman & Samuels (Rossman *et al.*, 1999)

71. Ascospores hyaline **72**

72. On decaying leaves and roots; perithecia solitary, concolorous, not collapsed when dry; ascospores 18–48 × 4–7 µm, smooth; anamorph *Cylindrocladium floridanum* .... *Calonectria kyotensis* Terashita (Crous and Wingfield, 1994)

72. On decaying wood; perithecia caespitose, red with a darker red ostiolar area, cupulate or not collapsed when dry; ascospores 28.5–37 × 7.5–9.5 µm anamorph not known .... '*Nectria*' *pseudadelphica* Rehm

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A Reassessment of the Taxonomy of Some Tropical Sooty Moulds

3

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Introduction

The sooty moulds are a group of tropical and sub-tropical fungi, numbering over 200 species, that live on plant surfaces forming dense, dark mats of hyphae so that the leaves appear to be covered with soot-like material. The general term sooty mould was first used by Berkeley and Desmazières (1849) in their detailed study of some members of *Fumago* Pers. on herbarium material, including specimens on coffee leaves from Ceylon, on citrus leaves in the Azores and Madeira, and on citrus species in their own conservatories in France. The term was popularized in US plant pathology literature starting with Weber's paper in 1897. The presence of sooty moulds is often associated with insect infestations of plants, the honeydew serving as a nutrient source for the saprobic community (Hughes, 1976). Included within this community are species which are commercially and agriculturally important. For example, *Caldariomyces fumago* Woron. is used to produce the enzyme chloroperoxidase for industrial purposes (Pickard *et al*., 1991) and '*Capnodium citri*' Pers. is a saprobic association with sucking insect infestations on *Citrus* spp. and a wide variety of ornamental plants (Reynolds, 1999).

To date, the taxonomy of this group of saprobic phylloplane fungi has been very unsatisfactory. The current confusion is caused by some basic problems pertaining to the sooty moulds. They exist as a community on plant surfaces, comprising many different mitosporic and ascosporic states. Their mycelium is uniformly darkly pigmented with melanin, and they share spore dispersal strategies adapted to wet leaf surfaces (Hughes, 1976; Reynolds, 1999). They share many other physical features that are adaptations to the

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phylloplane habitat, and this creates superficial morphological similarities, especially in the mitosporic structures. The morphological characters common to the sooty moulds have been used by workers in the 1800s and 1900s to establish taxonomic groupings, and names given to families of sooty moulds include the *Capnodiaceae* Sacc. and *Chaetothyriaceae* Hans. This has given rise to the 'polymorphic' species concepts that have confused sooty mould taxonomy (McAlpine, 1896). The first descriptions of sooty moulds from herbarium specimens, named as *Fumago* species by Persoon (1822), recorded great variability in morphological structures including the presence of 'small tubes' on the leaf surfaces of several deciduous tree species (Tulasne and Tulasne, 1863; Zopf, 1878). The descriptions of the specimens we now would recognize as *Caldariomyces* were from *Citrus medica* L. leaves from southern Europe. In a recent study of over 270 herbarium specimens of the *Caldariomyces fumago* and *Leptoxyphium graminum* Speg. sooty moulds, over 54% of the collections came from India, Malaysia, Cuba, New Borneo and the Philippines. Of the remaining specimens studied, all but four came from other tropical or sub-tropical regions, and the four collected in temperate regions were actually isolated from hothouse environments or seed, confirming this group to be Mediterranean to tropical in distribution (Olejnik, 1999).

Some of the known mitosporic structures ascribed to members of the sooty moulds are illustrated in Fig. 3.1 and a summary of their interpretation is shown in Fig. 3.2a–f. Figure 3.1a illustrates the cover of dense sooty mould that citrus trees may support. Individual leaves may be partly or completely covered (Fig. 3.1b). The mitosporic structure may be more or less fringed by elongate hairs (Fig. 3.1c,d) and may be tightly or loosely confined (Fig. 3.1d,e). The centrum, the mitospore production site (*sensu* Luttrell in Reynolds, 1981), varies in location between the different species. In *Leptoxyphium, Caldariomyces* (Fig. 3.2a) and *Ciferrioxyphium* Bat. & H. Maia (Fig. 3.2d), the centrum is at the apex of an elongated stipe, although in *Caldariomyces* and *Leptoxyphium* the structure is interpreted as an elongated pycnidium. The mitosporic centrum in *Polychaeton* Pers. (Fig. 3.2c) is at the base or in the mid-section of the stipe, whilst in *Conidiocarpus* Woron. (Fig. 3.2b) the centrum is in the mid-section and the stipe produces percurrent proliferations to form a series of mitosporic structures, again interpreted as pycnidia. The commonest mitosporic centrum seen in *Aithaloderma* H. & P. Syd., shown in Fig. 3.2e, is a stalkless pycnidium. Other species of sooty moulds have long, thin mitosporic structures where the location of the centrum is not characterized by a swelling, e.g. *Scolecoxyphium* Cif. & Bat. (Fig. 3.2f). The differences between these structures are ill-defined and contribute to the taxonomic confusion.

The phylogenetic and subsequent taxonomic relationships between mitosporic and ascosporic species are also confused and incomplete. The ascomata in the sooty mould group are black, ovoid to ellipsoidal or dome-shaped perithecia that may have setae. They contain a type of bitunicate ascus with

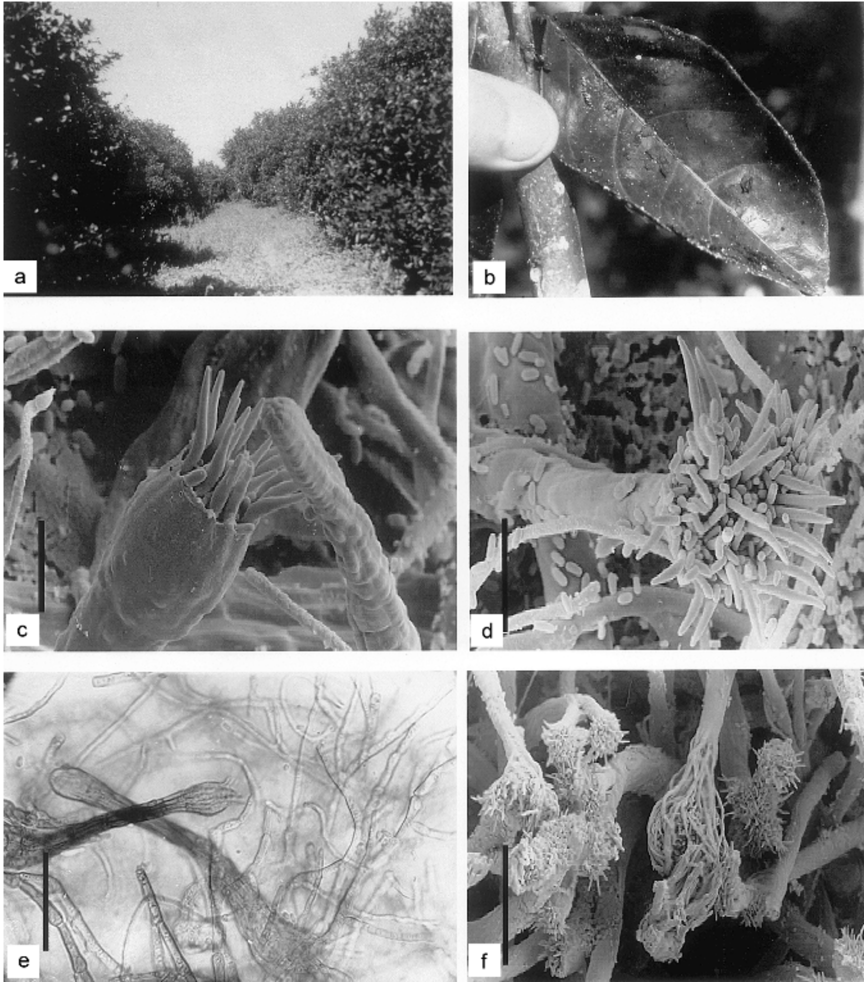

Fig. 3.1. Light and electron micrographs of mitosporic centra of sooty moulds. a, Sooty mould on leaves in a citrus plantation; b, sooty mould on citrus leaf; c, *Leptoxyphium graminum* Jena 886, bar = 10 µm; d, *Leptoxyphium graminum* DAOM 137632, bar = 10 µm; e, *Caldariomyces fumago* ATCC32110, bar = 40 µm; f, *Leptoxyphium graminum* DAOM 149569, bar = 40 µm.

eight hyaline to brown septate spores (Fig. 3.2g,h). Associated with these ascosporic structures are mitosporic structures, some of which are clearly recognizable as pycnidia (e.g. *Aithaloderma*, Fig. 3.2e), whilst others are *Ciferrioxyphium*-like structures (Fig. 3.2d), interpreted as synnemata.

The need to clarify the taxonomy of this group is now pressing. Attempts to improve on the commercial production of chloroperoxidase by screening related strains have proved unpromising. Species of *Scorias* Fr., *Ciferrioxyphium*

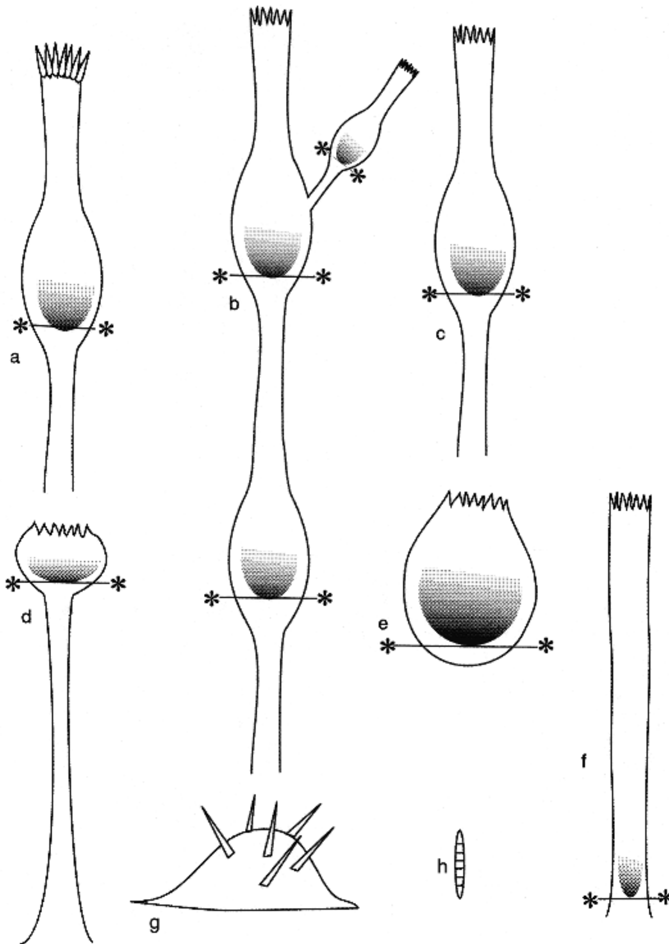

Fig. 3.2. Mitosporic and ascomatic centra of the sooty moulds (after Hughes, 1976). Mitosporic centra: a, *Leptoxyphium/Caldariomyces*; b, *Conidiocarpus*; c, *Polychaeton*; d, *Ciferrioxyphium* (occasional mitosporic state of *Aithaloderma*); e, common mitosporic state of *Aithaloderma*; f, *Scolecoxyphium*; g, ascoma centrum of a sooty mould, *Aithaloderma* sp. with setae (after Hughes, 1976); h, septate ascospore. ∗ — ∗ denotes location of mitotic centrum.

and *Polychaeton* have been tested, but none produced the enzyme (Hashimoto and Pickard, 1984). The results of a taxonomic study of the *Caldariomyces*/*Leptoxyphium*/*Aithaloderma* group are reported here and are integrated into the wider concept of a revised taxonomy of the sooty moulds.

Methods

A total of 272 isolates, including herbarium specimens and living cultures, was studied and used as taxonomic ‘units’. Herbarium specimens were characterized on the basis of micro-morphological data. Living cultures were coded on growth and morphology, substrate utilization and enzyme assays, creating a series of 168 binary variables. Macro- and microscopic structures were also coded for all samples as a series of 34 binary variables. Continuous characters were encoded as a series of additive binary variables. Variable codings have been listed by Olejnik *et al.* (1999).

Principal components analysis, hierarchical clustering, with average linkage as a clustering method, and discriminant analysis were carried out using an SPSS PC package (SPSS Inc., 1993).

Results

Initial principal components analysis for the living isolates failed to show any clear differences between isolates of *Caldariomyces* and *Leptoxyphium* (Olejnik *et al.*, 1999). Accepting the pre-existing identifications, combined data from all herbarium specimens again failed to separate *Leptoxyphium* and *Caldariomyces*. Furthermore, inclusion of the herbarium specimens of the mitosporic *Ciferrioxyphium* and the putative ascosporic state of *Leptoxyphium*, *Aithaloderma* (Hughes, 1976) created three clear groupings: one was a combination of *Leptoxyphium* and *Caldariomyces*, the second contained only *Aithaloderma*, and the third was a mixture of *Leptoxyphium* and *Aithaloderma* isolates (Olejnik *et al.*, 1999).

Re-examination of the herbarium specimens revealed that, of samples identified originally as belonging to the form genera *Caldariomyces* or *Ciferrioxyphium*, over 40% appeared to have been misidentified and should have been placed within the form genera *Polychaeton* or *Conidiocarpus*. Of the herbarium specimens named as *Leptoxyphium*, fewer than 20% had been correctly assigned. Many of the samples misidentified as *Leptoxyphium* had mitosporic structures more closely resembling *Polychaeton* specimens. Re-analysis of the data using principal components analysis, based on more accurate identifications, further strengthened the congeneric nature of *Leptoxyphium* and *Caldariomyces*, and separated off most *Polychaeton* specimens (Fig. 3.3). However, *Conidiocarpus* was less well resolved and further work is needed, especially on *Polychaeton* and *Conidiocarpus* specimens.

A preliminary examination of the herbarium specimens of *Aithaloderma* spp. also revealed a substantial level of misidentification of the ascosporic structures. However, removing the misidentified *Leptoxyphium* and *Caldariomyces* specimens from the analysis and reassigning the mitosporic herbarium specimens to their correct identifications, reinforced the difference between *Aithaloderma* and the *Caldariomyces*/*Leptoxyphium* group (Fig. 3.4).

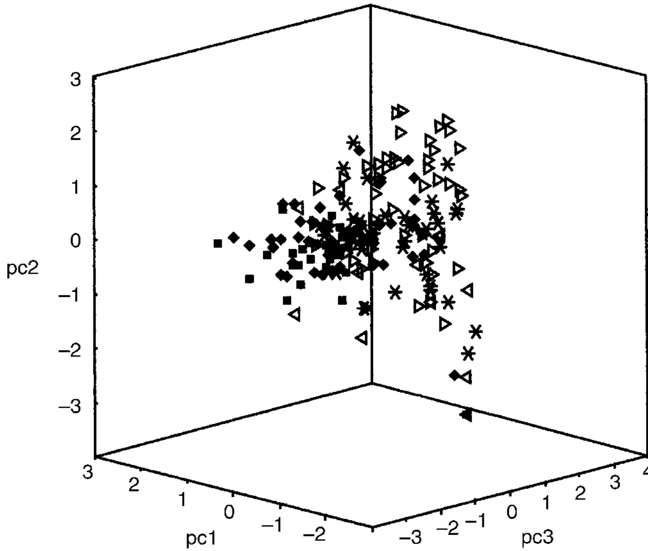

Fig. 3.3. Three-dimensional scatter plot of the first three factor scores provided by a principal components analysis of re-identified herbarium specimens. ◆ *Caldariomyces*; ■ *Leptoxyphium*; ▷ *Polychaeton*; ◁ *Conidiocarpus*; * unknown.

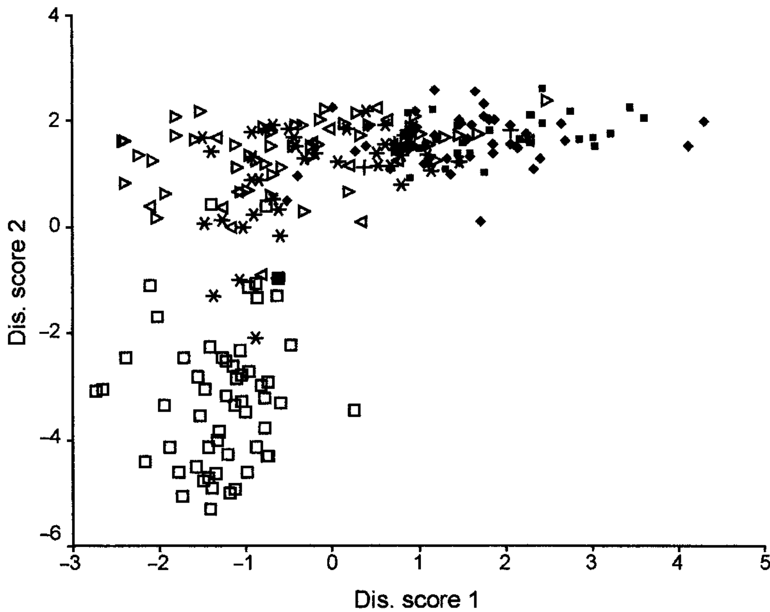

Fig. 3.4. Two-dimensional scatter plot of discriminant scores of 272 samples of *Aithaloderma, Caldariomyces/Leptoxyphium, Polychaeton* and *Conidioxyphium.* ◆ *Caldariomyces*; ■ *Leptoxyphium*; ▷ *Polychaeton*; ◁ *Conidiocarpus*; * unknown; □ *Aithaloderma*; + *Ciferrioxyphium.*

Discussion

These re-analysed results concur with the opinion of Hughes (1976) and Olejnik *et al.* (1999) that *Caldariomyces* and *Leptoxyphium* are poorly differentiated and likely to be congeneric. It is of some concern that there were many misidentifications of the herbarium specimens, particularly among those assigned to *Leptoxyphium*, which have added to the confusion that exists in the taxonomy of the sooty moulds. While it appears that three of the mitosporic taxa, *Leptoxyphium*, *Caldariomyces* and *Ciferrioxyphium*, are congeneric, specimens described as *Polychaeton* or *Conidiocarpus* are extremely variable and need further study.

Hughes (1976) stated that the mitosporic state of *Aithaloderma*, *Ciferrioxyphium*, shares many features with *Leptoxyphium*. However, the mitosporic structure of *Ciferrioxyphium* is termed a synnema with a mitosporic locus at the apex, whereas the mitosporic structures of *Leptoxyphium* are termed elongate pycnidia with a mitosporic locus either at the base or placed sub-apically within the structure. The author's studies strongly suggest that the structure seen in *Caldariomyces*/*Leptoxyphium* is a synnema rather than a pycnidium, in agreement with the work of Roquebert and Bury (1988). Although a semantic argument, defining the mitotic structure of *Caldariomyces*/*Leptoxyphium* as a synnema would strengthen the affiliation of *Aithaloderma* to this group. However, all our analyses to date indicate clear differences between the *Caldariomyces*/*Leptoxyphium* group and the *Aithaloderma* specimens. The latter must remain separated from the mitosporic clades until further research work shows that the two groups can be linked.

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Lignicolous Freshwater Higher Fungi with Reference to their Teleomorph and Anamorph Stages 4

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Introduction

This chapter reviews our knowledge of filamentous, tropical freshwater fungi with special reference to those growing on submerged wood. There is a rich and diverse range of tropical freshwater fungi and the observed mycota depends on the substrata examined. Senescent and fallen leaves support mainly Ingoldian fungi, while woody substrata support a different range of species, with many ascomycetes. These differences also extend to the teleomorph/anamorph relations, with discomycetes forming the majority of the teleomorphs of the Ingoldian fungi. However, teleomorphs of lignicolous fungi are generally pyrenomycetes.

Webster (1992) reviewed the teleomorph/anamorph relationships of Ingoldian fungi, listing 22 *Ascomycota*, five *Basidiomycota* and one tetraradiate conidial zygomycete (*Entomophthorales*). Shearer (1993) also listed the connections for freshwater ascomycetes, which included 33 for Ingoldian fungi, while Wong (1996) compiled and added a further nine. Most of these were reported from temperate regions with only four connections known from tropical zones: *Corynascus sepedonium* (Emmons) v. Arx / *Sepedonium*-like sp.; *Gnomonia papuana* Sivan. & D. Shaw / *Sesquicillium* sp.; *Hymenoscyphus malawiensis* P.J. Fisher & Spooner / unnamed hyphomycete, and *Microascus lunasporus* Jones / *Scopulariopsis lunaspora* Jones (Hyde *et al*., 1997).

Shearer (1989) reported five new species of *Pseudohalonectria* of which *P. phialidica* Shearer is the only species that yields an unnamed hyphomycete in culture. However, this connection was not listed by Shearer (1993). Webster *et al*. (1995) collected and isolated *Tricladium indicum* Sati & Tiwari from a

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foam sample in South Africa and this gave rise to *Cudoniella indica* Webster, Eicker & Spooner (teleomorph) when incubated in sterile water. This is the first reported connection of a *Tricladium* with this teleomorph genus. Furthermore, *T. indicum* developed from cultures of *Cudoniella* ascospores. Hyde and Goh (1998) reported a new connection between *Anthostomella aquatica* K.D. Hyde & Goh and *Geniculosporium sporodochiale* K.D. Hyde & Goh from tropical freshwater habitats, with both stages being found on submerged wood, and also confirmed in culture studies. Sivichai *et al.* (1998b) were the first to link a *Monotosporella* anamorph with *Ascotaiwania sawada* H.S. Chang & S.-Y. Hsieh, while Ranghoo and Hyde (1998) also described a *Monotosporella* anamorph for *Ascotaiwania mitriformis* Ranghoo & K.D. Hyde. Ranghoo and Hyde (1998) described the new genus *Ascolacicola* and linked the type species *Ascolacicola aquatica* Ranghoo & K.D. Hyde with *Trichocladium uniseptatum* Berk. & Broome. In 1998, Descals *et al.* reported two teleomorph links within the genus *Anguillospora*, namely *Pezoloma* sp. (*Leotiales*) as the teleomorph of *Anguillospora furtiva* Webster & Descals and an *Orbilia* sp. (*Leotiales*) as the teleomorph of *A. rosea*.

Goh (1997) noted that, in a number of teleomorph–anamorph connections, many of the anamorphic genera are heterogeneous. Some anamorphic genera have teleomorphs in various genera: e.g. *Tricladium* spp. with teleomorphs in *Cudoniella*, *Hydrocina* and *Hymenoscyphus*. Conversely, some teleomorph genera have a range of anamorph stages: e.g. *Massarina* spp. with anamorphs in *Anguillospora, Clavariopsis* and *Tumularia*.

Many related fungal states found in tropical freshwater habitats are poorly reported while the number of new species of teleomorphs and anamorphs is increasing dramatically. Fifty-six teleomorph/anamorph relationships have been recorded, of which 26 are discomycetes, 13 have been assigned to the *Loculoascomycetes* and 12 belong to unitunicate genera, while five anamorph species have basidiomycete teleomorphs (Sivichai, 2000).

In this survey of the lignicolous freshwater fungi of Thailand, > 236 species were observed (108 ascomycetes, 8 basidiomycetes, 120 anamorphic fungi). Three hundred and fourteen sporulated on cornmeal agar medium (CMA) in a cooled illuminated cabinet and 21 teleomorph–anamorph connections were established.

Materials and Methods

Fungi were isolated from submerged wood collected from freshwater streams at various sites in Thailand, and from four timber species (*Alstonia scholaris* R. Br., *Anisoptera oblonga* Dyer., *Dipterocarpus alatus* Roxb. and *Xylia dolabriformis* Benth.) exposed at Khao Yai National Park. The methods used have been described previously (Sivichai *et al.*, 1998a,b, 2000; Sivichai and Hywel-Jones, 1999).

Results

Of the 236 freshwater fungi collected, 343 different strains were established (from 2400 single-spore isolates), of which 206 sporulated on CMA. Six of these have known connections with four previously reported from terrestrial habitats: *Melanochaeta hemipsila* (Berk. & Broome) E. Müller (Fig. 4.3) with *Sporoschisma saccardoi* Mason & S. Hughes (Goh *et al.*, 1997) (Fig. 4.3); *Nectria chaetopsinae* Samuels with *Chaetopsina fulva* Samuels; *Nectria chaetopsinae-polyblastiae* Samuels with *Chaetopsina polyblastiae* Samuels (Samuels, 1985) and *Tubeufia cylindrothecia* (Seaver) Höhnel with *Helicomyces roseus* Link (Barr, 1980) and two from freshwater habitats: *Ascotaiwania sawada* with a *Monotosporella* anamorph stage (Sivichai *et al.*, 1998b) and *Hymenoscyphus varicosporoides* Tubaki with a *Varicosporium* anamorph stage (Tubaki, 1966).

A further 14 connections were made with teleomorphs in 14 pyrenomycetes, four loculoascomycetes and three discomycetes. Most of the anamorph stages can be referred to typical terrestrial genera, with only two Ingoldian freshwater species. Of the 21 connections listed in Table 4.1, 15 are referred to named taxa, while for four species only one stage can be referred to a named taxon.

Discussion

Webster (1992) listed 27 teleomorph/anamorph connections for freshwater fungi of which 15 (*c.* 55%) had a discomycete teleomorph. These were predominantly from temperate regions and had Ingoldian fungi as the anamorph. Similarly, of the 288 freshwater ascomycetes, predominantly temperate, reported by Shearer (1993), 112 belonged to the discomycetes and 176 were assigned to other ascomycete groups. Teleomorph/anamorph connections were reported for 33 (*c.* 11%) of the 288 species. However, significantly, of these 33, 20 (*c.* 60%) were from discomycetes while 13 (40%) were from other ascomycete groups.

Of the discomycete connections reported from temperate areas (Webster, 1992; Shearer, 1993), only three (*Hymenoscyphus malawiensis*; *Pezoloma rhodocarpa* P.J. Fisher & Spooner and *Cudoniella indica*), have been recorded from tropical regions. Hyde *et al.* (1997) also noted that they found only two discomycetes in 5 years of collecting freshwater lignicolous fungi. This suggests that discomycetes are probably more common in temperate regions or are favoured by lower temperatures for sporulation, and agrees with data from this study showing that discomycetes are not the most frequently isolated group in freshwater habitats in the tropics. In the insect-associated fungi, Evans (1982) noted that by far the greater majority of entomogenous fungal species in tropical forests belong to the genus *Cordyceps* (*Clavicipitales*; *Ascomycotina*) in contrast to temperate habitats where *Entomophthora* species

Table 4.1. Teleomorph/anamorph connections established in this study and means of confirmation.

Connections		Confirmation		
Teleomorph	Anamorph	Ascospores	Conidia	Both
Anthostomella taiwanensis	*Geniculosporium*-like*	+	–	–
Ascotaiwania sawada	*Monotosporella* sp.*	+	–	–
Delitschia sp. 1	*Phomopsis* sp. 1-like	+	–	–
Diaporthe sp. 1	*Phomopsis* sp. 2-like*	+	–	–
Hymenoscyphus varicosporoides	*Tricladium* anamorph of *H. varicosporoides*	+	–	–
Hysterographium sp. 1	*Phomopsis* sp. 3-like	+	–	–
Melanochaeta garethjonesii	*Sporoschisma uniseptatum**	+	–	–
Melanochaeta hemipsila	*Sporoschisma saccardoi*	+	–	–
Microascus sp.1-like	Unidentified hyphomycete*	+	–	–
Nectria chaetopsinae	*Chaetopsina fulva*	+	+	+
Nectria chaetopsinae-polyblastiae	*Chaetopsina polyblastiae*	+	–	–
Nectria sp. 1	*Cylindrocarpon* sp. 1*	+	–	–
Nectria sp. 2	*Chaetopsina* sp. 2*	+	+	+
Oxydothis sp.1-like	*Phialophora* cf. *cyclaminis**	+	–	–
Tubeufia cylindrothecia	*Helicomyces roseus*	+	–	–
Tubeufia sp. 1	*Aquaphila albicans*	+	–	–
Teleomorph unidentified	*Dactylaria* sp. 2*	–	+	–
Teleomorph unidentified	*Ellisembia brachypus**	+	–	–
Teleomorph unidentified	*Phaeoisaria clematidis**	+	+	+
Unidentified discomycete sp. 7	Unidentified hyphomycete	+	–	–
Unidentified discomycete sp. 9	Sporodochial sp. 1	+	–	–

* Teleomorph–anamorph connections reported for the first time.

(*Entomophthorales*; *Zygomycota*) predominate. However, the *Entomophthorales* were also found in the tropics but more frequently in the winter (November to February) and when temperatures are lower, e.g. 12–15°C. This is a topic worthy of further research in the current evaluation of freshwater fungal diversity. Consideration must be given to the mode of dispersal of ascospores. Discomycetes may be better adapted to terrestrial or amphibious/marginal aquatic habitats for ascospore release.

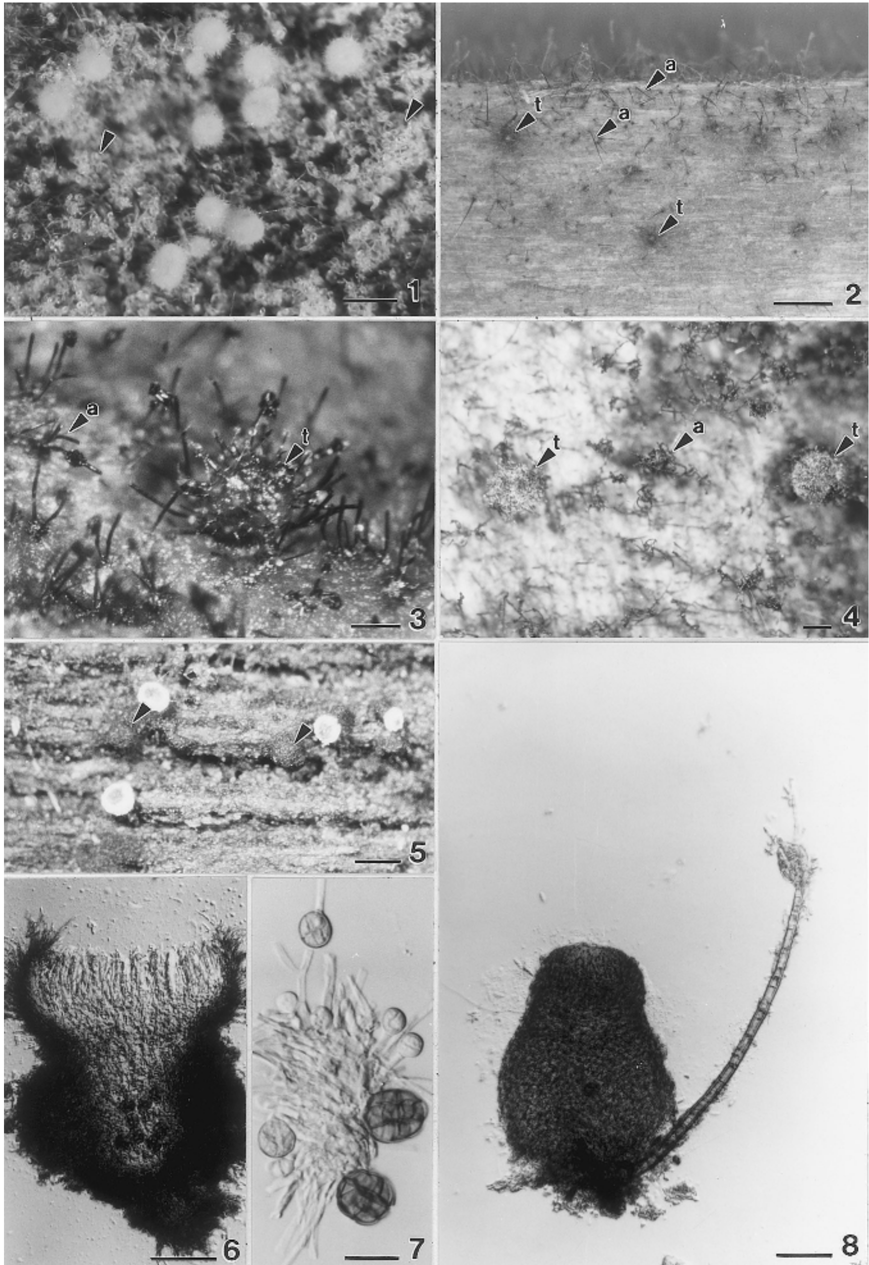
In contrast to the above, most of the teleomorph/anamorph connections in this study involved pyrenomycete ascomycetes (14; 67%), with only four loculoascomycetes (19%) and three discomycetes (14%). The four loculoascomycete connections included: one *Delitschia*, one *Hysterographium* and two *Tubeufia* species. All of these were new connections, apart from *Tubeufia cylindrothecia* (Fig. 4.1), which was already linked with *Helicomyces roseus*. The anamorphs connected with *Delitschia* and *Hysterographium* were the first reports of anamorphs for these genera.

Three discomycete connections were made, two reported for the first time (Figs 4.5–4.7). *Hymenoscyphus varicosporoides* was first reported from Japan (Tubaki, 1966) with a '*Varicosporium*' anamorph. However, Tubaki also indicated that the anamorph stage could be assigned equally well to *Tricladium* or *Polycladium*. Then, Webster *et al*. (1995) established the connection between *Cudoniella indica* with *Tricladium indicum* as the anamorph. The anamorph of *Hymenoscyphus varicosporoides* is also similar to *T. indicum*, but this was not discussed by Webster *et al*. (1995). Thus it is important to establish whether the apical ring of the teleomorph's asci gives a positive or negative reaction with I/KI. Further studies are in progress by the authors to determine the correct assignment of the teleomorph.

Most of the teleomorph/anamorph connections established were with pyrenomycetes and isolations were made from ascospores which yielded the anamorph stage in culture. Of the 21 connections in Table 4.1, many are linked to known anamorphic genera, although a *Microascus* sp. was linked to an unidentified hyphomycete. Of the 11 pyrenomycete connections, only two can be assigned to species level (*Sporoschisma uniseptatum, Phialophora* cf. *cyclaminis*). The others await further study for complete identification. Of the 11 connections, four can be assigned to species level (*Anthostomella taiwanensis, Ascotaiwania sawada, Melanochaeta garethjonesii* (Fig. 4.4), *Phaeoisaria clematidis* (Fig. 4.2)), five to generic level, while two remain to be identified. Future work will be directed to the full identification of these taxa and their description as new taxa.

Only two teleomorph/anamorph connections of Ingoldian hyphomycetes were recorded: *Hymenoscyphus varicosporoides* with a '*Tricladium* anamorph of *H. varicosporoides*' and *Nectria* sp. with a *Cylindrocarpon* sp., with teleomorphs belonging to discomycetes and pyrenomycetes, respectively. The ratio of Ingoldian fungi connections in this study was low (0.09) and this may be due to the examination of woody substrata (as most occur on senescent, fallen leaves) and the temperature used for their incubation (20°C).

In conclusion, the current study has produced many teleomorph/anamorph connections. This can be accounted for by: (i) the diversity of fungi collected, a total of 236 species; (ii) the large number of samples/collections studied (638 herbarium specimens were deposited); (iii) the high number of isolations made (from *c*. 2400 single spore-isolates), of which 206 sporulated in culture; (iv) previous studies of freshwater fungi being in temperate regions, where fungal diversity is lower compared with tropical regions; and (v) substrata being retrieved throughout the year at regular intervals, thus enabling sampling of early to late colonizers. This material was incubated routinely under laboratory conditions for 3 months, and often up to 12 months. Lamore and Goos (1978) showed that species richness of freshwater fungi was higher when collected in the rainy season. Two obvious examples in the present study are *Tubeufia cylindrothecia* and *Helicomyces roseus*. The latter is a common species which occurred over the whole period of the study, but *T. cylindrothecia*



Figs 4.1–4.8. Examples of teleomorph–anamorph connections on test blocks exposed at Khao Yai National Park, Thailand.

Continued

was mostly found in the dry season (April and May) when the water at the 29.2 km test site, in Khao Yai National Park, Thailand, dried out. Previous investigations focused on the isolation of anamorph conidia, whereas, in the present study, most of the connections were obtained from ascospores and nearly 60% of the cultures obtained sporulated, yielding 51% anamorphs, 8% teleomorphs, and 26% teleomorph/anamorphs. It can be argued that discomycete teleomorphs are less frequent in the tropics than in temperate waters, while pyrenomycetes are more frequent.

This study has also shown that teleomorphs usually require prolonged incubation before they develop fully and mature on the substrata. Of the sterile strains obtained, some may be Ingoldian fungi that require aeration, or submergence in water, before they produce conidia. All the strains isolated appeared to be homothallic, thus ensuring sporulation of the ascomycetes (e.g. *Ascotaiwania sawada, Nectria chaetopsinae* and *Nectria* sp. 2 (Fig. 4.8)). However, some of the non-sporulating cultures may be heterothallic and this aspect warrants further study.

Acknowledgements

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Figs 4.1–4.8. *Opposite*

Fig. 4.1. *Tubeufia cylindrothecia* and its anamorph *Helicomyces roseus* with white ascomata and coiled conidia (arrowed). Scale bar = 500 μm.

Fig. 4.2. Unidentified teleomorph and its anamorph *Phaeoisaria clematidis* with colony of the teleomorph (arrowed t) and anamorph (arrowed a). Scale bar = 1000 μm.

Fig. 4.3. *Melanochaeta hemipsila* (arrowed t) and its anamorph *Sporoschisma saccardoi* (arrowed a). Scale bar = 200 μm.

Fig. 4.4. *Melanochaeta garethjonesii* (arrowed t) and its anamorph *Sporoschisma uniseptatum* (arrowed a). Scale bar = 200 μm.

Figs 4.5–4.7. Unidentified discomycete sp. 9 and its anamorph sporodochial sp. 1.

Fig. 4.5. White apothecia of discomycete with discrete masses of brown conidia (arrowed). Scale bar = 500 μm.

Fig. 4.6. Section through an apothecium which developed from an acervulus. Scale bar = 80 μm.

Fig. 4.7. Conidiophores on hyaline hyphae with developing conida. Scale bar = 10 μm.

Fig. 4.8. *Nectria* sp. 2 and its anamorph *Chaetopsina* sp. 2 with a pyriform ascoma and conidiophore. Scale bar = 50 μm.

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The *Pandanaceae* – Does it Have a Diverse and Unique Fungal Biota? 5

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Introduction

During the past 10 years several studies have been undertaken to discover and describe the diversity of microfungi associated with the *Pandanaceae*. This chapter provides a summary of current knowledge and attempts to answer the question of whether or not the *Pandanaceae* supports a diverse and unique fungal biota.

The *Pandanaceae*

The *Pandanaceae* is a monocotyledon family, predominantly tropical but extending as far south as New Zealand, and as far north as Nepal. The family occurs throughout the Pacific, South-East Asia, and South Asia to western Africa. It contains only three genera but 800–900 species: *Freycinetia* Gaud., about 200 species, *Pandanus* Parkins., 600–700 species, and *Sararanga* Hemsl., two species.

Freycinetia species are usually lianes with scrambling or climbing stems and adventitious, adhesive roots clasping around trunks. The leaves are usually linear, rarely over 2 m long, and dead leaves often remain attached. The genus is distributed from Sri Lanka, throughout South-East Asia and Malesia to Taiwan, New Zealand, and the Pacific islands in the east. Towards its geographical limits in the east and south of the Pacific, there is often only

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a single species in each country. For example, in New Zealand there is only *F. baueriana* ssp. *banksii* (Cunn.) Stone, with the other subspecies, *baueriana*, endemic to Norfolk Island. In the Cook Islands, *F. wilderi* Martelli ex. Wilder is the only species, and in Hawaii it is *F. arborea* Gaudich.

Pandanus species or screwpines are usually trees or shrubs with adventitious prop-roots, never clasping but produced from stems and stem bases. The leaves are usually linear, often more than 2 m in length; they may remain attached for a long time following leaf death, and large amounts of dead leaves may accumulate under plants. The genus is characteristically found on the coast, sometimes in thick groves, but extending to montane forests. *Pandanus* is distributed throughout the range of the family but with only 24 species in Africa.

Sararanga species are trees growing to 20 m, producing adventitious prop-roots. The genus, which is typically found in open lowland swamps, is restricted to the Philippines, Papua New Guinea and the Solomon Islands.

The green leaf fibre of *Freycinetia* and *Pandanus* is sometimes used for plaiting into mats, baskets, fans, sails, and garments or, where abundant, for house thatch. Stems and aerial roots are used as close-fitting coverings for implements, for making fish traps, and for tying thatch onto house roofs. Many species have edible flowers or edible leafy flower bracts. Some species of *Pandanus* (e.g. *P. veitchii* Hort., or the widespread maritime species, *P. tectorius* Parkins.) are cultivated for edible fruit or as ornamentals; some of these have variegated or striped leaves.

Fungi described from the *Pandanaceae*

Prior to 1996, 169 species of fungi, mainly ascomycetes and mitosporic fungi (Table 5.1), had been described from the *Pandanaceae* (McKenzie and Hyde, 1996). However, there had been no systematic study of fungi on this family of plants. Subsequently, a further 18 ascomycetes and 14 mitosporic fungi have been described on *Pandanus*, and three ascomycetes and one hyphomycete on *Freycinetia* (Table 5.2).

Table 5.1. Number of fungi described from the *Pandanaceae*, prior to McKenzie and Hyde (1996).

	Number of fungal species	
	Freycinetia	*Pandanus*
Ascomycetes	15	59
Basidiomycetes	4	15
Hyphomycetes	13	25
Coelomycetes	3	35
Total	35	134

Table 5.2. Fungi described from the *Pandanaceae* since McKenzie and Hyde (1996).

Ascomycetes on *Pandanus*

Anthostomella minutoides K.D. Hyde, *Nova Hedwigia* 62, 315 (1996) – Java.
A. petrinensis Dulym., P.F. Cannon & Peerally, *Mycological Research* 102, 1319 (1998) – Mauritius.
A. theobromina Dulym., P.F. Cannon & Peerally, *Mycological Research* 102, 1322 (1998) – Mauritius.
Astrocystis cepiformis Dulym., P.F. Cannon & Peerally, *Mycological Research* 102, 1328 (1998) – Mauritius.
A. fimbriata Dulym., P.F. Cannon & Peerally, *Mycological Research* 102, 1326 (1998) – Mauritius.
A. rarissima Dulym., P.F. Cannon & Peerally, *Mycological Research* 102, 1327 (1998) – Mauritius.
Fasciatispora pandanicola K.D. Hyde, *Nova Hedwigia* 61, 261 (1995) – Java.
Linocarpon appendisporum K.D. Hyde, *Botanical Journal of the Linnean Society* 123, 116 (1997) – Irian Jaya.
L. breve K.D. Hyde, *Botanical Journal of the Linnean Society* 123, 119 (1997) – Irian Jaya.
L. falciformisporum K.D. Hyde, *Botanical Journal of the Linnean Society* 123, 123 (1997) – Irian Jaya.
L. fasciatum Dulym., P.F. Cannon & Peerally, *Mycological Research* 102, 1332 (1998) – Mauritius.
L. pandanicola K.D. Hyde, *Botanical Journal of the Linnean Society* 123, 129 (1997) – Irian Jaya.
L. spathulatum Dulym., P.F. Cannon & Peerally, *Mycological Research* 102, 1333 (1998) – Mauritius.
L. sulcatum Dulym., P.F. Cannon & Peerally, *Mycological Research* 102, 1336 (1998) – Mauritius.
Meliola kapoorii Hosag. & Raghu, *Meliolales of India,* 229 (1996) – India.
M. pandanacearum Hosag. & T.K. Abraham, *Indian Phytopathology* 51, 303 (1999) – India.
Nipicola pandani K.D. Hyde, *Nova Hedwigia* 63, 419 (1996) – Hong Kong.
Stictis pandani Whitton, K.D. Hyde & McKenzie, *Fungal Diversity* 2, 172 (1999) – Australia.

Hyphomycetes on *Pandanus*

Acrodictys lamma Whitton, McKenzie & K.D. Hyde, *Fungal Diversity* 4, 163 (2000) – Hong Kong.
A. triarmatus Whitton, McKenzie & K.D. Hyde, *Fungal Diversity* 4, 166 (2000) – Mauritius.
Bahusutrabeeja dubhashii Bhat, *Indian Journal of Forestry* 17, 129 (1994) – India.
Cryptophiale pandanicola Dulym., P.M. Kirk & Peerally, *Mycotaxon* 73, 313 (1999) – Mauritius.
Dictyochaeta fimbriaspora Whitton, McKenzie & K.D. Hyde, *Fungal Diversity* 4, 138 (2000) – Philippines.
D. microcylindrospora Whitton, McKenzie & K.D. Hyde, *Fungal Diversity* 4, 141 (2000) – Hong Kong.

Continued

Table 5.2. *Continued*

D. multisetula Whitton, McKenzie & K.D. Hyde, *Fungal Diversity* 4, 143 (2000) – Australia.
D. seychellensa Whitton, McKenzie & K.D. Hyde, *Fungal Diversity* 4, 148 (2000) – Seychelles.
Fuscophialis suttonii Dulym., W.P. Wu & Peerally, *Mycoscience* 39, 285 (1998) – Mauritius.
Paraceratocladium triseptata Dulym., W.P. Wu & Peerally, *Mycoscience* 39, 288 (1998) – Mauritius.
Spadicoides mauritiana Dulym., P.M. Kirk & Peerally, *Mycotaxon* 73, 319 (1999) – Mauritius.
Sporidesmium paradecorosum Dulym., W.P. Wu & Peerally, *Mycoscience* 39, 290 (1998) – Mauritius.
Troposporopsis atroapicis Whitton, McKenzie & K.D. Hyde, *Fungal Diversity* 3, 176 (1999) – Hong Kong (+ Australia, Mauritius, Philippines).

Coelomycete on *Pandanus*
Rubikia splendida Dulym., Minter & Peerally, *Mycological Research* 102, 1242 (1998) – Mauritius.

Ascomycetes on *Freycinetia*
Anthostomella kapiti Whitton, K.D. Hyde & McKenzie, in Lu & Hyde, *A World Monograph of Anthostomella,* 102 (2000) – New Zealand.
A. manawatu Whitton, K.D. Hyde & McKenzie, in Lu & Hyde, *A World Monograph of Anthostomella*, 119 (2000) – New Zealand.
A. okatina Whitton, K.D. Hyde & McKenzie, in Lu & Hyde, *A World Monograph of Anthostomella,* 138 (2000) – New Zealand.

Hyphomycete on *Freycinetia*
Dictyochaeta renispora Whitton, McKenzie & K.D. Hyde, *Fungal Diversity* 4, 146 (2000) – Philippines (+ Australia, Brunei).

McKenzie (1991a–c, 1995) and McKenzie and Kuthubutheen (1993) carried out an intensive study of hyphomycetes associated with 12 species of *Freycinetia* from 11 countries. Close attention was paid to *F. baueriana* ssp. *banksii* in New Zealand, with 39 collections examined for hyphomycetes. Dulymamode *et al.* (1998a–e, 1999) have recently described several new species of ascomycetes and mitosporic fungi, found in Mauritius, on one or other of the 14 endemic species of *Pandanus*. Whitton (1999) studied microfungi associated with members of the *Pandanaceae*. He examined nine species of *Freycinetia*, 23 species of *Pandanus*, and one species of *Sararanga* from 11 countries. Some new species have been described from this study (Whitton *et al.*, 1999a,b, 2000a,b; Lu and Hyde, 2000).

If the purported ratio of 5.7 species of fungi for every vascular plant species is correct (Hawksworth, 1991), then the *Pandanaceae* should support 5000 or so unique species of fungi. Is this possible? The family does not

form ectomycorrhizas, and monocotyledonous plants, in general, support few basidiomycetes. Obvious pathogenic associations appear to be rare; there are relatively few leaf spots, and only two rusts are known on the *Pandanaceae* (McKenzie and Hyde, 1997). Endophytic fungi associated with the *Pandanaceae* have not been studied. Of the 205 fungal species described to date on members of the *Pandanaceae*, less than 10% are macroscopic. Thus, most of the known mycota on the *Pandanaceae* is microscopic and saprotrophic, and it is found mainly on dead leaves, or sometimes on dead 'bark'. Approximately 450 species of fungi are now known to occur on the *Pandanaceae* (Whitton, 1999). How many of these species are unique to the *Pandanaceae* or are restricted to only one or a few species of *Freycinetia* or *Pandanus*? As soon as a fungal species is found on a second member of the *Pandanaceae*, or on a different host substratum, then it can count as only 0.5 of a fungus towards the 5.7 figure of Hawksworth (1991). While many of the microfungi may be restricted to the *Pandanaceae*, it is hard to imagine that they would be restricted to only a single species of *Freycinetia* or *Pandanus*.

How many species of fungi are unique to this plant family? Matsushima, for instance, recorded five hyphomycetes on *Pandanus tectorius* from Taiwan (Matsushima, 1980) and one from Japan (Matsushima, 1987), and two on *Pandanus* sp. from Australia (Matsushima, 1989). None of these species are restricted to *Pandanus*, and some (*Bipolaris australiensis* (M.B. Ellis) Tsuda & Ueyama, *B. hawaiiensis* (M.B. Ellis) J.Y. Uchida & Aragaki, *Curvularia lunata* (Wakker) Boedijn, and *Nigrospora sphaerica* (Sacc.) E.W. Mason) are common and widespread on a broad range of substrata.

Fungi on *Freycinetia baueriana* ssp. *banksii* in New Zealand, a Temperate Area

In New Zealand, the only representative of the *Pandanaceae* is the endemic *Freycinetia baueriana* ssp. *banksii*. Approximately 150 species of fungi, of which about 75 are ascomycetes and 65 are hyphomycetes, have been collected on this plant. Approximately two-thirds of these have been determined to species (53 ascomycetes, 10 basidiomycetes, 37 hyphomycetes). *F. baueriana* ssp. *banksii* is the host plant from which 14 fungal species have been described. Of these, five species are known from only the type collections (*Anthostomella manawatu* Whitton, K.D. Hyde & McKenzie, *A. okatina* Whitton, K.D. Hyde & McKenzie, *Crepidotus parietalis* E. Horak, *Odontia flexibilis* G. Cunn., *Puccinia freycinetiae* McKenzie). Only three species occurring in more than one collection (*Anthostomella kapiti* Whitton, K.D. Hyde & McKenzie – 3 specimens, *Chalarodes bisetis* McKenzie – 11 specimens, and *Stachybotrys freycinetiae* McKenzie – 35 specimens) have been found exclusively on *F. baueriana* ssp. *banksii*. Two species, *Chaetosphaeria aotearoae* S. Hughes (*Melanochaeta aotearoae* (S. Hughes) E. Müll., Harr & Sulmont) and *Stictis subiculata*

P.R. Johnst., are known on other hosts in New Zealand and elsewhere. *S. subiculata* also occurs on *Pandanus* in Australia, while the anamorph of *M. aotearoae* is the widespread *Sporoschisma mirabile* Berk. & Broome. Two species described from *F. baueriana* ssp. *banksii* also occur on other species of *Freycinetia* in some Pacific island countries (*Stachybotrys breviuscula* McKenzie – New Caledonia, and *Zebrospora bicolor* McKenzie – Cook Islands, New Caledonia, Samoa), while *Guedea novaezelandiae* S. Hughes occurs on *Pandanus* in Brunei, and *Nectria freycinetiae* Samuels on *Pandanus* in Hong Kong. One other fungus, *Coronospora novaezelandiae* Matsush., which is commonly found on *F. baueriana* ssp. *banksii* (31 collections), was described by Matsushima (1985) from a single collection on the New Zealand nikau palm (*Rhopalostylis sapida* H. Wend. & Drude). Of the other fungi found on *F. baueriana* ssp. *banksii* and determined to species level, three were described elsewhere on the *Pandanaceae* – *Clypeosphaeria stevensii* Syd. was described from *Freycinetia* sp. in Hawaii, *Echidnodes pandani* (Rostr.) Hansf. (*Asterina pandani* Rostr.) from *Pandanus* sp. in Thailand, and *Linocarpon pandani* (Syd. & P. Syd.) Syd. & P. Syd. (*Linospora pandani* Syd. & P. Syd.) in the Philippines. Another seven species of ascomycetes and five species of hyphomycetes are awaiting description as new species. The other approximately 125 species occur on a broad range of plants, either in New Zealand or overseas. Thus, ten described species of fungi are known so far to be restricted to *F. baueriana* ssp. *banksii*, along with 12 as yet undescribed species. It is probable that more intensive collecting will lead to the discovery of some of these fungi on other plant species, or perhaps on other species of the *Pandanaceae* in other countries. Detailed examination of fungi collected on *F. baueriana* ssp. *banksii*, but determined only to genus, may also reveal new species. However, the situation in New Zealand is perhaps unique. New Zealand is very isolated and it has only a single member of the *Pandanaceae*. It could be expected that this isolation has led to the evolution of fungi which are restricted to this one species of *Freycinetia*.

Fungi on *Pandanaceae* in the Tropics

In a further study of hyphomycetes on *Freycinetia*, McKenzie (unpublished data) examined 26 collections of *Freycinetia* from Indonesia, Malaysia, and nine Pacific island countries. He found 64 genera of hyphomycetes; 23 of these had not been recorded on *F. baueriana* ssp. *banksii* in New Zealand, but only eight of the genera were unknown in New Zealand. It is, perhaps, notable that *Chalarodes bisetis*, *Coronospora novaezelandiae*, and *Stachybotrys freycinetiae*, three species which are commonly found in New Zealand on *Freycinetia*, were not found in collections from these tropical countries. Conversely, *Chalarodes obconica* McKenzie, *Stachybotrys nephrodes* McKenzie, *S. parvispora* S. Hughes and *Sporidesmium freycinetiae* McKenzie are widespread in the tropical Pacific,

but were not found in New Zealand. Two fungi, *Stachybotrys breviuscula* and *Zebrospora bicolor*, were found both in New Zealand and elsewhere.

Whitton (1999) made a widespread study of microfungi on the *Pandanaceae*, also including ascomycetes. He found 225 species of fungi (149 mitosporic in 78 genera, and 76 ascomycetes in 27 genera) on 33 species of the *Pandanaceae*, an average of 6.8 species of fungi per species of host plant. He found five new genera of mitosporic fungi (*Dichotophora*, *Nakatopsis*, *Ramocapitis*, *Sporotretophora* and *Troposporopsis* (Whitton, 1999; Whitton *et al.*, 1999b)), two new ascomycete genera (*Callerascus* and *Flexuoniesslia*), and 54 new species of fungi. Thus, about 25% of all species identified were new to science, and there was a ratio of 1.6 new species per host species studied.

There are published records of at least 65 species of mitosporic fungi on *Freycinetia* spp. (Whitton, 1999). Of these, 17 species have been described with *Freycinetia* as the type substratum, a further ten are putatively new species (Whitton, 1999), and 38 were originally described from other substrata, including one described from *Pandanus*. Of the 27 species described or currently awaiting description from *Freycinetia*, eight are known from more than one species of *Pandanaceae*, leaving 19 so-called 'unique species'. Similarly, 61 ascomycetes have been recorded on *Freycinetia*, 18 with *Freycinetia* as the type substratum, eight putatively new species, and 35 described from other substrata, including four described from *Pandanus*. Of the 26 species described or to be described from *Freycinetia*, eight are known from more than one species of *Pandanaceae*, leaving 18 'unique species' (Table 5.3).

Table 5.3. Ascomycetes and mitosporic fungi recorded from the *Pandanaceae*.

	Total no. of species	Type on *Pandanaceae*	Type on other plant species	Undescribed species	'Unique' species*
On *Freycinetia*					
Mitosporic	65	17	38	10	19
Ascomycetes	61	18	35	8	18
On *Pandanus*					
Mitosporic	198	66	106	26	76
Ascomycetes	118	70	35	13	61
On *Sararanga*					
Mitosporic	5	0	4	1	1
Ascomycetes	2	0	2	0	0
Total	449	171	220	58	175

* Those species known only on the *Pandanaceae*, and on only one member (species) of the *Pandanaceae*.

Corresponding figures for mitosporic fungi on *Pandanus* are: 198 species known on *Pandanus*, 66 described from *Pandanus*, 26 putatively new species, and 106 described from other substrata. Of the 92 species so far described or to be described from *Pandanus*, 16 are known from more than one species of *Pandanaceae*, leaving 76 'unique species'. For ascomycetes on *Pandanus*: 118 species are known, 70 have been described from *Pandanus*, 13 are putatively new species, and 35 were described from other substrata (including two described on *Freycinetia*). Of the 83 species described or to be described from *Pandanus*, 22 are known from more than one species of *Pandanaceae*, leaving 61 'unique species'.

Only two species of ascomycetes and five mitosporic fungi have been recorded on *Sararanga*, all from the Philippines. The two ascomycetes were originally described from palms in Australia. Four of the mitosporic fungi were described from other substrata, but one species, *Cryptophiale hamulata* ined., is to be described as a new species, presently unique to *Sararanga*.

The diversity of fungi known from *Freycinetia* appears to differ from that inhabiting *Pandanus*. Of the 133 species of fungi known from *Freycinetia* (Table 5.4), 44 species are known also to inhabit *Pandanus*, giving a species composition overlap of 33%. Of the overlapping species, 36 are known from other substrata besides the *Pandanaceae*, suggesting that they are the less fastidious, more ubiquitous species. It can only be surmised whether the apparent difference in fungal species colonizing *Freycinetia* is due to morphological, ecological, or geographical differences. Differences in distribution of *Freycinetia* and *Pandanus* exist, but to a large extent wherever *Freycinetia* is found so is *Pandanus*. It is unlikely, therefore, that geography plays a major role in fungal species composition differences between *Freycinetia* and *Pandanus*. Morphologically there are some differences; generally the leaves of *Freycinetia* are smaller than those of *Pandanus*, and the leaves of the latter are often in contact with the soil and with other decaying plant litter.

Two species of *Pandanus* (*P. furcatus* and *P. tectorius*) occur in Hong Kong. Whitton (1999) found a difference in overall fungal species composition

Table 5.4. Number of fungal species and myxomycetes published as occurring on the *Pandanaceae*.

	Freycinetia	*Pandanus*	*Sararanga*	Overlap between *Freycinetia* and *Pandanus*
Ascomycetes	61	118	2	20
Basidiomycetes	6	17	0	0
Mitosporic fungi	65	198	5	23
Oomycetes	1	1	0	1
Myxomycetes	0	2	0	0
Total	133	336	7	44

between these two species of *Pandanus*. *P. furcatus* had 45 species of microfungi associated with its decaying plant parts, whilst *P. tectorius* had 35 species, with only ten species in common. Reasons for the apparent disparity in species composition between these two host substrata include:

1. Morphological differences – *P. furcatus* produces long leaves, and has a relatively thin cuticle and no trunk or branches. The leaves typically remain attached for some time following senescence, and are often in contact with the soil as soon as they die, if not before. *P. tectorius* has a distinct trunk, many branches, and much shorter leaves with a thicker cuticle. Because of the short leaves and trunk-forming habit, the leaves, which also remain attached for some time following senescence, typically do not come in direct contact with the soil until they fall from the plant.
2. Environmental differences – *P. furcatus* is restricted to southern China, and is typically found along the edges of streams in areas covered by forest. It tolerates low light levels and encounters high humidity, better developed soils, very little direct wind, and no salt spray. *P. tectorius* is found along coastlines throughout South-East Asia, southern China, northern Australia, the Pacific, and Japan. It is subjected to direct sunlight, salt spray, coastal storm and tidal events, desiccation from coastal winds, and shallow, poorly developed soils.
3. Ecological/varietal differences – *P. furcatus* shows very little morphological variation throughout its geographical range, whereas *P. tectorius* is divided into many varieties based on morphological variations (Stone, 1982, 1988).

When fungi known on *P. furcatus* and on *P. tectorius* from other parts of the world are combined with those known on these hosts in Hong Kong, there was still a difference in the number of fungal species recorded on these two hosts, and there was still only a small number of overlapping species. However, *P. tectorius* now has the higher number of fungal species – 88 species, compared to 45 on *P. furcatus*, with only 15 species in common. The higher number of species recorded on *P. tectorius* presumably reflects its broader geographical range, with more samples having been investigated, and the morphological/ varietal differences in this species.

Of the 35 species of fungi known from *P. tectorius* in Hong Kong, and the 61 species reported from this host in other parts of the world, there is an overlap of only eight species. More collections from the whole geographical (and varietal) range of *P. tectorius* are needed to ascertain whether or not the difference in fungal diversity is a true phenomenon, or if it is due to insufficient sampling.

Conclusions

The *Pandanaceae* certainly supports a diverse fungal biota, with approximately 450 species known on this family. However, less than 40% of these fungi are 'unique' to a single member of the *Pandanaceae*, and with time it is likely that

many of these will be found on other members of the *Pandanaceae*, or on other host families. The 175 'unique' fungi provide a ratio of only 0.2 species of fungi per host species, a long way short of the 5000 species which would be required by the 5.7:1 ratio suggested by Hawksworth (1991) in a calculation of possible world fungal diversity. However, every collection taken during the investigation by Whitton (1999) revealed at least some fungi that previously had not been known from the *Pandanaceae*. This suggests that the species accumulation curve has not flattened out and, therefore, indicates that current knowledge of microfungi on the *Pandanaceae* is incomplete. While the likelihood of any one type of substratum being a true indicator of overall biodiversity is slim, and an integrated approach using various different substrata and habitats is perhaps preferable (Hawksworth *et al.*, 1997), there is still a challenge to find, delimit, and describe the as yet undiscovered fungal treasures associated with the *Pandanaceae*.

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Aspects of Graminicolous Downy Mildew Biology: Perspectives for Tropical Plant Pathology and Peronosporomycetes Phylogeny 6

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Introduction

The *Peronosporomycetes* (*Oomycetes*) include important tropical plant pathogens. Several genera have a profound effect on local and regional economies due to their effect on gramineous crops. The graminicolous downy mildews (GDMs) cause extensive losses to tropical crops such as maize, sorghum, pearl millet and sugar cane (Shaw, 1981 and references therein). Knowledge of their biology and taxonomy is essential for the control of these pathogens within agricultural systems. This chapter will review aspects of tropical GDM biology, and consider their probable phylogenetic position and importance in tropical mycology and plant pathology. Compilation of data for this review is constrained by deficiencies in both host and pathogen nomenclature; thus difficulties occur when evaluating the identity of potential hosts, the geographic ranges of the pathogens and whether the pathogens affect hosts throughout their natural and cultivated distributions. The host family *Poaceae* has a complicated taxonomic and nomenclatural history, resulting in a proliferation of binomials, many of which are illegitimate. Host binomials have been corrected to current usage in this chapter. Many of the pathological data have been derived from crop plants. This has resulted in a serious imbalance of information on disease incidence. The frequent omission of epidemiological data (as opposed to occurrence) from wild-type infections is lamentable. Renfro and Bhat (1981) commented on this and the situation has not improved since then. Such data could have considerable impact on our understanding of pathogen taxonomy and the potential for future economically damaging epiphytotics. The obligate parasitism of the majority of the

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GDMs (Shaw, 1981; Dick, 2001b and references therein) makes study *ex situ* either problematic or impossible, depending upon the nature of the study.

Taxonomy of the Graminicolous Downy Mildews

The GDMs (*Sclerosporales*) were traditionally placed within the subclass *Peronosporomycetidae* as 'sister' to the *Peronosporales* (Shaw, 1978, 1981). The present authors believe this placement is incorrect and place them within the *Saprolegniomycetidae* as 'sister' to the *Leptolegniaceae* (Dick *et al.*, 1999) based on a suite of morphological, epidemiological, ultrastructural and biogeographical characters. This assertion is supported by analysis of rDNA restriction sites (Klassen *et al.*, 1988), 28S rDNA molecular data (Riethmüller *et al.*, 1999) as well as currently unpublished molecular data of the authors. The *Sclerosporales* (Dick *et al.*, 1984) contain two families (*Sclerosporaceae* and *Verrucalvaceae*) comprising 24 species and one variety. Revision may establish some synonymy, especially within the genus *Peronosclerospora*. A complete list of currently accepted or used binomials is provided in Table 6.1.

Aspects of Graminicolous Downy Mildew Biology

Host preference and range

The majority of hosts of GDMs are found within the primarily tropical subfamilies *Chloridoideae* and *Panicoideae* of the *Poaceae*. Differences in core host range between *Peronosporales* and *Sclerosporales* strongly indicate the deep phylogenetic divergence between the orders (*contra* Shaw, 1981). A summary of the known host ranges of the GDMs is shown in Fig. 6.1. The *Peronosporales* are mainly pathogens of dicotyledonous herbs of temperate regions (Dick, 2001c). Notable exceptions (Table 6.1), parasitizing the subfamily *Panicoideae* of the *Poaceae*, are: *Albugo ipomoeae-panduranae*, *Bremia graminicola* and *B. graminicola* var. *indica* on species of *Arthraxon* (Naomoff, 1913; Patel, 1948; Tai, 1979); *Plasmopara oplismeni* on *Oplismenus compositus* (Viennot-Bourgin, 1959) and *Plasmopara penniseti* on *Pennisetum glaucum* (Kenneth and Kranz, 1973 as *Pennisetum typhoides*). Additionally, *Peronospora destructor* and *P. fugitai* infest various *Liliaceae* s.l. (Constantinescu, 1991). These host–pathogen relationships are derived and should not be considered ancestral. It is notable that there are no known host transferrals by the GDMs from the *Poaceae* to the dicotyledons; indeed, they are highly restricted in their host range with only a few reports of *Sclerophthora macrospora* parasitizing *Cyperaceae* (Erwin and Ribiero, 1996, including references on *Cyperus* spp.).

Table 6.1. Species of the *Sclerosporales* (types in bold print) and graminicolous *Peronosporomycetidae* (*Peronospora* and graminicolous *Pythium* species excluded). Authors, publication dates and type hosts are provided; synonyms not included. * Indicates non-type host.

Species	Type host
SCLEROSPORACEAE M.W. Dick 1984; in Dick *et al.*, 1984	
Sclerospora J. Schröt. 1879	
Sclerospora butleri W. Weston 1933	*Eragrostis aspera* (Jacq.) T. Nees
***Sclerospora graminicola* (Sacc.) J. Schröt.** 1886	*Setaria viridis* (L.) P. Beauv.
Sclerospora iseilematis Thirum. & Naras. 1949	*Iseilema laxum* Hackel ex Duthie
Sclerospora northii W. Weston 1929	*Saccharum maximum* Trin.
Sclerospora secalina Naumov 1949	*Secale cereale* L.
Peronosclerospora (S. Ito) Hara 1927; in Shirai, 1927	
Peronosclerospora dichanthiicola (Thirum. & Naras.) C.G. Shaw 1978	*Dichanthium annulatum* (Forrsk.) Stapf
Peronosclerospora globosa Kubicek & R.G. Kenneth 1984	*Eriochloa contracta* Hitchc.
Peronosclerospora heteropogonis Siradhana *et al.* 1980	*Hetropogon contortus* (L.) Roemer & Schultes
Peronosclerospora maydis (Racib.) C.G. Shaw 1978	*Zea mays* L.
Peronosclerospora miscanthi (T. Miyake) C.G. Shaw 1978	*Miscanthus japonicus* Anderss.
Peronosclerospora noblei (W. Weston) C.G. Shaw 1980	*Andropogon australis* Spreng.
Peronosclerospora philippinensis (W. Weston) C.G. Shaw 1978	*Zea mays* L.
***Peronosclerospora sacchari* (T. Miyake) Hara;** in Shirai, 1927	*Saccharum*. sp.
Peronosclerospora sorghi (W. Weston & Uppal) C.G. Shaw 1978	*Sorghum arundinaceum* (Desv.) Stapf
Peronosclerospora spontanea (W. Weston) C.G. Shaw 1978	*Zea mays* L.
Peronosclerospora westonii (M.C. Sriniv. *et al.*) C.G. Shaw 1978.	*Iseilema laxum* Hackel ex Duthie
Peronosclerospora zeae Yao 1991	*Zea mays* L.
VERRUCALVACEAL M.W. Dick 1984; in Dick *et al.*, 1984	
Pachymetra B.J. Croft & M.W. Dick 1989; in Dick *et al.*, 1989	
***Pachymetra chaunorhiza* B.J. Croft & M.W. Dick** 1989; in Dick *et al.*, 1989	*Saccharum officinarum* L.

Continued

Table 6.1. *Continued*

Sclerophthora Thirum., Shaw, C.G. & Naras. 1953	
Sclerophthora cryophila W. Jones 1955	*Dactylis glomerata* L.
Sclerophthora farlowii (Griffiths) R.G. Kenneth 1979	*Chloris virgata* Sw.
Sclerophthora lolii R.G. Kenneth 1963	*Lolium rigidum* Gaudin
***Sclerophthora macrospora* (Sacc.) Thirum. *et al.*,** 1953	*Alopecurus* sp.
Sclerophthora rayssiae R.G. Kenneth *et al.*, var. *rayssiae* 1964	*Hordeum vulgare* L.
Sclerophthora rayssiae R.G. Kenneth *et al.* var. *zeae* Payak & Renfro 1967	*Zea mays* L.
Verrucalvus P. Wong & M.W. Dick 1984; in Dick *et al.*, 1984	
***Verrucalvus flavofaciens* P. Wong & M.W. Dick** 1984; in Dick *et al.*, 1984	*Pennisetum clandestinum* Chiov.
PERONOSPOROMYCETIDAE Dick 1984; in Dick *et al.*, 1984	
Albugo ipomoeae-panduranae (L.) G. Meyer	**Arthraxon hispidus* (Thunb.) Makino
Bremia graminicola Naumov var. *graminicola* 1913	*Arthraxon ciliaris* P. Beauv.
Bremia graminicola Naumov var. *indica* M.K. Patel 1948	*Arthraxon lancifolius* (Trin.) Hochst.
Peronospora destructor (Berk.) Casp.	**Liliaceae s.l.*
Peronospora fugitai S. Ito & Tokun.	**Liliaceae s.l.*
Phytophthora cyperi (Ideta) S. Ito 1935; in Ito and Tokunaga, 1935	*Cyperus malaccensis* Lam.
Phytophthora cyper-bulbosi Seethal. & K. Ramakr. 1953	*Cyperus bulbosus* Vahl
Phytophthora fragrariae Hickman var. *oryzobladis* J.S. Wang & J.Y. Lu 1978	*Oryza sativa* L.
Phytophthora japonica Waterhouse 1974	*Oryza sativa* L.
Phytophthora leersiae H.H. Ho & H.S. Chang 1992	*Leersia hexandra* Sw.
Phytophthora lepironiae Sawada 1919	*Lepironia mucronata* Rich.
Plasmopara oplismeni Vienn.-Bourg. 1959	*Oplismenus compositus* (L.) P. Beauv.
Plasmopara penniseti R.G. Kenneth & J. Kranz 1973	*Pennisetum glaucum* (L.) R. Br.
Pythiogeton zeae H.J. Jee *et al.* 2000	*Zea mays* L.

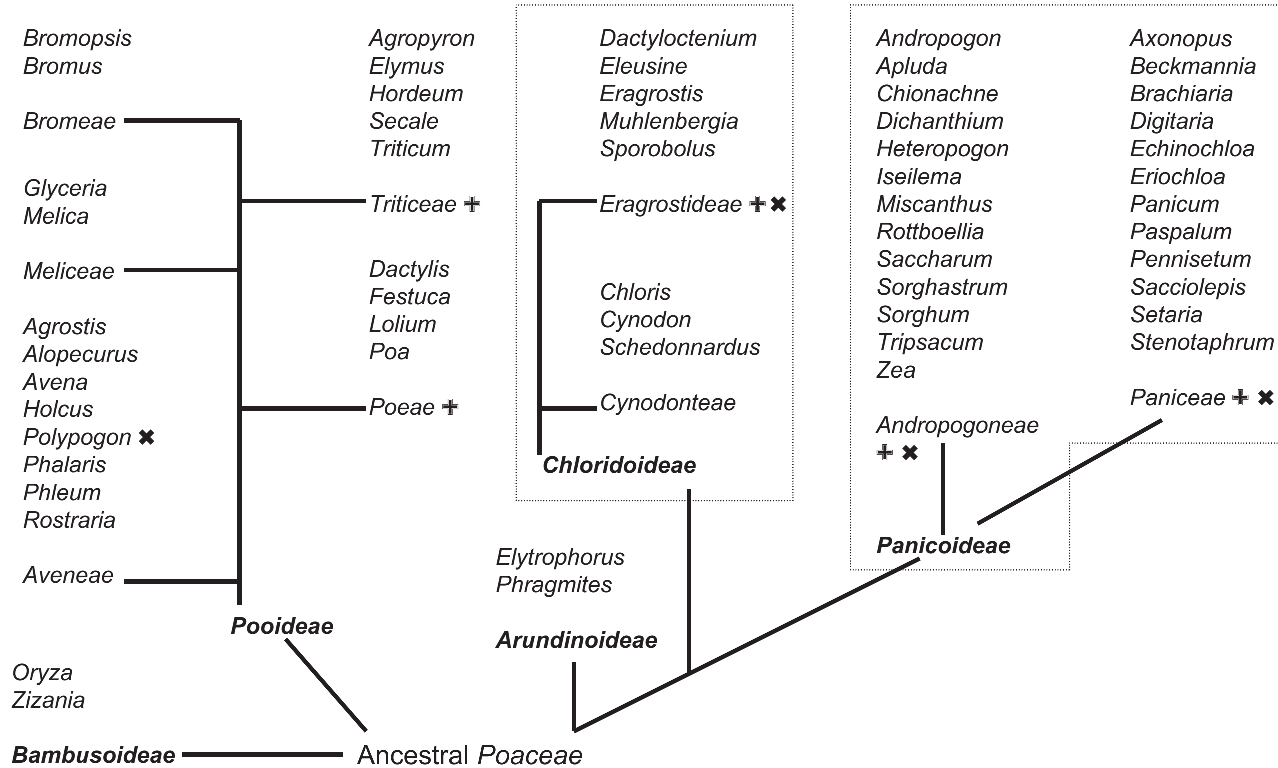

Fig. 6.1. Host genera of graminicolous downy mildews (records of artificial inoculation excluded). All subfamilies and tribes of the *Poaceae* detailed are parasitized by *Sclerophthora*; ✚ tribe parasitized by taxa assigned to *Sclerospora*; ✖ tribe or genus parasitized by *Peronosclerospora*; ··············· subfamilies with C_4 photosynthetic pathways.

Morphology and ultrastructure

The obligate biotrophic parasitism of *Peronosporales* and *Sclerosporales* differs widely not only in host selection but also in the morphology and morphogenesis of the pathogens. The asexual conidiosporangiophores of *Sclerosporales* and *Peronosporomycetidae* are alike in gross morphology, reflecting similarities in propagule dispersal. However, there are considerable differences in detailed morphology and morphogenesis. *Peronospora* conidiosporangiophores are dichotomous, isodiametric and persistent, unlike the pseudo-dichotomous (or simple), inflated and evanescent conidiosporangiophore of the *Sclerosporales*. These differences are indicative of convergent evolution rather than phylogenetic radiation (Dick *et al.*, 1984; Dick, 2001c). Sexual reproductive organs (antheridia and oogonia) of the *Peronosporomycetes* are highly important for evaluation of phylogenetic relationships (Dick, 1995, 2001a,b). Details of oosporogenesis have been important in establishing differences between the two main subclasses of the *Peronosporomycetes* (Dick *et al.*, 1984, 1989). Oospores of the *Peronosporomycetidae* develop centripetally whereas those of the *Saprolegniomycetidae* develop centrifugally (Dick, 2001a). Safeeulla and Thirumalachar (1955) demonstrated centrifugal oospore nuclear development in *Peronosclerospora sorghi* (described as *Sclerospora andropogonis-sorghi* (Kulkarni) Kulkarni). Centrifugal oospore nuclear development, characteristic of the *Saprolegniomycetidae*, has also been observed in *Sclerospora graminicola* (McDonough, 1937) and *Sclerophthora macrospora* (McDonough, 1947). This contrasts with oosporogenesis in *Albugo* (Safeeulla and Thirumalachar, 1955) and *Phytophthora infestans* (Graham, 1954), in which oospores develop centripetally. The matrix of the oospore ooplast is highly distinctive within the *Sclerosporales* and is comparable to the granular fluid ooplast of the *Saprolegniales*, unlike the solid translucent ooplast of the *Peronosporomycetidae* (Dick *et al.*, 1984). The oogonia of the *Pythiales* and *Peronosporales* are thin-walled, unlike those of the *Sclerosporales*, which are thick-walled and similar to those of the *Saprolegniales* (Dick *et al.*, 1989).

Epidemiology, physiology and ecology

There is considerable functional convergence of asexual propagules of the *Sclerosporales*, *Pythiales* and *Peronosporales*, reflecting environmental selection pressures. The conidia and conidiosporangiophores of *Peronospora* and *Plasmopara* are not truly homologous with those of *Peronosclerospora*. The conidiosporangiophores of *Peronosclerospora* are evanescent while those of *Peronosporales* are highly persistent. Similarities in epidemiological function are convergences; dissimilarities in function, supporting ultrastructural and morphological data, indicate a deep phylogenetic divide between the

Sclerosporales and the *Peronosporomycetidae* (Dick *et al.*, 1989; Riethmüller *et al.*, 1999). The dispersal and germination of conidia of *Peronospora* and *Peronosclerospora* display considerable differences in epidemiology; the conidia of *Peronospora* may remain viable for several weeks (Pegg and Mence, 1970) and are light-sensitive (Fried and Stutville, 1977). Conversely those of *Peronosclerospora* are viable for and germinate in only a few hours (Dogma, 1975), are non-reactive to light, nocturnally dispersed and germination is dependent on high relative humidity (Dogma, 1975; Bock *et al.*, 1998). While both genera have a wide range of temperature tolerance, conidial formation is usually inhibited by temperatures over 27–30°C; *Peronospora* species may successfully sporulate and germinate at lower temperatures (Pegg and Mence, 1970), 0°C compared to a minimum of 10°C for *Peronosclerospora* (Bock *et al.*, 1998).

Losses of anabolic sterol metabolic pathways have been reported within the *Peronosporomycetes* (Warner *et al.*, 1983) and the GDMs may require exogenous sources of sterols. Hosts growing in areas of high solar incidence frequently exhibit raised carbohydrate production via photosynthesis. This is conducive to the production of secondary metabolites (flavonoids and sulphonated flavonoids from phenylalanine, sterols, essential oils and alkaloids from carotenoid precursors). The loss of ability (and subsequent unlikelihood of restoration) to utilize certain inorganic forms of nitrogen and sulphur by certain groups of *Peronosporomycetes* is still accepted (Cantino, 1955). The *Peronosporomycetidae* are able to use SO_4^{2-}, and their ability to metabolize different inorganic NO_3^- sources is variable. The *Saprolegniomycetidae*, however, are unable to utilize SO_4^{2-}- or NO_3^--containing substrates. While the *Sclerosporales* have not been experimentally demonstrated to be unable to synthesize SO_4^{2-}, their association with grasses that retain substantial photosynthetic 'sinks' of organic sulphonated flavonoids is notable.

The *Verrucalvaceae* are intermediate between the *Peronosporomycetidae* and the *Saprolegniomycetidae* in terms of response to the fungicidal isoxazoles (Hymexazol) and phenylamides (Metalaxyl) (Kato *et al.*, 1990). The *Verrucalvaceae* are relatively resistant to Metalaxyl, as are the *Saprolegniaceae* and *Leptomitaceae*, unlike the susceptible *Phytophthora* (although resistance is known to occur in some populations of *P. infestans;* see Erwin and Ribiero, 1996). It is noteworthy that Kato *et al.* (1990) observed similarities of response in axenic culture between *Verrucalvus* and *Pachymetra* and that of *Leptolegnia caudata*. This close relationship is supported by unpublished 18S rDNA data of the authors and Riethmüller *et al.* (1999). However, Kang and Lee (1987) noted a 133% yield increase and reduced disease incidence in a rice crop infested with *Sclerophthora macrospora* following application of Metalaxyl. Similarly, others (Eastwood and Malein, 1998; Panicker and Gangadharan, 1999) found that *Peronosclerospora* was also susceptible to Metalaxyl. These apparently contradictory results are unexplained but may be due to differing methodologies or extrinsic factors; it is possible that the noted responses may

have been affected by synergistic effects on mycopathogens within soil or plant tissue. With respect to Hymexazol, *Sapromyces* and *Phytophthora* behaved similarly, whereas the *Verrucalvaceae* had responses more similar to those of *Pythium* (Kato *et al.*, 1990). *Peronosporales* are known to be susceptible to phosphonate fungicides (including fosetyl-Al) (Cohen and Coffey, 1986), unlike *Peronosclerospora sacchari*, where resistance to fosetyl-Al is known (Eastwood and Malein, 1998). In *Peronosclerospora sorghi*, reduction in sporulation but not germination has been observed (Panicker and Gangadharan, 1999) following treatment with fosetyl-Al.

While most downy mildews are unculturable, a few species have been grown in biphasic systems using host tissue culture (Dick, 2001a). Others, such as *Verrucalvus* and *Pachymetra*, are culturable with difficulty (Dick *et al.*, 1984, 1989). Claims that *Sclerospora graminicola* (Tiwari and Arya, 1969) and *Sclerophthora macrospora* (Tokura, 1975) have been maintained axenically require careful reinvestigation as the original cultures do not appear to be extant. The *Pythiogetonaceae* (*Peronosporomycetidae*) are similar to sclerosporaleans in being either highly fastidious (*Pythiogeton*) or unculturable (*Medusoides*) (Voglmayr *et al.*, 1999). Similarity of culturability of organisms should not be considered a reflection of phylogenetic relatedness, but potentially a reflection of independent substrate specialization; therefore, the obligate nature of the *Peronosporales* should not necessarily be considered as a synapomorphic trait with the *Sclerosporales*.

Evolution of the *Poaceae*

Clayton and Renvoize (1986) suggested that the origins of the grasses may have been in Gondwana on what is now northern South America and western Africa with the subfamily *Bambusoideae* evolving first; this is supported by molecular data that place the herbaceous neotropical tribes *Streptochaeteae* and *Anomochloeae* as basal to the *Bambusiodeae* (Clark *et al.*, 1995) and *Poaceae* as a whole. The first radiation of the *Poaceae* possibly occurred during the late Cretaceous and early Tertiary (Maastrichtian – mid-Eocene, 70–40 m.y. BP) (Jacobs *et al.*, 1999) although clear fossil evidence is not present until the Palaeocene (65–57 m.y. BP) (Dick, 1988, 2001c). This early radiation of primarily C_3 photosynthetic pathway-utilizing bambusoid, oryzoid and primitive pooid clades (Kellogg, 1998) was predominantly in mesic forest/savannah ecotones with relatively low levels of insolation (Clayton and Renvoize, 1986; Jacobs *et al.*, 1999).

South American Oligocene (35–23 m.y. BP) fossils of grinding hypsodont mammalian teeth imply the formation of early grasslands and the spread of *Poaceae* out of the forest/savannah ecotone (Dick, 1988, 2001c; Jacobs *et al.*, 1999). Clear vertebrate and palaeobotanical evidence of widespread grass-dominated ecosystems in northern continents did not occur until the mid- to

late Miocene (9–5 m.y. BP) (Clayton and Renvoize, 1986; Jacobs *et al.*, 1999). The late Miocene spread of C_4 grasses possibly involved a decrease in atmospheric CO_2 and heralded the establishment of modern seasonality and rainfall patterns. The Himalayan uplift (20 m.y. BP), which drained the Tethys Ocean remnants and resulted in colder montane and Siberian steppe climates to the north, would have forced the early C_4 tropical grasses southwards into Africa, India, and South-East Asia. C_4 grasses are abundant from approximately 15 m.y. BP, undergoing a dramatic expansion in the lower latitudes of North America, South America, East Africa, and Pakistan between 9 and 4 m.y. BP. The modern temperate pasture grasses may have radiated from tropical and warm temperate regions as the climate cooled and habitats were opened up by the grazing mammals (Dick, 1988, 2001c). The later radiation of mainly C_4 photosynthetic pathway-utilizing arundinoids, chlorids, centothecoids and panicoids (Kellogg, 1998) displaced many of the genera of the earlier radiation to the forest understorey/margins and cooler temperate regions. Throughout this period Australasia was isolated, drifting northwards with a previously temperate-adapted biota. From the Oligocene, land bridges permitted tropical panicoid grasses a southward migration into Australia, which continued through the Quaternary.

Coevolution of the *Sclerosporales* and *Poaceae*

There are no known fossils of sclerosporaleans. Although both GDMs and downy mildews of dicotyledonous plants are of relatively recent origin, the antecedents of the latter are probably more ancient (Dick, 1988, 2001a,b). Comparison of the present host ranges of the downy mildews and the relative antiquity of their hosts, together with what is known of host ranges for *Pythium* and *Phytophthora*, presents some problems. The affinity of *Phytophthora* for woody dicotyledonous hosts and the distinct, later origin of the pasture grasses makes it possible to suggest that, contrary to accepted phylogenies (Shaw, 1978, 1981; Barr, 1983), some sections of *Pythium* (with many taxa associated with grasses) may have evolved later than *Phytophthora*. This receives some support from mitochondrial genome analyses (Belkhiri and Dick, 1988), which indicated that *Phytophthora* lacks the inverted repeat characteristic of *Pythium* (Klassen *et al.*, 1987). While it is realistic to propose a late Cretaceous origin for *Phytophthora*, the origin of some sections of *Pythium s.l.* may have been with the pasture grasses in the Tertiary. It is probable that GDMs evolved during the mid- to late Miocene (9–5 m.y. BP) at the same time as the second main radiation of the *Poaceae*. This may be inferred by the predominance of hosts from within the subfamily *Panicoideae* (Fig. 6.1). Notably the paucity of hosts from the pooids and the oryzoids indicate that these particular host–pathogen relationships developed relatively late in *Poaceae* evolution (probably secondarily derived following anthropogenic

disturbance). The probable inability of the GDMs to synthesize inorganic sulphur suggests that they require organic sulphur substrates; these are particularly abundant as sulphonated flavonoids in the C_4 pathway-utilizing grasses (but not the non-sulphonated flavonoids of the dicotyledons). Reliance on other aspects of host metabolism, such as sterol pathways and secondary metabolites other than sulphonated flavonoids, may play an essential role in GDM physiology and coevolution with the grasses. As yet there are no data to indicate such metabolic relationships. Investigation of these biochemical pathways may prove informative for both systematists and crop protectionists.

The GDMs are primarily associated with the highly evolved tropical grazing grasses of the subfamilies *Chloridoideae* and *Panicoideae* (Fig. 6.2). The hypothesis that the putative ancestral hosts were within the bambusoids, oryzoids, and south hemisphere grasses can be dismissed. The subfamily *Panicoideae* has tropical evolutionary origins and is currently centred in South-East Asia (Clayton and Renvoize, 1986). The *Panicoideae* contains species with leaf anatomy of both kranz (C_4 photosynthesis) and non-kranz kinds. The kranz anatomy can be of both MS and PS kinds (for details see Clayton and Renvoize, 1986). Kranz MS species have evolved metabolic pathways that ‘sink’ high levels of carbohydrates arising from their habitats, including marshes, ruderal and arid habitats. These are all habitats with high insolation. The occurrence of *Sclerospora* and *Sclerophthora* in the non-kranz genus *Oryza* is apparently anomalous on presently available information, but future studies may reveal metabolic similarities between the wetland *Oryza* and the *Panicoideae*.

Present-day geographical distributions disguise evolutionary origins and may merely confirm the opportunism of these parasites. The recent transcontinental host–pathogen interaction of *Peronosclerospora* with *Zea* is one example of this. The relationship between *Peronosclerospora* and the tribe *Andropogoneae* in Melanesia (Figs 6.1, 6.2) is probably of a primary nature (Weltzein, 1981). *Saccharum* (including *Erianthus*) consists of 35–40 Old World warm temperate and tropical species; the sugar cane species have their centre of diversity in Melanesia (Clayton and Renvoize, 1986). Wild sugar cane species and other *Andropogoneae* are often infected with *Peronosclerospora* in habitats with minimal anthropogenic disturbance (Renfro and Bhat, 1981). The possibility that a high diversity of pathogen taxa infecting *Saccharum* and other *Andropogoneae* in wild-type habitats is secondary must be considered to be minimal. The tribe *Paniceae* is probably primarily C_3 (Clayton and Renvoize, 1986) and the high prevalence of these taxa as hosts for *Peronosclerospora* cannot be discounted. It may be that *Peronosclerospora* coevolved with ancestral C_3 *Paniceae* during the mid- to late Miocene (9–5 m.y. BP) in the region now occupied by the Himalayas. Subsequent cooling of this region (Dick, 1988, 2001c) and the evolution of C_4 *Panicoideae* may have forced *Peronosclerospora* southward into Melanesia. It is notable that there are two apparent centres of

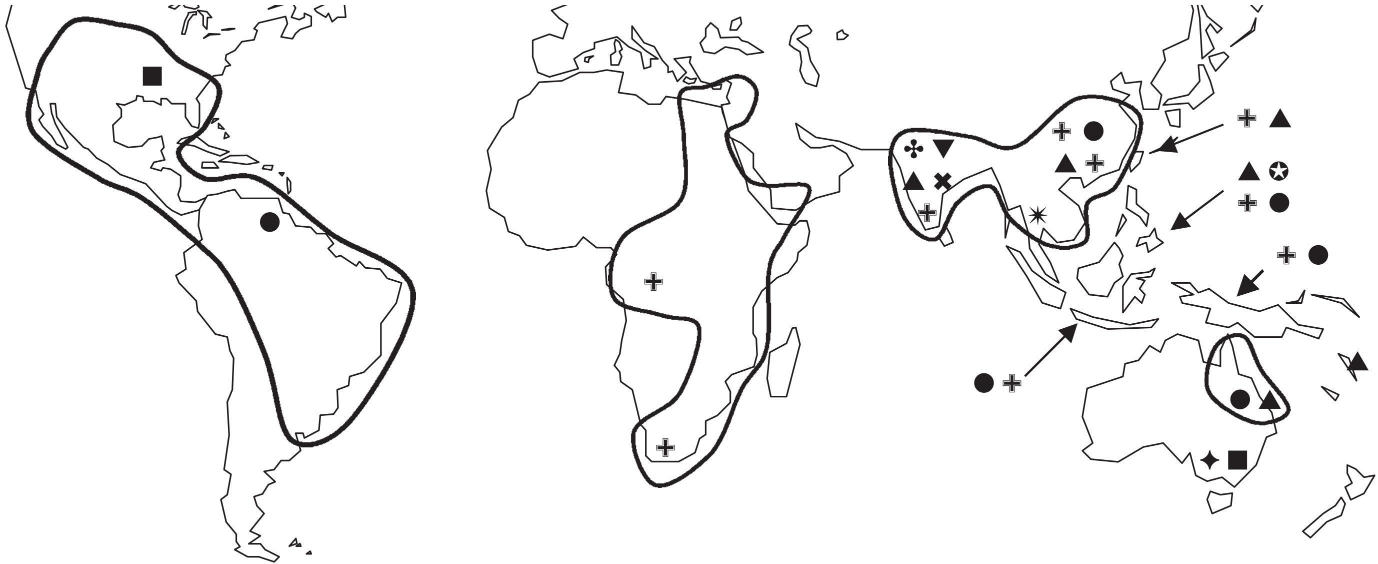

Fig. 6.2. Known distribution of *Peronosclerospora* species: ✣ *P. dichanthiicola*; ■ *P. globosa*; ▼ *P. heteropogonis*; ● *P. maydis*; ✦ *P. noblei*, ✙ *P. philippinensis*; ▲ *P. sacchari*; — *P. sorghi*; ✪ *P. spontanea*; ✖ *P. westonii*; ✳ *P. zeae*.

diversity for *Peronosclerospora*, one in subcontinental India (*P. dichanthiicola*, *P. heteropogonis* and *P. westonii*) and the other in eastern Melanesia and Australasia (*P. globosa*, *P. maydis*, *P. miscanthi*, *P. noblei*, *P. sacchari* and *P. spontanea*). The remaining species (*P. philippinensis* and *P. sorghii*) are of widespread distribution and their origins are conjectural. The suggestion that *Peronosclerospora* may have originated at the site of the Himalayan orogeny allows for a sympathetic explanation of the origin of *Sclerospora*. Whilst the tropical conidial *Peronosclerospora* migrated southwards the temperate zoosporangial *Sclerospora* may have evolved to the north and west of the Himalayan orogeny and the Indian subcontinent (Fig. 6.3). Although *Sclerospora graminicola* is well characterized, other members of the genus are poorly known. *S. iseilematis, S. northii* and *S. secalina* are oogonial and have no known asexual phase; therefore evaluation of their relationship to the other genera is problematic other than defining them as GDMs. *Sclerospora butleri* has previously been assigned to *Basidiophora* (*B. butleri* (Weston) Thirumalachar and Whitehead, 1952). This placement has been questioned by some later authors (Kenneth and Kranz, 1973; Dick *et al*., 1984) and later rejected from *Basidiophora* (Barreto and Dick, 1991).

The current known distribution of *Pachymetra* and *Verrucalvus* in Australia (Dick e*t al*., 1984, 1989) presents problems for a sclerosporalean origin (but not a later diversification) on the Indian/Asian convergence zone. It is possible that they may have migrated south at the same time as *Peronosclerospora*, but their poor dispersal capacity (airborne conidia are unknown in either taxon) allows only a most remote possibility that they were able to cross Wallasia during the Miocene. *Pachymetra* is known from native Blady Grass, *Imperata cylindrica* (R.C. Magarey, 1989, Queensland, Australia, personal communication), a widespread Old World tropical panicoid grass, as well as sugar cane. It is possible that either *Pachymetra* or *Verrucalvus* may be present in wild-type habitats in South-East Asia; collections from this region as well as further sampling in eastern Australia are desirable. If either genus is to be found in South-East Asia the possibility arises that both are of recent anthropogenic origin in Australian agricultural systems. This is an essential element for future research to provide an understanding of the pathogenicity of these potentially globally damaging organisms.

The origins of *Sclerophthora* are possibly distinct (but not independent) from those of *Sclerospora* and *Peronosclerospora*. Unlike the latter genera, *Sclerophthora* has no predisposition to grasses of the *Panicoideae* or *Chloridoideae* (Fig. 6.1) and has been noted from over 140 taxa throughout the *Poaceae* (see Kenneth, 1981, including references). This may reflect cryptic speciation within the current species concept and further investigation may enable patterns of host–pathogen relations to be evaluated (Shaw, 1981). *Sclerophthora macrospora* is found in most warm temperate regions of the world. The others (*S. cryophila*, *S. lolii*, *S. rayssiae* and *S. rayssiae* var. *zeae*) are centred on the Middle East and drier regions of India (Fig. 6.3). *S. cryophila*, on *Dactylis*, an

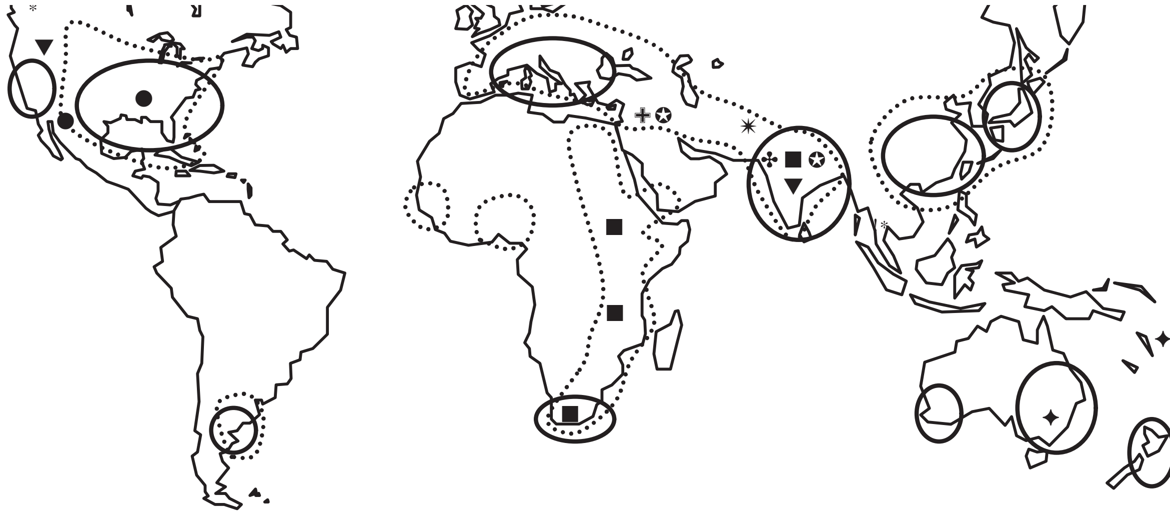

Fig. 6.3. Known distribution of graminicolous downy mildews of the genera *Sclerospora* and *Sclerophthora*: ■ *Sclerospora butleri*; ••••• *Sclerospora graminicola*; ✣ *Sclerospora iseilematis*; ✦ *Sclerospora northii*; ✳ *Sclerospora secalina*; ▼ *Sclerophthora cryophila*; ● *Sclerophthora farlowii*; ✙ *Sclerophthora lolii*; — *Sclerophthora macrospora*; ✪ *Sclerophthora rayssiae*.

introduced Old World genus, has outliers of anthropogenic origin in North America. *Sclerophthora farlowii* (Griffiths) R.G. Kenneth in Arizona may be a remnant of the circum-tethyan flora, but it is more likely that it is indigenous to the Old World, its presence in America being of anthropogenic origin. It is also possible that *S. farlowii* is not a *Sclerophthora*; Shaw (1981) noted that R.G. Kenneth 'by careful re-examination of the original collections of *S. farlowii* has found the anamorph thereof' (Kenneth, 1979, 1981). This is considered unlikely by the authors as the asexual organs of *Sclerosporales* are evanescent and impermanent in herbarium exsiccatae. Conidiosporangiophores are therefore unlikely to have survived adequately to allow description for approximately 70 years since Griffiths' original description.

Graminicolous Downy Mildews and *Phytophthora*

Several taxa ascribed to *Phytophthora s.l.* are probably misplaced and a critical reappraisal of all aspects of their biology may reveal alternative relationships. *P. verrucosa* was described from glasshouse crops of *Meconopsis* and *Lycopersicon* (Foister, 1940). While this host range is clearly anomalous for GDMs, similarities in oogonial morphology suggest that *P. verrucosa* may be more appropriately associated with the GDMs (Dick *et al.*, 1984, 1989). *Phytophthora cyperi*, *P. cyper-bulbosii* and *P. lepironiae*, pathogens of tropical *Cyperaceae*, are likewise probably not *Phytophthora s.s.*; each has sexual reproductive organs similar to those of *Sclerophthora* (Dick *et al.*, 1984, 1989). Like GDMs, they are apparently unculturable axenically (Erwin and Ribiero, 1996). Their host range on tropical monocotyledons is also atypical of many *Phytophthora* species. Great emphasis is often placed upon the presence of amphigynous antheridia as a defining character of *Phytophthora*. The presence of amphigyny in several of the preceding species has been considered sufficient for inclusion within *Phytophthora*. However, there are two other genera where amphigyny has been observed, *Trachysphaera* and *Peronophythora*. The possibility that differing morphogenetic events in amphigynous antheridial maturation occur within *Phytophthora* species suggests that the condition may not be homologous (Stevenson and Erwin, 1972; Hemmes and Ribeiro, 1977). Therefore the inclusion of taxa within *Phytophthora* (as currently circumscribed) solely because amphigynous antheridia are present is inappropriate. It is possible that, following critical evaluation of biochemical, epidemiological, ultrastructural and molecular data in conjunction with a wide range of *Peronosporales* and other *Pythiales*, *Phytophthora s.l.* could be untenable in its current form (Dick *et al.*, 1999; Dick, 2001a,b).

Conclusion

GDMs are not closely related to the *Pythiales* or the *Peronosporales* but to the water moulds of the *Saprolegniomycetidae*, especially the *Leptolegniaceae*. The warm temperate and tropical distribution and the phytogeography of their hosts is incompatible with an evolutionary relationship with the cool temperate *Peronosporales.* The absence of a similarity in core host range between the *Peronosporales* and *Sclerosporales* suggests differing origins. The large suite of fine morphological and ultrastructural differences indicate convergent adaptation to similar ecological niches. This is derived from a presumed need to access substrates rich in secondary metabolites, including sterols or sulphonated flavonoids. Considerable differences in epidemiology and fungicidal response occur, which arise from constraints of biochemistry. Investigation of all aspects of tropical *Peronosporomycetes* biology will further resolve relationships between GDMs and the *Peronosporomycetidae*. It is especially important that published molecular data are complemented by other gene loci and wider taxonomic sampling. Resolution of these relationships will enable directed strategies of control to be developed for these important tropical plant pathogens.

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Invasive Neotropical Pathogens of Tree Crops 7

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Introduction

Since the early 1990s increasing attention has been drawn towards the environmental and socio-economic problems resulting from biological invasions on a global scale (Cronk and Fuller, 1995; Kaiser, 1999; Mack *et al.*, 2000; Pimentel *et al.*, 2000). In such invasions, alien or exotic, non-indigenous organisms, either deliberately or accidentally introduced, become adapted to and subsequently dominate these new habitats, with inevitable and often profound detrimental impacts on the flora and fauna of both natural and agricultural ecosystems. Most of the available literature and popular reviews on the subject pertain to biological invasions involving either plants (D'Antonio and Vitousek, 1992; Lonsdale, 1999), invertebrates (Howarth, 1985; Porter and Savignano, 1990), vertebrates (Maciolek, 1986; Dobson, 1988) or a combination of these (Mooney and Drake, 1986; Simberloff *et al.*, 1997). In particular, in the general overviews of invasive species little mention has been made of the actual and potential importance of alien fungal plant pathogens, despite recent high-profile examples, such as *Phytophthora cinnamomi* Rands in Australia (Weste and Marks, 1987); *Phytophthora infestans* (Mont.) de Bary in Europe (Fry *et al.*, 1992); *Cryphonectria parasitica* (Murr.) Barr (chestnut blight) and *Discula destructiva* Red. (dogwood anthracnose) in the USA (Anagnostakis, 1987; Daughtrey and Hibben, 1994).

This chapter focuses on neotropical fungal pathogens of two of the major commodity crops, rubber and cacao, at least one of which has the potential to change 'the political history of the world' (Disraeli's view of potato blight; in Ramsbottom, 1953), tracing their past and present impacts on agriculture

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in the region and highlighting the continuing threat they pose to other Latin American countries, as well as to other continents. In essence, it is revisiting the subject of catastrophic or threatening plant diseases (the term invasive being of relatively recent coinage), so admirably reviewed by Klinkowski (1970) and Thurston (1973). Following the example set by the latter author, the chapter covers plant diseases of 'potential international importance' but those which are 'at present limited to a few countries or a continent' and which 'are important in developing countries' (Thurston, 1973).

South American Leaf Blight of Rubber

Historical

All the natural rubber of commerce is now derived from the neotropical euphorbiaceous tree, *Hevea brasiliensis* (A. Juss.) Muell. Arg., which is cultivated predominantly in the Palaeotropics, far removed from its native range (Purseglove, 1968). *H. brasiliensis* is indigenous to the tropical rainforests of the Amazon basin, occurring only south of the river and extending to Peru and Bolivia (Schultes, 1956). The intriguing history of the early rubber germplasm collections, their establishment in the Far East and the eventual creation of the Asian plantation industry in the early 1900s, was admirably synthesized by Purseglove (1968), and was retold in a more dramatic and expansive form by Dean (1987) and Davis (1997). The descendants of these earlier Brazilian collections were returned in the late 1800s to the Neotropics as part of a colonial programme to develop rubber plantations, particularly in north-east South America, Central America and the Caribbean. It was from the Guianas and Trinidad that the first indications that a serious disease was affecting the crop began to surface; and, in the period 1910–1917, a number of colonial pathologists investigated the problem, which resulted in the identification of the causal agent and an insight into its biology (Petch, 1914; Stahel, 1917, 1927). Indeed, Holliday (1970a) described the publication by Stahel (1917) as being still the original source of much of our current knowledge of the disease. Within a relatively short time, it was realized that the disease posed a serious threat to the booming Asian rubber industry (Belgrave, 1922).

The pathogen had been collected earlier by E. Ule in western Amazonia (Brazil and Peru) on wild *Hevea* spp., and the teleomorph was subsequently described by P. Hennings, as *Dothidella ulei* P.Henn., in 1904 (see Holliday, 1970a). However, the hyphomycete anamorph was not described until 1912 by J. Kuyper in Surinam, and the two were not linked until several years later (Petch, 1914; Stahel, 1917). Stahel (1917) erected the new genus *Melanopsammopsis* to accommodate the teleomorph, but von Arx subsequently transferred this to the genus *Microcyclus* Sacc. (von Arx and Müller, 1975).

The nascent rubber industries in both Guyana and Surinam were literally nipped in the bud by the new disease as the early yield data starkly revealed, with rubber production falling in Guyana alone from over 20,000 lb in 1920 to less than 2000 lb in the following year (Holliday, 1970a). As Maclaren (1924) commented, South American leaf blight, which first appeared in the Colony in 1909, 'reduced the vitality of the trees to a low ebb'.

Causal agent

Microcyclus ulei (P. Henn.) von Arx (*Dothideales, Mycosphaerellaceae*)
Anamorph: *Fusicladium macrosporum* Kuyper
A pycnidial form, *Aposphaeria ulei* P. Henn., has also been described but it appears to play no role in the infection cycle (Chee, 1978), and it is assumed, but not confirmed, that the pycnospores function as spermatia and that the fungus is heterothallic (Holliday, 1970a; Cannon *et al.*, 1995).

The superficial, black, globose ascostromata form mainly on the upper leaf surface, often crowded and associated with shot-hole symptoms. The ellipsoidal, hyaline, 1-septate ascospores, produced in clavate, bitunicate asci, are typical of the genus *Mycosphaerella* Johanson. As discussed by von Arx (1983) and Cannon *et al.* (1995), only the excessive development of stromatic tissue in *Microcyclus*, resulting in erumpent ascostromata, separates it from *Mycosphaerella*. Indeed, some of the *Mycosphaerella* spp. associated with pine trees have similar prominent ascostromata (Evans, 1984).

The *Fusicladium* anamorph appears as olive-grey to greenish black, powdery, velvet-like masses on the lower leaf surface, which consist of simple conidiophores producing predominantly 1-septate, cylindrical, irregular and twisted conidia singly from the tip. Von Arx (1983) remarked that *Fusicladium* is 'much alike' *Passalora*, the genus assigned to it by G. Massee in 1913 (Holliday, 1970a), and, furthermore, he considered that *Fusicladium* was more referable to anamorphs of *Venturiaceae* than *Mycosphaerellaceae*. Clearly, a re-examination of its taxonomic position would be justified.

Both the conidia and ascospores are infective and penetrate directly through the leaf cuticle following formation of appressoria (Stahel, 1917), and foliage up to 10 days old is especially susceptible. Conidia may be produced on the leaf within a week of infection, but the fungus can also infect and sporulate on petioles, green stems, inflorescences and fruits. The palisade layer of infected leaves may be 3–4 times thicker than that of healthy leaves, whilst petioles and green stems can be up to twice the normal size due to the overproduction of cortical cells (hyperplasia), which often results in growth distortions (hypertrophy) (Stahel, 1917; Fig. 7.1). Repeated infection results in severe defoliation, canopy dieback and death, even of mature trees (Holliday, 1970a). Ascospores survive in the leaf litter and provide the main inoculum source to renew disease cycles at the beginning of the rainy season

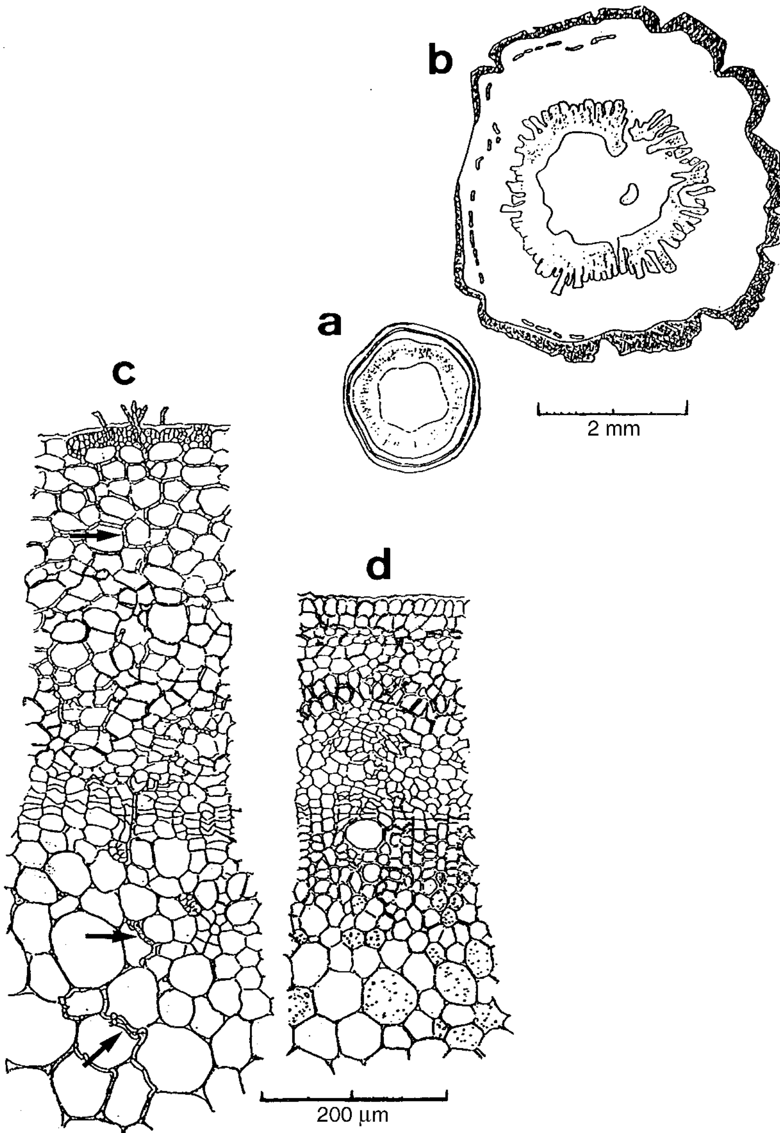

Fig. 7.1. Transverse section through a healthy petiole (a) of *Hevea brasiliensis* and one infected with *Microcyclus ulei* (b), showing the tissue disorganization and gross increase in petiole diameter following infection. Mycelium of *M. ulei* colonizes the upper and lower cortex (arrows), grows intercellularly (c) and appears to disrupt the cambium, leading to overproduction of cells (hyperplasia), of larger size than normal (hypertrophy), compared to a healthy petiole (d). After Stahel (1917).

(Chee, 1976), although their precise role and overall importance are still uncertain.

A number of races, as well as two morphological types, of the pathogen have been identified in Brazil (Langford and Townsend, 1953; Chee *et al*., 1986; Hashim and Almeida, 1987).

Distribution and impact: actual and potential

The pathogen is specific to *Hevea* and attacks at least four of the 12 species in the genus: *H. brasiliensis, H. benthamiana* Muell. Arg., *H. guianensis* Auld. and *H. spruceana* (Benth.) Muell. Arg. Between them, these species cover the whole of the Amazon and Orinoco river basins, extending from Guyana in the north to Bolivia in the south, and from the Atlantic coast to the eastern Andes (Fig. 7.2). The spread of the disease from 1908 onwards into emerging rubber plantations in Bolivia, Brazil, Colombia, Ecuador, Guyana, Peru, Surinam and Venezuela has been attributed to infection from wild *Hevea* trees in the surrounding forests (Hilton, 1955; Holliday, 1970a). Prior to this, most of the world's rubber was collected from scattered forest trees in the Brazilian Amazon, which made fortunes for a few and kept many in virtual slavery (Dean, 1987). It has been speculated that these sparse forest populations were the resistant survivors of much more extensive original populations. However, this sporadic distribution (estimated at six trees per acre; Thurston, 1973) is typical of tropical trees in primary forest ecosystems, be it rubber, cacao or Brazil nut. As Hilton (1955) correctly deduced, 'the immunity of many of the jungle trees [*Hevea*] is due to the fact that being isolated from their neighbours they have never been exposed to high concentrations of infective material'. The pathogen spread rapidly from these forest foci, with a dramatic impact on the monocultures of *H. brasiliensis*. In Guyana, for example, the disease was first reported in 1909 (Rands, 1924), and within a decade led to the abandonment of plantation rubber in that country. Rands (1924) also noted that the disease impact in Surinam was even greater, probably due to the plantations being closer to the forest zone, and thus nearer to natural infection foci on *H. guianensis*, as well as being away from the drying coastal winds. As Stahel (1917) reported, by 1916 trees of all ages were dying from the disease, and the destruction of infected wild *Hevea* populations, as well as chemical spraying, proved to be both ineffective and uneconomic.

Leaf blight was first recorded outside its natural range in 1916 when J.B. Rorer identified *M. ulei* in Trinidad (in Lamont *et al*., 1917). Much later, the pathogen reached and invaded Central America, appearing first in Costa Rica (Stevenson, 1935), and shortly after in Panama (Hilton, 1955), and some 10 years later in Mexico (Martin, 1948). Subsequently, *M. ulei* was reported in Honduras (Waite and Dunlap, 1952) and Guatemala in 1955 (Holliday, 1970a). The pathogen has purportedly been present in the Brazilian State of

Fig. 7.2. Distribution of *Microcyclus ulei* on wild and plantation rubber (as shown by dotted line). The important collecting sites of wild, resistant *Hevea* germplasm (Leticia, Iquitos, Acre, Madre de Dios), and the research bases where rubber clonal gardens were established (Belém, Tingo Maria, Turrialba, Villa Arteaga), are included in the map. In addition, the failed, abandoned Ford plantations on the Rio Tapajoz (Belterra and Fordlandia) are also marked. The wild hosts in the Amazon–Orinoco basins are: *H. benthamiana* (northerly); *H. brasiliensis* (southerly); *H. guianensis* (throughout); *H. spruceana* (lower and middle Amazon). After Holliday (1970a).

Bahia since the late 1930s and reached its most southernly distribution in São Paulo State in the 1960s (Holliday, 1970a). Thus, there were successive invasions or disease fronts in Latin America: in the Guianas (1914–1923); Brazil (1930–1943); and Central America (1935–1955), with serious socio-economic impacts, all of which strongly indicated that any attempts at establishing a plantation industry in the Neotropics, and especially within the native range of *Hevea*, would be fraught with difficulties. Henry Ford ignored these early warnings, probably because, as Holliday (1989) sardonically commented, he considered that 'history is bunk'. However, his efforts at planting rubber on a huge scale in the Brazilian Amazon from 1927 to 1943, firstly at Fordlandia (> 8000 ha) and then at Belterra (> 12,000 ha), near to the original collecting sites of the Asian rubber material in the previous century

(Fig. 7.2), proved to be one of the most costly failures of any agricultural project. Despite the importation of selected, high-yielding stock from Sumatra and Malaysia, and the use of new grafting techniques which involved top-budding with disease-resistant *H. guianensis* and *H. spruceanum*, the plantations succumbed to disease (Dean, 1987; Hecht and Cockburn, 1990). Indeed, a significant part of the US$20 million investment was used in top-budding more than 2 million trees, occupying 600 workers over a 4-year period (Davis, 1997).

The pathogen has now reached its invasive limit in the Neotropics; wherever rubber has been planted *M. ulei* has caught up with its host, either naturally by airborne inoculum from indigenous forest trees, or, in the case of exotic plantations, by human agency (Martin, 1948; Altson, 1955; Hilton, 1955). As discussed earlier, the threat posed by *M. ulei* to plantations in the Old World was recognized at an early stage when Belgrave (1922) speculated on the possible arrival and the potential impact of the pathogen in Malaysia. Due to the short-lived nature of the main infective propagules (conidia), it was considered highly unlikely that the fungus could reach palaeotropic plantations, either through natural or human means. However, the extremely short survival period (15 h) originally proposed (Stahel, 1917) has now been shown to be erroneous and, in low light intensity, the conidia can survive up to 2 weeks (Holliday, 1969). Later, Hilton (1955) analysed climatic data and concluded that conditions in the Far East rubber-growing regions were suitable for pathogen development and, therefore, that, given the proven susceptibility and narrow genetic base of the material, epiphytotics would be inevitable. As Altson (1955) put it: 'the not improbable sequel might be the ruin of the present Malayan rubber industry'.

The fact that *M. ulei* has not reached African and Asian plantations has been attributed to the few importations of rubber germplasm which have been made directly from the Neotropics. Fortuitously, the original introductions had gone through a third country quarantine at Kew Gardens, at a time, of course, when leaf blight was unknown. Nevertheless, the chances of its accidental introduction have vastly increased in the last 20–30 years in line with increasing international trade and communications (Rao, 1973; Edathil, 1986).

Control

Prevention

Obviously, the best form of control is prevention, and Malaysia, in particular, has had long-established legislation in place, designed both to prevent or reduce the chances of importation of the pathogen through stringent quarantine, and to empower its eradication should the disease appear (Altson, 1955; Hilton, 1955). Pamphlets for early recognition of the disease were issued in 1953 and an action plan was developed to deal with any outbreak

area, including a guard belt extending to 400 m. This involved defoliation of the trees with aerial spraying of 2,4,5-T in oil, followed by felling and burning. Use of military-style flamethrowers was also seriously considered (Altson, 1955). These earlier pamphlets have been replaced by a colour brochure detailing the biology and aetiology of the fungus, as well as quarantine measures, advice for travellers and the phytosanitary treatments necessary to eradicate the pathogen from *Hevea* germplasm (Anon., 1986). Most of the new information in this document resulted from the work of Malaysian scientists seconded to the Neotropics. Earlier, Berg (1970) had summarized the quarantine measures in force and the legislation enacted by the various countries belonging to the Inter-African Phytosanitary Commission and the Plant Protection Committee for the Southeast Asia and the Pacific Region, in order to keep out South American leaf blight.

Chemical

Work in Trinidad identified several fungicides, including triadimefon, benomyl, mancozeb and chlorothalonil, which showed protectant as well as suppressant activity against *M. ulei* (Chee, 1978). Large-scale application of some of these compounds, using fogging and helicopter spraying, has been practised in both the Amazonian and Bahian regions of Brazil, often government-subsidized (Chee and Wastie, 1980). However, these authors recommended more basic research to resolve technical problems, especially relating to application. It is still not clear whether or not the use of fungicides is economically sustainable.

Biological

Stahel (1917) described a white fungal overgrowth on the ascostromata of *M. ulei* and assigned it to the genus *Botrytis*. However, an illustration depicts *Hansfordia*-like conidiophores, while others show clear mycoparasitism of the anamorph, with the '*Botrytis*' hyphae entwining and apparently penetrating the *Fusicladium* conidiophores. Hilton (1955) seems to have been the first to suggest that biotic factors could have an influence on the disease and that 'indigenous parasites' may be involved in its control, although he dismissed any role in this for the *Botrytis* sp. It is possible that this fungus is a specific mycoparasite of *M. ulei* and, therefore, that it merits evaluation as a potential biofungicide. Mycological surveys in the forest populations of *Hevea* may yield other exploitable biocontrol agents.

Resistance

The English botanist Richard Spruce has been credited with first drawing attention to the economic possibilities of rubber as a result of his work in Amazonia in the 1850s (Dickenson, 1996; Schultes, 1996), and, on the basis of his collections, six new species of *Hevea* were described (Smith, 1996).

Smith (1996) reflected that, if *M. ulei* should ever reach the Old World, 'a veritable scramble for resistance genes would ensue, and that the early work of Spruce on the taxonomy and distribution of the genus *Hevea* would become critical'. During the Second World War, the USA became acutely aware of the necessity to prospect in South America not only for alternative sources of rubber but also for higher-yielding and disease-resistant *Hevea* material. To this end, a series of experimental stations and survey bases were established in the Neotropics (Langford and Townsend, 1953). The myco-botanist R.E. Schultes undertook the surveys for *Hevea* germplasm, which ultimately spanned a 12-year period. Morphologically distinct, high-yielding and resistant ecotypes of *H. brasiliensis* were identified in the Upper Amazon, around Leticia in Colombia, and Madre de Dios in south-east Peru (Fig. 7.2). Rubber from the latter region, known as Acre fino, was also far superior in quality to that of commercial rubber from the Old World plantations (Schultes, 1970). These collections, including over 350 clonal selections made by R. Seibert in the forests of Madre de Dios, were established at the Turrialba Research Station in Costa Rica. However, in the 1950s, the US State Department decided that the rubber programme was superfluous to requirements, particularly since synthetic rubber was at that time replacing natural rubber, and, as a result, most of the Costa Rican collections were destroyed. As Davis (1997) eloquently and pithily phrased it: 'The clonal garden that had once served as a repository for germplasm of an entire continent was replaced by a field of sugarcane. The last of the Schultes and Seibert collection . . . was destroyed by a forgettable botanist from Scotland. As an act of folly it has few equals in botanical history.'

Witches' Broom Disease of Cacao

Historical

Cacao (*Theobroma cacao* L., *Sterculiaceae*) is endemic to the Amazon basin and, once again, the Upper Amazon region has been identified as its probable centre of origin or genetic diversity (Cheeseman, 1944). However, unlike rubber, there is a clear distinction between the botanical birthplace of cacao and its region of earliest use or domestication, which lies in Mesoamerica (Cuatrecasas, 1964; Schultes, 1984). Shortly after the conquest of Mexico, cacao was being exported to Spain and the foundations of a chocolate industry were laid. By the 17th century, chocolate houses were a popular feature in many European countries. During the 19th century, techniques were developed in The Netherlands and Switzerland to make the product more palatable, particularly by removing excess fat (butter) and adding milk, and thus even more popular. From the 1850s onwards, cacao was extensively planted in the Guianas, as well as in Ecuador, to supplement those plantations already well

established in Venezuela and in the Caribbean islands, especially Trinidad (Purseglove, 1968), and considerable wealth was generated from the crop on both sides of northern South America. The first documented record of a witches' broom disease attacking cacao was from Surinam in 1895 (Stahel, 1919; Baker and Holliday, 1957), although there is anecdotal evidence that witches' broom symptoms were described by early explorers in the Brazilian Amazon many years before (Silva, 1987).

What followed in Surinam, as the disease swept through the plantations, was a catalogue of misidentifications, detailed by Stahel (1915): firstly, as *Exoascus* (*Taphrina*) *theobromae* Ritzema Bos., G. Massee initially agreed with this finding but others suggested *Fusarium* and then *Lasiodiplodia*, although it should be remembered that these mycologists were dealing with only alcohol-preserved specimens. In 1908, the name *Colletotrichum luxificum* van Hall & Drost was proposed, which was finally accepted as the causal pathogen in the book by Massee (1910), *Diseases of Cultivated Plants and Trees*. This gave a thorough description of the disease symptoms, and of the '*Colletotrichum*' causal agent. Incredibly, as it seems now, relatively sophisticated and expensive control measures, including spraying with Bordeaux mixture and pollarding, were in operation throughout the Colony before the aetiology and epidemiology of the disease were known. Later, Rorer (1913) concluded that the causal agent was a basidiomycete fungus, after observing clamp connections in cultures from diseased tissues. Shortly afterwards, Stahel (1915) linked the small, pinkish-red mushrooms appearing on old brooms with the disease, and named the fungus *Marasmius perniciosus* Stahel. Once again, the names of Rorer and Stahel figured prominently in this pioneering work, and, as with South American leaf blight, Stahel's monograph on witches' broom disease (Stahel, 1919) remained the standard reference for many years (Holliday, 1989).

Subsequently, the disease was reported in Guyana (1906), Colombia (1917), Ecuador (1918), Trinidad (1928), Tobago (1939), Grenada (Holliday, 1952), and, most recently, in the Bahia region of Brazil (1989) (Pereira *et al.*, 1990). Almost 50 years previously, in an obscure taxonomic monograph, Singer (1942) had transferred *M. perniciosus* to the genus *Crinipellis* Pat. Although this transfer was acknowledged by Baker and Holliday (1957) in their treatise on witches' broom disease, they argued that the name *Marasmius perniciosus* was so well known that it should be conserved. For this reason, the adoption and common use of the correct binomial is of relatively recent origin and, indeed, some still appear to be unaware of the name change (Willson, 1999).

Causal agent

Crinipellis perniciosa (Stahel) Singer (*Agaricales, Tricholomataceae*)
C. perniciosa var. *ecuadoriensis* (Stahel) Pegler
C. perniciosa var. *citriniceps* Pegler

Pegler (1978) revised the species concept based on new collections on both cacao and liana from Ecuador (Evans, 1978), as well as on original material from Surinam, and recognized three varieties. However, this interpretation has been questioned recently (Griffith *et al.*, 1994), and morphological differences may warrant separation at the subspecies level. Earlier, Dennis (1951) redescribed the fungus (*C.* '*perniciosus*'), based on Trinidadian material, and he also included as a synonym an unpublished species, *Marasmius scalpturatus* Berk. & Curt., from Berkeley's collection in Herb. K, originally obtained from Cuba on 'dead twigs' in 1858. This specimen was re-examined by the present author and the twigs appeared to be malformed. Tissues from this material were later analysed at Kew and the wood structure was found to be characteristic of the genus *Theobroma* (Pegler, 1978). However, witches' broom disease has never been reported from Cuba, but it is possible that this specimen came from long-abandoned and ancient Spanish colonial plantations and that the fungus had been inadvertently imported with planting material, probably from Venezuela, the origin of the favoured Trinitario selections (Purseglove, 1968; Thorold, 1975). Lack of interest in cacao, and of mycologists in Cuba, may explain why the disease has not been recognized since and reported.

Basidiospores are the only infective propagules of *C. perniciosa*. These are liberated during the hours of darkness as the temperature drops and the humidity rises, and they appear to penetrate directly through the cuticle, or via stomata (Stahel, 1919). Infection of unhardened flushes (shoots), flowers and young pods (cherelles) results in hypertrophy and hyperplasia in these actively growing, meristematic tissues, with severe growth abnormalities: vegetative brooms (terminal and lateral); flower or cushion brooms; parthenocarpic and immature pods (strawberry- and carrot-shaped); and distorted or indurated, mature pods (Stahel, 1919; Holliday, 1952; Baker and Holliday, 1957; Thorold, 1975; Evans, 1978; Rudgard, 1989).

The mycelium of *C. perniciosa* initially grows intercellularly, and visible symptoms may not appear until 5–6 weeks after infection; some 6 weeks later, the host tissues die as they become invaded intracellularly. Evans (1980) proposed that the life cycle could be divided into two well-defined, genetically and physiologically independent phases: a biotrophic phase represented by a thick, convoluted or irregular mycelium, without clamp connections, only found *in planta* or in *in vivo* callus tissues, considered to be monokaryotic; and a necrotrophic phase, in which the dying host tissues are invaded by a vigorous, thin, regular mycelium with clamps, considered to represent the dikaryotic condition. Stahel (1915) illustrated both these mycelial forms. On average, basidiomata are produced 5–6 months after tissue death, but this will vary between climatic zones and, typically, in western Ecuador up to a year may elapse between infection and sporulation (Evans, 1981a). It is not surprising, therefore, that opportunistic fungi colonizing the necrotic brooms and pods were originally thought to be the causal agents of witches' broom disease.

In addition to the morphological forms or varieties described above, a complex of physiological races or pathotypes has been identified from liana (*Bignoniaceae*) and solanaceous hosts, and from other *Theobroma* species (Evans, 1978, 1981a; Bastos and Evans, 1985; Bastos and Andebrahn, 1986; Wheeler and Mepstead, 1988; Griffith and Hedger, 1994).

Distribution and impact: actual and potential

Crinipellis perniciosa has a strikingly similar natural range to rubber blight, occurring on wild cacao and other *Theobroma* spp. throughout the Amazon and Orinoco river basins (Fig. 7.3). Almost certainly, the invasions of cacao plantations in the Guianas were initiated from inoculum sources within this lowland forest ecosystem. Baker and Holliday (1957) described and illustrated the symptoms on a range of *Theobroma* hosts. However, it is rare to see conspicuous symptoms, such as vegetative brooms, on wild cacao trees, and

Fig. 7.3. Distribution of *Crinipellis perniciosa* on cacao (as shown by dotted line). The 19th-century record of *C. perniciosa* from Cuba is not included and requires verification. The area between Leticia and Iquitos in the Upper Amazon is the purported centre of origin or diversification of cacao, where resistance to *C. perniciosa* has been reported (Pound, 1943).

parthenocarpic pods (chirimoyas) are the most common and obvious evidence of infection.

In germplasm collections in both Ecuador and Brazil, closely related *Herrania* spp. have also been shown to be susceptible (Evans and Barreto, 1996). In Pará State (Brazil), *Theobroma grandiflorum* (Spreng.) Schum., or cupuaçu, is commonly cultivated and highly prized for its aromatic pulp, which is used in a variety of beverages and dishes, but trees are invariably laden with conspicuously large brooms, justifying its local name of 'mãe da vassoura de bruxa' (mother of witches' broom).

From its appearance in the Guianas just over a century ago, witches' broom disease decimated the cacao plantations in both Surinam and Guyana, and it has been cited as the sole cause of the decline and subsequent abandonment of the once flourishing cacao industry in these countries (Stahel, 1919; Thorold, 1975). For example, Padwick (1956) showed that there was a catastrophic drop in cacao exports in Guyana, from over 70,000 t in 1906, when the disease was first reported, to almost zero by 1923. Witches' broom did not reach Trinidad until 1928, when exports of cacao stood at nearly 60 million lb (30 million kg, Padwick, 1956), and, although its initial impact was relatively slow, with a lag phase of 5–6 years, by the end of the 1930s the disease was significantly affecting cacao production and exports fell to less than 17 million lb (8 million kg) in 1939. During the war years, exports averaged less than 10 million lb (5 million kg) per annum, although additional factors compounded this decline (Thorold, 1975). A similar story emerges from Ecuador, as the vast cacao haciendas of the Pacific region, which produced over 40,000 t of high-quality Arriba cacao in 1915, were invaded by several diseases, including witches' broom, from the 1920s onwards. A decade later, yields amounted to less than 15,000 t (Evans *et al.*, 1977). As Baker and Holliday (1957) pointed out, the Andes proved to be an effective barrier to natural dispersal of the pathogen from its Amazonian range, thereby allowing the cacao industry of western Ecuador to prosper for almost 80 years, based on the susceptible 'Nacional' variety. Its arrival was probably aided by man, as also seems to have been the case in Trinidad (Baker and Holliday, 1957).

Cacao has been cultivated in the Brazilian Amazon for centuries (Silva, 1987) but not on a large scale, although it was apparently the dominant species in floodplain forest of the lower Amazon and is considered to represent the remnants of old plantings (Smith, 1996). *C. perniciosa* is also endemic in the forests of the Amazon and Orinoco river basins of Colombia, Peru and Venezuela, and the disease seems to have progressed and invaded from these relatively isolated foci to wherever cacao plantations were established.

During the 1970s, Brazil opened up the Amazon basin for colonization with the installation of the Trans-Amazonian highway. Cacao was considered to be a priority crop because of its high market value, ease of transportation, ecological benefits and sustainability. The government empowered the

Brazilian cacao organization (CEPLAC) to develop cacao centres ('polos cacaueiras'), from Pará State in the east to Rondonia in the west. With new technology and 'resistant' varieties, the ravages of witches' broom disease were judged to be past history and, as a result of this Amazonian initiative (potentially > 300,000 ha of additional cacao), Brazil would soon take the lead as the world's major cacao producer. This scheme failed badly when the extremely productive Rondonian plantations in particular succumbed rapidly and heavily to witches' broom disease (90–100% pod losses were not uncommon), mimicking the situation in the 'new age' plantations of coastal Ecuador established in the 1960s and subsequently abandoned in the 1970s (Evans, 1981a). Whether this catastrophic loss of resistance was due to environmental factors, virulent pathotypes or inoculum pressure has never been resolved satisfactorily.

The Amazonian venture caused disquiet in Bahia, which was also voiced by the cacao industry in general, since it was argued that increased cacao production in Amazonia would inevitably result in the pathogen reaching the principal cacao-growing region of the Neotropics. 'If this disease would ever hit Bahia's cocoa area its production would decline severely in the following 5 years. This forecast might well cause the market to explode' (IOCC, 1984). From epidemiological data, it was concluded that *C. perniciosa* would not be capable of reaching Bahia through natural dissemination due to ecological barriers (Evans, 1981a), and that the real threat was accidental introduction of planting material, either cacao or the increasingly popular cupuaçu (*T. grandiflorum*). A public awareness campaign was initiated and a *cordon sanitaire* was established in 1978, covering all the major airports and roads out of Amazonia (Pereira *et al.*, 1997). However, in 1989, after more than 200 years of escape, the pathogen caught up with its coevolved host and, as predicted by IOCC (1984), its impact was swift and dramatic, as it spread through the 600,000 ha of almost contiguous cacao. This was despite the bimodal rainfall pattern in Bahia, rather than the unimodal Amazon type, which some observers had claimed would not favour disease development. Initial attempts to eradicate the disease through mechanical removal of brooms, fungicide application (from air and ground), and the killing and burning of infected trees all proved to be unsuccessful and the strategy turned to containment (Pereira *et al.*, 1997). Despite elaborate and costly control measures, *C. perniciosa* has continued its rampage through Bahian plantations, even achieving notoriety in a national soap opera, surely a mycological first! Pre-disease annual yields of over 400,000 t have now been reduced to less than 150,000 t, discouraging farmers and threatening the long-term future of this region as a major cacao producer. The pathogen has almost reached the limits of its neotropical invasion, although the recent arrival of *C. perniciosa* in Panama poses a threat to those cacao-growing countries (Costa Rica, Honduras and Mexico) lying to the north.

Control

Prevention

There can be no doubt that the arrival of witches' broom disease was human-assisted. Whether it was an accidental introduction on infected planting material, and this is feasible given that seed transmission can occur, albeit at an extremely low frequency (Cronshaw and Evans, 1978), or deliberate, as popular belief would have it, will always remain a mystery. Through efficient quarantine procedures and adherence to well-documented technical guidelines (Frison and Feliu, 1989), the need for which was recognized many decades ago when cacao germplasm (especially Pound's Upper Amazon material) was being moved through Kew Gardens on its way to Africa and Asia, movement of *C. perniciosa* to the Palaeotropics is certainly preventable.

Cultural

As described by Evans (1981a), and even more recently by Pereira *et al.* (1997) in Bahia, the difficulties of removing all diseased tissues, and therefore infection foci ('hidden inoculum'), are an impossible task, particularly in mature cacao plantations containing highly susceptible hybrids. However, if tailored to or synchronized with the disease cycle in each climatic region, and carried out judiciously, with the destruction of all prunings, then cultural control can be extremely valuable by helping to reduce inoculum potential and, thereby, increasing the effectiveness of other control measures (see Resistance).

Chemical

Past experiences, notably in Trinidad (Baker and Holliday, 1957) and most recently in Bahia (Pereira *et al.*, 1997), demonstrate that, although certain fungicides are highly active against *C. perniciosa*, the economics and mechanics (particularly timing) of spraying are too challenging for effective and sustainable control. However, it may be economical to limit the target spray to the trunk region in those areas where yields are high and pod production is, or can be, concentrated on the lower trunk (Evans *et al.*, 1977).

Biological

Specific mycoparasites, *Cladobotryum amazonense* Bastos, Evans & Samson and *Lecanicillium acerosum* Gams, Evans & Zare, have been described from basidiomata of *C. perniciosa* collected in the Brazilian Amazon (Bastos *et al.*, 1981; Zare and Gams, 2001). In addition to physically overgrowing the pileus and preventing spore release, *C. amazonense* also produces an extracellular, heat-stable toxin, which lyses the basidiospores and which, potentially, could be exploited as a mycochemical fungicide (Simmonds *et al.*, 1992). However, further progress has been halted in the mire of patenting rights, and interest has now switched to another, newly described, Amazonian fungus, *Trichoderma*

stromaticum Samuels & Pardo-Schultheiss, isolated from cacao brooms (Samuels *et al.*, 2000). This mycoparasite actively colonizes the mycelium of *C. perniciosa* within the broom tissues and inhibits basidioma production. Currently, it is being mass-produced and marketed in Bahia as Tricovab® by the Ministry of Agriculture. This fungus is particularly promising since it is a coevolved parasite of *C. perniciosa* and, as a consequence, is adapted to invading broomed tissues. If problems relating to product quality, formulation and spray techniques can be addressed, there is cause for optimism concerning the future role of *T. stromaticum* as a biocontrol agent of witches' broom disease.

Resistance

The pioneering surveys of wild and semi-domesticated cacao populations by F.J. Pound in the Upper Amazon demonstrated that, as with rubber, there are good sources of disease resistance in this region (Pound, 1943). The resultant clones and their hybrids from these collections formed the basis of new planting in Trinidad, Ecuador and Brazil (Baker and Holliday, 1957; Evans, 1981a), and, at least in the former two countries, may have contributed to a gradual lowering of inoculum potential and thus to reduced disease incidence over the intervening years. Nevertheless, as previously discussed, in certain regions, such as Rondonia, this material has not performed too well. It may, however, still prove to be useful in the long term, especially if highly susceptible clones and their hybrids are identified and removed from future breeding programmes and, ideally, from existing plantations, in order to provide a firm foundation for a broader integrated pest management approach (Purdy and Schmidt, 1996).

Frosty (*Monilia*) Pod Rot of Cacao

Historical

The early history of this disease is still somewhat anecdotal, with reports as long ago as 1851 from the Antioquia region of Colombia of pods being covered by a powdery, velvet-like fungus (Baker *et al.*, 1954; Thorold, 1975). Jorgensen (1970) quotes from the diary of one of the cacao hacienda owners in western Ecuador in 1895 describing similar frosty pod rot-like symptoms – 'pods become white while maturing on the trees . . . inside is watery . . . . The beans are rotten and useless'. At this time, Ecuador was by far the biggest cacao producer and alarm bells must have sounded since one of the foremost cacao experts, C.J.J. Van Hall, toured the cacao-growing region, centred in Los Rios Province, seemingly to report on the disease situation. He delimited two pod disease types: 'mancha' (rot), causing decay of the whole pod; and 'helada' (frost), with abnormal growth of both pods and beans (Van Hall, 1914). Both these conditions can be induced by frosty pod rot and it can be concluded that he was describing different stages of the same disease, and that this was the first sci-

entific report of the 'new' pod disease. However, he failed to identify the causal agent and subsequent attempts are cited by Jorgensen (1970) from two unpublished 1916 reports to the Ecuadorian agricultural board: one identifying it as of cryptogamic origin (a common belief was held that the condition was abiotic, because of a sudden drop in temperature) and the other actually uses the name *Monilia* but determined the cause as *Phytophthora*. Finally, the experienced J.B. Rorer (see previously) was contracted by concerned plantation owners in 1917 to investigate the problem. Following surveys in coastal Ecuador, he forwarded specimens of the pod fungus to R.E. Smith (University of California), who identified it as a species of *Monilia*, close to *M. fructicola* (Wint.) Honey, a serious disease of stone fruit in the USA. Over the next 8 years, Rorer made periodic visits to Ecuador, working around the Quevedo region of Los Rios Province, to monitor the disease and to undertake fungicide trials (Rorer, 1926). For this reason, Quevedo disease, along with watery pod rot ('podredumbre-acuosa'), was one of the first common English names for the disease. No further identification of the causal agent was attempted until specimens were sent from Ecuador by E. Parodi to R. Ciferri – 'a versatile and prolific mycologist and plant pathologist' (Holliday, 1989) – in Italy, who considered it to be an undescribed *Monilia* species, close to *M. seaveri* Reade, which he named *M. roreri* Cif. in recognition of Rorer's pioneering work (Ciferri and Parodi, 1933). He suspected this to represent the anamorph of an unknown *Sclerotinia* species, and, indeed, Wellman (1972) later included it within a section on sclerotial diseases in his treatise on neotropical plant diseases. Investigations in Ecuador during the 1970s led to the hypothesis that *M. roreri* was an anamorphic basidiomycete, based on comparative symptomatology with *C. perniciosa* and the mushroom-like odour of the white pseudostroma formed on and within pods. Subsequent SEM studies of conidiogenesis and TEM sections of hyphae proved this to be the case and a new genus was proposed (Evans *et al.*, 1978; Evans, 1981b). As with witches' broom disease, however, some have shown a reluctance to adopt the new generic name, preferring to keep *Monilia* for the scientific as well as the common name.

Causal agent

Moniliophthora roreri Evans, Stalpers, Samson & Benny
The presence of dolipore septa in the hyphae and the retrogressive development of basipetally maturing chains of mitospores readily separate this fungus from *Monilia* species – ascomycete anamorphs with acropetalous conidial chains – as well as from other genera of mitosporic fungi. The theory that *M. roreri* and *C. perniciosa* are closely related, if not actually two evolutionary branches of the same taxon (Evans, 1981b), is nearer to being proved; preliminary molecular data show them to be very similar, based on comparisons of their rRNA (G.W. Griffith, Ascot, UK, 2001, personal communication).

Conidia are the only infective propagules and penetrate either directly through the epidermis or via stomata. In the field, only pod tissues appear to be susceptible, although, under high inoculum potential in greenhouse conditions, it has been found that germinating beans and shoots can become infected, leading to growth abnormalities (Evans, 1981b). In addition to its ability to cause hormonal imbalances in the host, another characteristic which *M. roreri* shares with *C. perniciosa* is the long incubation period from initial penetration to external symptom appearance, in the form of chlorosis (premature ripening) and necrosis. This ranges *c.* 40–90 days but is dependent on a complex of factors, including: pod age; host cultivar; inoculum density; and even climate. It also explains why Rorer in Ecuador and various investigators in Colombia, even as late as the 1950s, failed to obtain infection from inoculation experiments using detached pods in the laboratory and only short observation periods in the field.

Pods infected at an early (cherelle) stage frequently develop lateral swellings and in extreme cases are totally distorted, symptoms which are indistinguishable from *C. perniciosa* infection. Moreover, internal development of both pathogens follows a similar pattern. In cherelle infections, the beans may fail to differentiate and the pod contents are replaced by gelatinous substances, hence the watery pod rot symptom, while in older pod infections there is overproduction of bean tissue leading to compaction and a denser, heavier pod, which becomes dry or indurated with age. As the infected pods ripen prematurely, the first signs to the farmer that the apparently 'healthy' pod is not as it seems, chocolate brown, irregular lesions appear on the pod surface, rapidly followed by a white pseudostroma, on which develops a cream-coloured, spore bloom, which becomes greyish-tan and powdery with age, imparting a frosted ('helada') appearance.

The period from chlorosis to sporulation is 3–8 days and it is only as the pseudostroma emerges that a positive identification can be made of the causal agent. Up to this point, *C. perniciosa* and *M. roreri* infections are indistinguishable, which may explain previous erroneous records of *M. roreri* (Evans, 1986). The dry, powdery spores are readily dislodged and freely carried in the convection currents and by wind, probably over considerable distances. Conidia have been detected in quantity, particularly during the day (11.00–18.00 h) in volumetric spore traps situated *c.* 1 km from the nearest inoculum source (Evans, 1981b). The spore wall thickens with age and this may account for spore longevity, since viable conidia have been collected from mummified pods in the cacao canopy up to 9 months after infection.

Distribution and impact: actual and potential

During his surveys in Ecuador, Rorer (1926) also recorded this disease on *Theobroma bicolor* Humb. & Bonpl. and *Herrania balaoensis* Preuss. The fungus

had also been found on *T. gileri* Cuatr. in north-west Colombia (Baker *et al.*,1954), which led Holliday (1970b) to speculate that this was the original wild host. *T. gileri* was originally described from north-west Ecuador (in Cuatrecasas, 1964), and a disease very similar to *M. roreri* was described from the pods. The type locality was visited recently by the present author and *M. roreri*-infected pods, showing severe mycoparasitism, were collected. It would seem probable, therefore, that *T. gileri* does represent the coevolutionary host of *M. roreri*, with its endemic range extending from western Ecuador to north-west Colombia. It is likely that most species of *Theobroma* and *Herrania* are susceptible, judging from the results of a survey in a comprehensive germplasm collection in western Ecuador (Evans, 1981b). Almost certainly, *M. roreri* invaded the burgeoning cacao plantations of coastal Ecuador from these forest foci, on the lower, western slopes of the Andean cordillera, sometime during the 19th century. However, the Andes proved to be a powerful barrier to eastward movement and Amazonian Ecuador remained free of the disease until relatively recently (Pound, 1943; Evans *et al.*, 1998). The development of a trans-Andean oil pipeline in the 1970s opened up the previously remote eastern region of Ecuador to colonists and cacao cultivars were imported from western Ecuador; the subsequent arrival of *M. roreri* was inevitable and predictable (Evans, 1981b). Evans (1986) postulated that it was only a matter of time before the pathogen reached the Brazilian Amazon, although progress could be slow due to the scattered distribution of wild and planted cacao from the Napo to the Amazon river. In fact, the pathogen has moved rapidly southwards along the eastern slopes of the Andes to reach northern Peru in 1989 and, by stepwise movements through the isolated cacao-growing valleys of the Huallaga, Apurimac and Ene rivers, it arrived in the Cuzco region in 1996 (Evans *et al.*, 1998). If it moves across the Sierra Madre de Dios, the pathogen then poses a direct threat to the Brazilian plantations in Rondonia, as well as to Bolivian cacao (Fig. 7.4). Previous records in the 1950s of *M. roreri* in both Peru and Bolivia can be discounted, and pod infections were almost certainly due to *C. perniciosa* (Evans, 1981b).

Movement of *M. roreri* from its possible western Colombian forest habitats was much earlier: firstly, eastwards to Zulia State of western Venezuela, probably in the 1940s, and northwards to eastern Panama in the 1950s (Orellana, 1956) and Costa Rica in the 1970s (Enriquez and Suarez, 1978). Dissemination may have been by airborne conidia, but the pathogen was probably introduced accidentally by man into Panama (Orellana, 1956). Thus, *M. roreri* is still on a invasive front, with recent reports of its arrival in Guatemala and Honduras (W. Phillips, Reading, UK, 2000, personal communication), and it now threatens not only Mexico to the north but also Bolivia in the south, as well as the much more extensive and economically important plantations in the Brazilian Amazon and Bahia.

Wherever *M. roreri* has invaded, crop production has been severely affected. From its appearance in epiphytotic proportions in Ecuador (*c.* 1916)

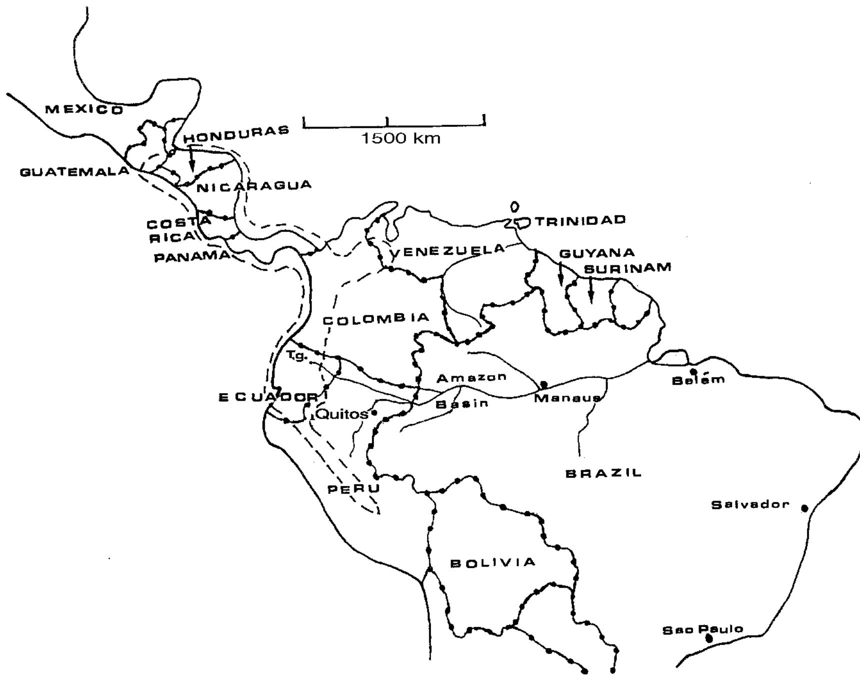

Fig. 7.4. Distribution of *Moniliophthora roreri* on cacao (as shown by dotted line). The records from Nicaragua, Honduras and Guatemala are recent (W. Phillips, Reading, UK, 2000, personal communication). A purported centre of origin of the pathogen on its forest host, *Theobroma gileri*, is marked T.g. on the western slopes of the Ecuadorian Andes.

until the arrival of witches' broom disease (after 1920), annual losses have been estimated at 10,000 t or 20–30% of the total exports. More recent data from several of the principal cacao-growing zones put pod infection due to *M. roreri* at 20–43%, with similar figures being quoted for Colombia, with an annual revenue loss of US$21 million (Evans, 1981b). From its detection in Costa Rica in 1978, cacao production decreased rapidly, with up to 60–90% pod loss (Evans, 1986). Until 1990, the major cacao disease in Peru was witches' broom, with a complex of *Phytophthora* diseases a poor second. The situation changed rapidly and dramatically thereafter, with an overall fall in production of 40–50%, and even total crop loss in some areas, leading to the abandonment of farms (Evans *et al.*, 1998).

There is ample evidence from the eastern Andes and Peru that the pathogen once free of natural barriers moves quickly and efficiently by airborne conidia. Evans *et al.* (1998) estimated that it took 5–7 years to reach north-east Peru from the Napo region of Ecuador, a distance of some 500–600 km. It is not too dissimilar a distance to the Rondonian plantations

from south-east Peru, and, surely, it must be only a matter of time before it invades plantations in the south-east of Mexico from Guatemala. The critical ecological situation in Central America has also been highlighted recently, following a multidisciplinary workshop in Panama. The concern is that cacao growers on the Caribbean coast of both Panama and Costa Rica will abandon their farms due to increasing disease and uneconomic returns. The predicted land use change, from a forest-shaded, perennial crop to annual cash or subsistence crops could have a dramatic impact on the biodiversity of the region. An even darker scenario threatens Peru. If the alternative crops programme fails, and here cacao takes a leading role, then farmers will abandon commodity crops and return to cocao-growing. If *M. roreri* cannot be contained or controlled, then the economic returns from cacao will provide little incentive to continue with this crop.

Control

Prevention

The talcum powder-like qualities of the spores, combined with their longevity, have made *M. roreri* a formidable invader once it escaped the relatively narrow confines of its origin, on the western slopes of the Andes. If there are no extensive geographic barriers then there is little that can be done to prevent the entry of this pathogen into new territories. Evans (1986) considered that natural spread to the Bahian region from a hypothetical Amazonian source is a remote possibility, given that there are over 1500 km of land without cacao and most of this is semi-arid, thorn forest (Fig. 7.4).

The chances of long-distance dissemination by man, however, are considerable because of the cryptic, latent period within the pod, which can fool even professional collectors, as evidenced by interceptions at Kew Gardens (Evans, 1986). Once these ripening pods are opened and found to be 'useless', sporulation on the cut surface is both rapid and abundant. The powdery spores could also adhere to budwood or similar planting material and remain viable for many months. However, since all such material is routinely passed through intermediate quarantine stations, which follow the Food and Agricultural Organization guidelines (Frison and Feliu, 1989), then this route to the Old World plantations has effectively been blocked. Could it cross the Atlantic, as purportedly happened with coffee leaf rust, *Hemileia vastatrix* Berk. & Broome (Bawden *et al.*, 1971)? This is open to debate and speculation.

Cultural

Fulton (1989) pondered on the fact that, since the infective stage of *M. roreri* is limited to those spores produced on the pod surface, then field management of the disease through good cultural practices, particularly crop sanitation,

should be highly effective. He then outlined the reasons why this has not proved to be the case. For example, the cryptic nature of the disease deceives the farmer as to its potential impact on crop loss. If he is not vigilant, then the extremely rapid switch from apparently healthy to necrotic pod, with massive sporulation (estimated at 44 million spores cm^{-2}; Evans, 1981b), creates infection foci which are then difficult to eradicate and, in fact, removal at this stage may only exacerbate the problem as the disturbed pods release clouds of spores. Evans (1981b, 1986) had advocated removal of all mummified pods in the canopy during the intercrop or dry season in order to reduce and delay the build-up of infection during the following season. However, erratic cropping cycles, poor follow-up sanitation (diseased pods must be removed or buried) and contamination from badly managed, neighbouring farms can all prejudice cultural control.

Chemical

As with witches' broom disease, the maintenance of a continuous fungicide cover on rapidly expanding, susceptible pods is a daunting task, illustrated by Rorer's experiences over 8 years in Ecuador as he battled to control the 'new' disease with sulphur and copper products: '. . . These [fungicide] experiments showed that many applications at frequent intervals were necessary to control the disease at all successfully and that the cost of the work was absolutely prohibitive' (Rorer, 1926). Nevertheless, copper-based protectants have proved to be economic in Ecuador when cropping is heavy but have failed to give acceptable returns in Costa Rica (Evans, 1986). Fulton (1989) has advocated the addition of oil to copper formulations in order to delay sporulation, enhance droplet spread and increase residual persistence.

Biological

It is only relatively recently that this control strategy has been explored and two different approaches are being evaluated, both based on the classical biocontrol premise that suitable agents will only be found in the natural range of the target pest. In the search for specific mycoparasites, wild populations of *Theobroma gileri* have been surveyed in remnant primary forest on the western slopes of the Ecuadorian Andes (700–800 m a.s.l.). *M. roreri*-infected pods have been located but the pathogen never appears to be a critical constraint. A range of mycoparasites colonizes the pseudostroma of *M. roreri* on the pod surface, including species of *Nectria* and their *Clonostachys* anamorphs, and appears to impact on and significantly reduce sporulation. The other, ongoing approach is to assess the potential of benign endophytes which have been found within the stem tissues of wild *T. gileri*, and which may confer induced resistance to malign endophytes, such as *M. roreri*, through passive exclusion or antagonism.

Resistance

All *Theobroma* and *Herrania* species would appear to be highly susceptible to *M. roreri*, although some resistance, in the form of reduced lesion size and low sporulation, has been detected in the 'Nacional' cacao of coastal Ecuador (Rorer, 1926), and the hybrids derived from it (Evans, 1981b). Resistance reported in other 'refrectario' trees has been ascribed to disease escape due to a cropping pattern in which most pods develop during the dry season when conditions are unfavourable for the pathogen. Based on this principle, crop manipulation techniques, using artificial stimulation of flowering combined with hand-pollination during the drier months of the year, have given promising results in Ecuador (Evans *et al.*, 1977).

Conclusions

The fungal pathogens comprising this trinity of neotropical plant diseases share many historical and biological traits. All rose to prominence at approximately the same time when countries in northern South America began to exploit their natural resources and cultivate indigenous forest tree species on a large scale, in particular rubber and cacao, mainly for export to Europe. Previously unknown natural enemies, especially fungal pathogens, emerged from the obscurity of their forest habitats to wreak havoc in these new monocultures. Over the intervening 80–90 years, they have been on an invasive front, catching up with their hosts in most if not all the neotropical regions where the crops have been introduced. The socio-economic and ecological ramifications have been and continue to be severe.

Perhaps not surprisingly, the same pathologists and mycologists figure prominently in the early history of these diseases and some, such as G. Stahel and J. Rorer, made outstanding contributions, which, in general, have stood the test of time. Given the uniqueness and complexity of the pathogens involved, and of their disease cycles, it was inevitable that other fungi would be implicated initially as the causal agents. In particular, the cryptic nature of the cacao diseases, with a prolonged incubation period from infection to symptom expression, compounded in the case of witches' broom by an even longer time lag to sporulation, created additional problems. The latter pathogen is now undoubtedly correctly placed in the genus *Crinipellis*, despite the prolonged and illogical resistance to the name change from *Marasmius*, where it had been accommodated for over 25 years. *Crinipellis* species are well represented in the Neotropics (Singer, 1942), and there is increasing evidence that some species are obligate, and benign endophytes. For example, basidiomata of *C. siparunae* Singer have been encountered regularly by the author in Amazonia on the trunks of living healthy trees of the genus *Siparuna*. This taxon was originally described from a *Siparuna* tree growing in the Leningrad Botanical Garden (Singer, 1942) and, undoubtedly, the fungus

had been imported with its host from Brazil. Whereas most endophytes colonize and grow within their hosts without causing abnormal reactions, the swollen, intercellular mycelium of *C. perniciosa* and also that of *M. roreri* appear to have a 'haustorial' or absorption function, sequestering nutrients which diffuse out of host cells. It is postulated that this 'leakiness' is due to the release of fungal metabolites which alter the permeability of the host cell walls, a side-effect of which is to provoke a hormonal imbalance, resulting in gross hyperplasia and hypertrophy (Evans, 1980). The hemibiotrophic nutrition of these two cacao pathogens is also shared by *Microcyclus ulei* and, during the biotrophic phase, tissue malformations are also induced in the rubber host (Fig. 7.1). Indeed, Stahel (1919) illustrated amorphous, haustorial-like structures within the cortical cells colonized by the pathogen. The necrotrophic phase is characterized by the rapid invasion and death of the host tissues by a saprotrophic mycelium. It has been hypothesized, for *C. perniciosa* at least, that these mycelial types are genetically and physiologically distinct (Evans, 1980). If this holds true for *M. roreri*, then meiosis must occur during sporogenesis and, controversially, the conidiogenous cell is, in fact, a modified basidium.

It took nearly 20 years for the frosty pod pathogen to be formally assigned to a genus, and a further 45 years for it to be recognized as an anamorphic basidiomycete and accommodated in a new genus. Hopefully, the story will be completed in the near future, using modern molecular techniques, when its basidiomycete status is confirmed in the genus *Crinipellis*, close to *C. perniciosa* (Evans, 1981b).

The correct taxonomic placement of the rubber leaf blight pathogen has been less problematic, although it took over 10 years to link the teleomorph and anamorph stages and nearly 50 years to determine its relationship to the genus *Microcyclus*. Nevertheless, von Arx (1983) has since expressed reservations about both the teleomorph and anamorph names. A more critical evaluation and comparison of both its morphological and molecular characters may reveal that this pathogen has close affinities with the genera *Mycosphaerella* and *Passalora*.

All three pathogens have destabilized economies wherever they have invaded in the Neotropics and, potentially, their impact in the Palaeotropics could be even more dramatic, none more so than South America leaf blight, which has been recognized for many years as the number one threat to natural rubber production. As Davis (1997) pointed out, the short-sightedness of bureaucrats has meant that the considerable investment made in collecting and selecting sources of disease-resistant material from Amazonia has been wasted, and there is no existing germplasm collection in the Neotropics on which to base a long-term breeding programme. Thus, South American leaf blight of rubber 'continues to hang like a Damoclean sword over the neck of the industrial world' (Davis, 1997).

The knock-on effects resulting from invasion by the cacao pathogens have

proved to be even more diverse and complex. The arrival of *M. roreri* in Central America, for example, has threatened the livelihood of the small-scale cacao farmers in the region. If they should decide to abandon the crop in the face of decreasing yields and low prices, then this could result in widespread deforestation as farms turn to annual cash or subsistence crops. With the loss of these forest corridors, bird migration would be seriously disrupted, perhaps permanently. At the other extreme of its current range in Peru, *M. roreri* is now posing a threat to the alternative crops programme, in which cacao occupies a key position. In the worst-case scenario, the farmers may become disillusioned and return to cocao cultivation as cacao-growing becomes uneconomic. The Peru experience has demonstrated that, in the 'pecking order' of cacao diseases, *M. roreri* occupies the top position, probably by virtue of its massive sporulation capacity, having rapidly ousted *C. perniciosa* as a major constraint to production (Evans *et al.*, 1998). This bodes ill, of course, for Bahia, where witches' broom disease on its own has seriously affected the economic viability of the entire region. Moreover, abandonment of the traditional old cacao farms, which are based on cultivation under remnants of the highly biodiverse Atlantic forest, could result in the loss of these forest refuges, home to many unique plants and animals.

Unlike rubber, however, invaluable cacao germplasm collections still exist, thanks largely to the efforts of the chocolate industry. However, their future is uncertain and, if the UK apple germplasm collection can be threatened with extinction, as it was some years ago, the risk to cacao collections must be that much greater. The long-term strategy of breeding for resistance to both diseases should eventually benefit Latin American, as well as African cacao-growing countries, if either of these pathogens should ever cross the Atlantic.

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Lichens of Tropical Forests 8

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Introduction

The tropical zone occupies about one-fifth of the earth's surface between the Tropic of Cancer and the Tropic of Capricorn. In this zone, tropical forests have been the dominant vegetation over large areas of the landmass since the Tertiary period. The forests extend in altitude from coastal mangroves, to lowland areas supporting tropical rainforest, to montane cloud forest, and in latitude from the tropical rainforest with *c.* 2000–3000 mm year^{-1} of rain northwards to deciduous savannah forests with extended dry periods in a monsoon climate. Of the 5 million ha of land area in this zone, *c.* 37% is still forested, despite an annual deforestation rate of 15.4 million ha or 0.8% (Food and Agriculture Organization of the United Nations (FAO),1997).

Total Numbers of Species in the Tropics

The diversity of tropical forests is well established for groups of larger organisms but for fungi and their lichenized counterparts there are still enormous gaps in our taxonomic and ecological knowledge. Aptroot and Sipman (1997) estimated that between one-third and one-half of the world's lichen diversity occurs in the tropics, but more precise figures or estimates are currently impossible to give because of the lack of suitable checklists. Even for extra-tropical regions the figures are difficult to give. *Ainsworth and Bisby's Dictionary of the Fungi* (Kirk *et al.*, 2001) accounts for 17,000–20,000 species of lichenized fungi (most of which are ascomycetes), but other estimates range

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from 18,000 (Nash and Egan, 1988) and 17,000–20,000 (Galloway, 1992), to 20,000–30,000 (Aptroot, 1997b).

The latest North American checklist has *c.* 3400 species (Egan, 1987), and the equivalent figure for Europe is probably slightly higher, given the more extensive coverage. Although there is no European checklist, most European countries have recent lichen checklists available in print or from web sites. Approximate species totals are: Austria (2100), British Isles (1760), Finland (1500), Germany (1700), Italy (2100) and Norway and Sweden combined (2300). These totals are remarkably similar to the estimated totals of 1500–2000 and 2000–2500 for Papua New Guinea (PNG) and Colombia, respectively (Aptroot and Sipman, 1997).

How reliable are these estimates for tropical regions? Aptroot (1997a) stated that 1079 species were reliably reported for New Guinea, but that a further 300 species were as yet unidentified among the collections made by himself and his colleagues. A brief and probably not totally comprehensive search of recent lichen literature for 1997–1999 revealed the addition of 105 species for New Guinea, including 55 species new to science; these figures exclude the report of many new additions by Aptroot *et al.* (1997), which were presumably included in the '1079'. A major contribution to these additional figures concerns *Pertusaria*, a large but hitherto little studied genus in tropical regions. A revision of Australasian species added 48 species to the lichen flora of PNG, 31 being new to science (Elix *et al.*, 1997; Archer and Elix, 1998a,b). Other important papers concerning the numbers of lichens in New Guinea are studies on *Parmotrema* (18 added species, six new to science) by Louwhoff and Elix (1999) and of miscellaneous genera (20 added species, three new to science) by Aptroot (1998). Given that the net additions (taking account of synonyms) to the 'well-known' British lichen flora have continued to average about ten species year^{-1} since the mid-1960s, the average of 35 species year^{-1} for 1997–1999 should easily result in an accepted total for New Guinea of exceeding 1500 by the year 2013.

Analyses of tropical lichen floras in most countries are difficult to do, given the dearth of modern checklists or 'floras'. Since 1997, the only checklists to appear for anywhere in the tropics are for Hong Kong (Aptroot and Seaward, 1999) and a catalogue of the lichens of the smaller Pacific Islands (Elix and McCarthy, 1998). However, checklists are in preparation for Colombia, Ecuador and Thailand, and the latest checklist for Australia (Filson, 1996) includes tropical species but does not specifically identify them. The Hong Kong list included only 261 species, but 176 were new to Hong Kong, 132 were new to China, and 43 were new to East Asia. Aptroot and Sipman's (1997) estimate that 50% of the tropical lichen flora is still unknown is probably a fair estimate overall, but for most tropical countries the percentage is far higher.

Site Diversity

'Within-site' diversity of lichens is surprisingly similar in both tropical and temperate conditions. Aptroot (1997a) reported 400–450 species (from all habitats) for each lowland, intermediate and high-altitude area in PNG. These species' totals equate well with recent site surveys carried out in areas little affected by atmospheric pollution in Scotland: 402 from Craig Clunie and Lion's Face Site of Special Scientific Interest in the North East Highlands (Coppins and Coppins, 1999), 467 from Glen Shira in the West Highlands (Coppins and Coppins, 1996), and 466 from the high ground of Ben Lawers National Nature Reserve in the Central Highlands (Gilbert *et al.*, 1988; Fryday, 1989). In all situations lichen diversity drops rapidly where forest destruction and/or atmospheric pollution is occurring.

Substrata

Although in temperate and boreal zones a large proportion of the lichen diversity is associated with terrestrial and saxicolous substrata, in tropical climates the highest lichen diversity is found among epiphytes. Where temperatures and rainfall are relatively stable for much of the year, foliicolous lichen diversity may be very high, contributing to *c.* 30% of the total lichen flora on trees and shrubs in lowland tropical rainforest (Aptroot, 1997a). In seasonal deciduous forests, diversity is highest on corticolous substrata, and foliicolous species are found only along watercourses (Wolseley and Aguirre-Hudson, 1997a). Within a tropical rainforest, much of the corticolous diversity is not readily accessible as the diversity increases markedly with distance from the ground. Sipman (in Gradstein *et al.*, 1996) pointed out that a reliable canopy inventory cannot be carried out by sampling fallen branches, because most of these branches are from the lower canopy and soon become covered in saprotrophic moulds. The best results are obtained from the study of recently fallen, or felled, trees, or by cutting and carefully lowering down selected branches (ter Steege and Cornelissen, 1988). Aptroot (1997a) recorded 173 lichen taxa on a recently fallen *Elaeocarpus* tree. Of these only 41 (24%) occurred on the lower trunk below 5 m. A recent, somewhat more sophisticated, technique has been employed in Project Surumoni in a tropical lowland rainforest in southern Venezuela, using a 40 m high tower crane mounted on a 120 m long railway, with a 40 m jib-boom (Komposch and Hafellner, 2000a). From their study of nine trees in a single 1.5 ha plot, Komposch and Hafellner (1999, 2000a) recorded 268 species. Of these only 9% occurred on the lower trunk, with the most diverse zone being the middle canopy. Accordingly, they believed that Aptroot and Sipman's (1997) estimation that the 'total number of corticolous and foliicolous lichens per km^2 of tropical rainforest may well be over 300' was cautious, and that the figure should be changed to 300 species ha^{-1}.

Foliicolous lichens

Evergreen leaves form a constantly renewable surface for colonization by both lichens and bryophytes, and, as the specimens have been more accessible than many epiphytes of tropical forests, advances in this area were made relatively early on. Santesson's (1952) monumental treatise on the taxonomy and identification of foliicolous lichens was a landmark, in which he reduced the accepted *c.* 1000 species to 250 and provided workable keys. With this foundation, other lichenologists, especially Antonin Vezda, Emmanuël Sérusiaux and Robert Lücking, have increased the number of foliicolous lichens to beyond 600 species, and have also provided good illustrations and updated keys to genera and species where necessary. A comprehensive reference list to the taxonomic works on foliicolous lichens is provided by Farkas and Sipman (1997) and the more important subsequent works are by Aptroot *et al.* (1997); Lücking (1997a,b, 1998a, 1999a); Lücking and Vezda (1998); Lücking and Cáceras (1999). An additional advantage for studying foliicolous lichens is that samples are easily collected – on the 'micro' scale, a whole substratum and its community of colonizers can be quickly plucked into a collecting packet for comfortable examination in the laboratory. However, this is countered by the need for good microscopical equipment and great patience and skill in sectioning and examining the individual components of the community! For ecological studies, experience is needed, in order to reliably identify juvenile, poorly developed, parasitized or sterile thalli. Taxonomists embarked on purely taxonomic studies have the luxury of discarding poor specimens, but this possibility is denied them if carrying out ecological investigations.

Saxicolous lichens

Although rich saxicolous lichen communities, and associated terricolous lichens, can be found on some high mountains within the tropics, rocky habitats within the rainforest zones are usually dismissed as 'poor'. The rocks are usually either too shaded for lichens or, if exposed, too eroded or sun-baked. Also, where rock surfaces are in an appropriate condition for lichen colonization, the flora is often composed mostly of normally corticolous species (e.g. species of *Graphidaceae*, *Thelotremataceae*, *Parmeliaceae*, *Heterodermia* or *Pertusaria*). However, careful scrutiny for suitable niches can be rewarding. The specialist lichens of rocks in and alongside streams and rivers in temperate regions are reasonably well known and appreciated, but in tropical rainforests have scarcely been mentioned. During a short stay in Khao Yai National Park, Thailand in 1997, such lichens were found within the evergreen rainforest zone. These included the widely distributed *Endocarpon adscendens* (Anzi) Müll. Arg., the recently described *Ionaspis tropica*

Aptroot from New Guinea, undescribed species of *Staurothele* and *Verrucaria*, and three new species of *Porina* (Boonpragob *et al*., 1998; McCarthy, 1999). Although, admittedly not likely to be as 'rich' as their temperate counterparts, the rocky habitats within rainforests have potential and should not be ignored. Their previous neglect could be a combination of such simple factors as the explorers being overwhelmed and diverted by the richness and newness of the corticolous and foliicolous habitats, and by not having a hammer and chisel in their collecting bag!

Taxonomic Literature and Identification Keys

In many areas the literature is still poor. There is an urgent need for taxonomic revisions of families of tropical lichens, particularly large families of crustose taxa, such as *Graphidaceae* and *Thelotremataceae*, both of which contribute significantly to biodiversity and may also be indicators of environmental conditions. These two families contain *c*. 1000 and 800 species, respectively. Some advances are being made in this direction, with the recent revision of *Acanthothecis* (*Graphidaceae*) by Staiger and Kalb (1999), although several studies are not yet published. A study of 767 collections of *Thelotremataceae* from Thailand and Malaysia by Homchantara (1999) recognized 124 species, 35 of which were unnamed and perhaps undescribed. Klaus Kalb and his students are carrying out further studies on these two families, including one on African thelotremes by Andreas Frisch.

A survey of the taxonomic literature on lichens from 1997 to 1999 revealed from 40 publications that 193 new species had been described from the tropics, including new species of macrolichens (e.g. Pooprang *et al*., 1999). Excellent though most of these papers are, only 14 of them provide keys to the genera or groups concerned. Global keys are given for *Acanthothecis* (Staiger and Kalb, 1999), foliicolous *Arthoniaceae* (Ferraro and Lücking, 1997), foliicolous *Chroodiscus* (Santesson and Lücking, 1999), *Fellhanera* p.p. (Lücking, 1997c), *Porina epiphylla* group (Lücking and Vezda, 1998) and the *Trichotheliaceae* (Lücking, 1998a). Regional keys have been provided for: foliicolous *Coenogonium* and *Dimerella* (Lücking, 1997a), *Lobaria* (Yoshimura, 1998) and *Peltigera* (Vitikainen, 1998) in the neotropics, the *Gomphillaceae* in Costa Rica (Lücking, 1999a), and *Anisomeridium* and *Pyrenula* (Aptroot *et al*., 1997), *Parmotrema* (Louwhoff and Elix, 1999), *Pertusaria* (Archer and Elix, 1998a) and *Stereocaulon* (Sipman, 1998) for New Guinea.

These keys are much welcomed but were written for the specialist. There are no widely available, illustrated keys to genera for the benefit of the generalist and the potential specialists of the future, especially those residing in tropical countries. The latter may well have been able to acquire the necessary microscopical and chemical equipment, and have full access to the Internet, but progress with identification is hampered by the lack of

literature. The production of a user-friendly, illustrated identification manual for the genera of tropical lichens should be considered a priority by the lichenological community. Such a manual would perhaps be best available from a web site, so as to be easily updated, and to avoid currency problems. An Internet location (www.mycology.net/lias/index.cfm) for specialist keys already exists in the form of LIAS (The Global Information System for Lichenized and Non-lichenized Ascomycetes), coordinated by Gerhard Rambold at Munich, and monographers are urged to contribute their data for the benefit of all.

Taxonomic studies, often undertaken in a rather piecemeal fashion, with short-term grants, would be much enhanced by long-term, well-coordinated, reliably funded international programmes. Lichenologists should consider setting up international specialist groups, as exist for many families of vascular plants, to improve communications and provide a better focus for funding.

Recent Ecological Studies on Lichens in Tropical Forests

The structural diversity of habitats in tropical forests was outlined by Richards (1984), related to cryptogams by Gradstein (1992) and described for canopy specialists by Rhoades (1995). The contrast between the dense shade of the ground and lower trunks and the exposed canopy branches is extreme. In the intermediate zones, there may be a great number of ecological niches with characteristic lichen communities. In the outer canopy, lichens may have to withstand high UV levels and also be subject to extremes of wetting and drying. In contrast, the trunk receives very low light levels, but humidity is ± 100% most of the time. Lichens, such as *Trypetheliaceae*, that dominate the outer canopy are often highly coloured by anthraquinones and other secondary products, which act as a sun and radiation screen for the photobiont, whereas lichens on the bole are pale or dark coloured and often have hydrophobic secondary products, which protect their surface from excess water (Kantvilas *et al.*, 1985; Wolseley, 1997). The high diversity along the vertical gradient is reflected in the species turnover (beta diversity) of rather distinct communities adapted to the micro-conditions. Cornelissen and ter Steege (1989) distinguished 13 cryptogamic communities along a vertical gradient in evergreen forest in Guyana. At high altitudes the forests have less vertical stratification and macrolichens are a conspicuous feature of the forest, whereas in lowland rainforest the diversity is dominated by crustose species, many of which present problems of identification. For this reason, much lichen research concentrated on high-altitude zones (Wolf, 1993a,b; Pentecost, 1998). The importance of crustose communities in the lowland rainforests of Guyana has been documented by Montfoort and Ek (1990), and changes in crustose communities from trunk to canopy in a lowland forest in Venezuela have been investigated by Komposch and Hafellner (2000a), who characterized six lichen zones, all of which were dominated by crustose

species, with foliose species occurring only on the lowest trunk (zone 1) and in the well-lit middle canopy of zone 5. There was no species overlap between zones 1 and 6, whereas between zones 1 and 2 there was a 23% species similarity. The pattern throughout lowland rainforests is remarkably similar, with minor variations according to local conditions (Sipman and Harris, 1989; Wolseley *et al*., 1998; Komposch and Hafellner, 1999), with *Thelotremataceae* dominant at lower levels together with *Crocynia*, *Eschatagonia* and species of *Porina* (*Trichotheliaceae*). Species of *Graphidaceae* and *Arthoniaceae* increase towards the upper zones, and in the outer canopy *Pyrenulaceae* and *Trypetheliaceae* become the dominant families. Crustose species increase in diameter with age and in undisturbed forests a single thallus may be a metre or more in diameter and encircle the trunk, suggesting that a lichen may colonize a young tree and grow with the tree (Fig. 8.1). On slow-growing trees the circumference is round, but on fast-growing trees the lichen thallus is stretched into an ellipse (Wolseley and Aguirre-Hudson, 1997a).

Fig. 8.1. Large smooth-barked emergent tree in the 50 ha Forest Dynamic Plot at Pasoh Forest Reserve at Negri Sembilan, Malaysia, showing large rounded thalli of *Thelotremataceae* and *Graphidaceae*.

Reproduction

Research on foliicolous lichens has led the way in ecological studies of lichens in tropical forests, due to accessibility of the habitat and the sound taxonomic basis available. In a study of the community ecology of foliicolous lichens, Lücking (1999b) demonstrated two major groups governed by microclimatic factors: one characteristic of the shady understorey and the other confined to light gaps. The shady understorey community is dominated by species of the *Arthoniaceae*, *Opegraphaceae*, *Trichotheliaceae* and *Pilocarpaceae*, which predominantly have photobionts of the *Trentepohliaceae*, thin thalli, abundant sexual reproduction, small ascospores produced in high numbers and pycnidial conidiomata. The light-gap community is dominated by species of *Gomphillaceae* and *Ectolechiaceae*, with *Trebouxia* as photobiont, thickly crystalline or whitish, dispersed thalli, frequent asexual reproduction, large ascospores produced in low numbers and specialized conidiomata, such as campylidia and hyphophores. The complexities involved in interpreting the ecological significance of these reproductive mechanisms are discussed in detail by Lücking (1999b), and are clearly a fascinating area for further research. It is well known that the proportion of species producing large, multi-celled ascospores is much higher in the tropical forests than in temperate or boreal regions (Sipman and Harris, 1989), but the ecological interpretation of this is far from clear. It is further complicated by observations that large, muriform ascospores are often secondarily divided into minute simple conidia within the asci (e.g. Hafellner and Bellemère, 1983; Sipman and Harris, 1989), or following release (as in *Agonimia pacifica* (Harada) Diederich).

Of the 268 species collected from nine trees in lowland rainforest at Surumoni, 94% were crustose species and 90% reproduced sexually, including 12% that also produced vegetative propagules (Komposch and Hafellner, 2000b). Komposch and Hafellner (2000b) further found that asexual propagation was highest at ground level (60% of observations), and continuously decreased upwards, being absent altogether in the outer canopy. In plots in the more open seasonal deciduous forests of northern Thailand, Wolseley (1997) found that asexual propagules were more frequent in fire-damaged areas, and that in undisturbed forest sexual reproduction was more frequent. On 20 trees in two 100 m plots in fire-damaged deciduous dipterocarp forest, 62% and 85% of lichens on trunks (up to 3 m), respectively, produced propagules, whereas in a dipterocarp forest protected from fire for 23 years, 53% were reproducing sexually, and in unburned seasonal forest, 58% of lichens were reproducing sexually (Wolseley, 1997).

Lichens as Indicators of Ecological Continuity

Detailed studies in the British Isles and other parts of western Europe revealed

that many species were confined to woodlands with a long ecological continuity and presence of mature trees. From these observations, lists of 'old forest indicator' species were drawn up, and indices of ecological continuity devised (Rose, 1976, 1992). The most important species in the fagaceous woodlands and forests of temperate western Europe are found in epiphytic communities belonging to the *Lobarion pulmonariae* alliance. Wolseley (1991) demonstrated the same pattern of association with ancient, little-disturbed forest for similar 'Lobarion' lichens in montane fagaceous (hill evergreen) forest zones in South-East Asia. These 'old forest' lichens are tolerant of some forms of management, e.g. conversion to wood pasture in Europe, and traditional slash and burn in Asia. However, they are intolerant of more drastic management, such as clear-felling or extensive burning. Very few of these 'old forest' lichens are to be found in secondary forest or in formerly cleared forest that has been re-established with native trees, even after a century or two. This is perhaps not surprising, as many of these lichens require the bark substratum provided by mature or ancient trees, and they are intolerant of dense shade as would occur following a coppice cycle or secondary forest development.

What is more surprising is that some foliicolous lichens are equally good indicators of ecological continuity, given that the lifespan of their substratum is limited to rarely more than 2 years (Lücking, 1992, 1995). From 99 sites within the rainforests of Costa Rica, Lücking (1995) compared species and 'form' diversity with environmental conditions, including leaf age, phorophyte (host) identity, seasonality, light intensity, relative humidity and altitude. The highest species number was found at low-altitude sites with a slight dry season, and these were in primary forest. The species total for primary forests was 283, with secondary forest supporting little more than half that number, and anthropogenic vegetation (plantations and fruit trees) 78 species. The results of further ecological studies have followed (Lücking, 1998b,c) and, with the information gained, Lücking (1997d) analysed the use of foliicolous lichens as bioindicators. He proposed that their use as indicators of altitudinal zonation and seasonality was rather restricted but that they had great potential as indicators of anthropogenic disturbance and microclimatic conditions.

Lichens as Indicators of Changes Induced by Fire

In the seasonal climate of Thailand, deciduous dipterocarp forests are widespread, often occurring in a mosaic with evergreen forests. Fire occurs naturally in the dry season, but its incidence has been increased by man, causing a shift in the balance of forest types in favour of the fire-tolerant deciduous dipterocarp forests. The lichen communities of these forest types were found to be markedly different in composition at the family and generic levels, the evergreen forests being dominated by lichen families with trentepohlioid algae,

while the deciduous dipterocarp forests were dominated by families with trebouxioid algae. Species diversity was high in both types of forest where ecological continuity was maintained, but, where there had been an environmental shift from evergreen to deciduous forest, lichen diversity was very low, with a marked absence of species characteristic of 'natural' deciduous dipterocarp forest, and the remnants of evergreen forest had species-poor communities. In sites where the deciduous forest was native and of long-standing continuity, lichen communities were highly diverse, with many species adapted to conditions of high light intensity, temperature and UV radiation, some having characteristic, highly coloured anthraquinone and xanthone pigments (Figs 8.2, 8.3). Conversely, in the evergreen forests the lichen communities were more characteristic of those found in rainforests throughout South-East Asia, and dominated by the *Thelotremataceae* pyrenocarps, and species of *Crocynia*, *Eschatagonia* and *Phyllopsora*. The disjunct nature of these communities, with adaptations to very different environmental conditions, suggests that the species-rich communities of both forest

Fig. 8.2. Fire-tolerant *Dipterocarpus obtusifolius* Teysm. ex Miq. in dry dipterocarp forest (DDF) in Doi Suthep National Park, Chiang Mai,Thailand, showing conspicuously coloured mainly foliose lichens *Pyxine coccifera* (Fée) Nyl. (red) and *Relicinopsis rahengensis* (Vain.) Elix & Verdon (yellow-green) above the fire zone.

Fig. 8.3. Quadrat on *Dipterocarpus tuberculatus* Roxb. in DDF in a protected area at Wat Phalad in Doi Suthep NP, showing high lichen diversity with a mixture of highly coloured fire-tolerant and pale-coloured fire-sensitive species, and presence of fertile crustose species.

types evolved under different climatic conditions. Those of the deciduous dipterocarp forests probably developed in the drier glacial periods of the Holocene when fires were frequent, and those of the evergreen forest are an extension of evergreen forest that was widespread in the Tertiary (Wolseley and Aguirre-Hudson, 1997b).

Lichens and the Ecosystem

The dominance of lichens in the montane forest contributes considerably to the biomass of the forest (Pentecost, 1998). The role of cyanobacteria in nitrogen fixation may also be important in these montane areas of high rainfall where soil nitrogen is rapidly leached (Forman, 1975). However, the importance of lichens to other microorganisms is still little understood.

Recent research in Borneo demonstrated a preference of the lichen-feeding termite *Hospitalitermes* for large emergent dipterocarps (Fig. 8.4). A

Fig. 8.4. Termites, *Hospitalitermes hospitalis* Haviland, feeding on lichens on the trunk of a tree in a recently logged rainforest site, Tabalong district, South Kalimatan, Indonesia. The termites leave their nest in the afternoon and travel in columns across the forest floor until they find a suitable tree on which to forage. The termites climb the tree and forage throughout the night, with workers returning to the nest carrying balls of food held in their mandibles.

colony will feed on lichens and bryophytes in the canopy of one tree for *c*. 4 days, chopping the epiphytes into food balls and carrying these back to the nest, before moving on to another tree (Jones and Gathorne-Hardy, 1995). Investigation of the food balls has identified lichen spores (Collins, 1979), and bryophyte, lichen and algal tissue. Loss of dipterocarps from logged forest may cause *Hospitalitermes* to feed on lichens and on other bryophyte and algal tissue on other trees. In recently logged forest in Kalimantan, *Hospitalitermes* was feeding on a range of crustose lichens on many different species and ages of tree, leaving highly grazed patches of crustose lichens with only fungal tissue remaining (Wolseley *et al*., 2001; Fig. 8.4). Other canopy feeders on lichens are oribatid mites, which are recorded in very high densities of 1000 to 10,000 mites m^{-2} of leaf in rainforest canopies in Queensland (Walter and O'Dowd, 1995). Apart from the food source, some species are known to camouflage themselves with lichen propagules (Stubbs, 1995). The role of both organisms in the lateral and vertical transport of lichen propagules is important in tropical rainforests, where there is often very little wind below the outer canopy and only rain contributes to the downward transport of propagules.

Forest Management and Factors Affecting Lichens

Although the loss of tropical forests is now occurring in all forest zones from lowland to montane, there is still little research on the effects of this on diversity of cryptogamic or invertebrate populations. Loss of lowland rainforest is still mainly due to logging, and between 1981 and 1990 averaged 4.6 million ha annum^{-1}; in moist deciduous forests the rate of loss was 6.1 million ha annum^{-1}, and in the dry forests, where there are few valuable timber trees, the loss was 2.2 million ha annum^{-1}. Montane and hill forest do not contain many useful timber trees, but a loss of 2.5 million ha annum^{-1} has been recorded (FAO, 1997), mainly due to the increasing demand for agricultural land.

Logging has become an increasingly technical process, dependent on large machinery and road access to all sites. In order to obtain selected timber trees, many other trees are lost or damaged. What effect does this have on cryptogamic diversity? In an area of lowland forest in Kalimantan, where the forest had been clear-felled, then slashed and burned for agricultural land for 1–2 years, and converted to plantation forest of exotic species, there was almost complete absence of forest species of lichens, bryophytes and termites. This was in marked contrast to a site logged in 1999, where lichen and termite diversity was not much lower than that of a plot logged 17 years before. In the 1999 plot, the topography was complex, so that pockets of forest remained, contributing to the availability of propagules, and, with increased light on the trunks, many of the canopy species were able to invade sites lower down the trunks. In the 1950s, selective logging of a site in Malaysia adjacent to a 50 ha plot of old-growth forest had encouraged the retention of trees as seed trees. In 1996, these trees were surrounded by a rapidly regenerating stand of young dipterocarps with a considerable diversity of lichen species, albeit few specialist species (Wolseley *et al.*, 1998). Although there is a need to describe primary site diversity in tropical forests and to define and protect the areas of outstanding species richness, there is also an urgent need to assess management practices in tropical forests so that best practices can be established.

Fire is also an increasing hazard in tropical forests that are not fire-adapted, especially in montane evergreen forests or in lowland forest established on peat. The increased use of fire by man has brought about the spread of deciduous forest at the expense of the evergreen forest in areas of monsoon climate with an extended dry period. Lichen species of the original forest are lost and, as lichens of the new forest type colonize slowly, the species composition of epiphytic lichens can be used to chart the time period over which the change has been occurring (Wolseley and Aguirre-Hudson, 1997b: Fig 8.5). Lichens are well established as indicators of the ecological continuity associated with old-growth forests in temperate Europe and America, and recent work suggests that this will be similar in the tropics, but there is still a

Fig. 8.5. Quadrat in fire-damaged seasonal evergreen forest in Huay Kha Khaeng Wildlife Sanctuary showing invasion by *Pyxine consocians* Vain. following death of the pyrenocarp.

considerable amount of taxonomic and survey work to be done to establish which species can be used to detect ecological continuity in tropical forests and to define indicators of environmental change.

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The Importance of Invertebrate-pathogenic Fungi from the Tropics

9

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Introduction

Fungal pathogens of invertebrates (mostly Insecta and Araneae are considered here)[1] have assumed increasing importance as potential biological control agents and as a source of novel secondary metabolites for industry in the last 20 years (Nisbet and Porter, 1989; Nisbet and Fox, 1991; Rossman, 1996). Despite this, little is known about the basic biology of these fungi. Samson *et al.* (1988) provided an overall picture of 'entomopathogenic fungi', with chapters on topics such as taxonomy, pathogenesis, ecology and biology, biotechnology and biological control. Consequently, these authors provided the most modern synthesis of the broad subject, and yet this work pre-dated the revolution provided by the development of molecular phylogenetic analysis as a tool for clarifying evolutionary relationships.

Importantly, in the last 10 years the significance of fungi in assessments of global biodiversity has also increased, largely due to Hawksworth (1991), who produced estimates of fungal diversity based on an analysis of a wide range of literature. At that date *c.* 68,000 species of fungi were considered to have been described for the world. Compared with plants and animals, where it is acknowledged that more than 85% of species diversity have been named, the fungi come poorly down the list with < 5% having been reliably named. The Hawksworth estimate of 1.5 million has often been considered conservative, although it has been challenged and reduced to one-third of that figure

[1]In this chapter, I refer to invertebrate-pathogenic fungi in recognition of the many species that are pathogenic to spiders and mites.

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(A. Aptroot, Hong Kong, China, 2000, personal communication). Even at one-third, there is still > 85% of global fungal diversity hidden from current view. For invertebrate-pathogenic fungi there is every indication that our knowledge of such fungi is even more poorly realized, and that these may contribute a still further hidden and large element of the overall fungal diversity on our planet. The aim of this chapter is to examine this component from the standpoint of the tropics, where fungi are a major component of biodiversity (Hawksworth, Chapter 1, this volume).

What are the Invertebrate-pathogenic Fungi?

Apart from work on a few species with broad host ranges, there are few studies that have fully examined details of pathogenesis in invertebrate-pathogenic fungi. Where studies have been done, these have been limited to a few 'model' species such as *Metarhizium anisopliae* (Metschn.) Sorokin, *Beauveria bassiana* (Bals.) Vuill. and *Nomuraea rileyi* (Farlow) Samson. For the rest, it is assumed they have a truly pathogenic life cycle, based on their restricted host ranges and relationships to those which have adopted a wider host range and have lent themselves to laboratory study. Invertebrate-pathogenic fungi include seemingly highly coevolved pathogens (e.g. *Cordyceps*), broad-range opportunistic pathogens (*M. anisopliae*) and opportunistic necrotrophs (*Conidiobolus coronatus* (Costantin) Batko).

Within the fungal kingdom at least five 'hot spots' have evolved associations with invertebrates. These are:

- *Zygomycota – Entomophthorales*
- *Zygomycota – Eccrinales*
- *Ascomycota – Laboulbeniales*
- *Ascomycota – Hypocreales*
- *Basidiomycota – Septobasidiales*

In keeping with modern phylogenetic studies, invertebrate-pathogenic *Chytridiomycota* and *Oomycota* are excluded, but see Samson *et al.* (1988). It seems that, when the jump is made from a presumptive plant host to an invertebrate host, there is great opportunity for rapid speciation of the fungi as they seek out new invertebrate hosts. This, not surprisingly, can be accounted for by the fact that invertebrates are the largest component of diversity on the planet.

Within the five 'hot spots', the ascomycete order *Laboulbeniales* is completely invertebrate-associated and currently numbers *c.* 2000 species. Few systematic studies have been made in the tropics and undoubtedly many new species of *Laboulbeniales* are waiting to be found here (Tavares, 1985; Weir and Hammond, 1997). A significant limit to further taxonomic work on this order is the present lack of culturability and opportunities for *in vitro* study.

With increasing emphasis on the use of molecular phylogenetics in many areas of systematics, this is a hindrance. However, this need not be limiting in the future, as techniques are available for securing DNA from minute samples, albeit at a cost. Further, it must surely be only a matter of time before efficient and routine methods are devised by which *Laboulbeniales* can be cultured *in vitro*, providing DNA in larger amounts.

A second, similarly species-rich 'hot spot' is the *Septobasidiales* (*Basidiomycota*). These are wholly restricted to one insect order, Homoptera, and are furthermore restricted to the immobile larval stages of scale insects. However, the large number of species recorded for the *Septobasidiales* is an indication of their restricted host ranges. As with the *Laboulbeniales*, absence of routine methods of isolation is currently a hindrance to further work on this order. The monograph of Couch (1938) still stands as a defining text on the subject. Although tropical to subtropical in distribution, there has been no modern systematic study of the order. This is long overdue.

A third 'hot spot' is the *Entomophthorales* (*Zygomycota*), which stands out as an order with many invertebrate pathogens. The name of the order means 'insect destroyer'. Most of the work on these has been from temperate (specifically North American and European) regions and, again, little systematic study has been done in the tropics. Evans (1982) implied diversity of *Entomophthorales* was low in the tropics 'in contrast to temperate habitats where *Entomophthora* species (*Entomophthorales*; *Zygomycota*) predominate'. There is, however, increasing evidence that the *Entomophthorales* are more numerous in the tropics than previously considered. Continuous study in Thailand shows that members of this order are found in the early morning, and there is every indication that death of an insect and sporulation are confined to the night and early morning, when temperatures are lowered and humidity high. This contrasts with temperate regions, where sporulation can occur over a period of days under favourable circumstances, making it more apparent. A full survey of the *Entomophthorales* in natural forests in the tropics is needed. Also within the *Zygomycota*, the *Trichomycetes* is a fourth 'hot spot' that has received comparatively little attention in the tropics. Like the *Laboulbeniales*, these are external parasites albeit in the gut. They do not cross the cuticle barrier and invade the haemolymph.

The 'hot spot' that has received by far the most attention is that within the *Hypocreales,* especially the *Clavicipitaceae*. It is this group that will be covered in further detail. Although most work has been done on the *Clavicipitaceae* (especially in the tropics), this chapter must still highlight what little is known. By inference, knowledge of the other four 'hot spots' in the tropics will be seen to lag further behind the *Clavicipitaceae*.

These five 'hot spots' account for *c*. 3000 of the currently accepted 75,000 fungal species known, i.e. *c*. 4% of total fungal species diversity described for the planet. However, there has been a disproportionately higher level of study on 'plant'-associated fungi compared with 'invertebrate'-associated

fungi. If the Hawksworth (1991) figure is correct then 'invertebrate' fungi may, in the future, account for *c*. 60,000 species, assuming they continue to represent 4% of total fungal biodiversity.

It is significant that all estimates of fungal biodiversity are currently based on plant–fungus ratios. In the future, fungus–invertebrate ratios may provide a more reliable estimate of overall fungal biodiversity. However, with both groups, fungi and invertebrates, the current problem is that there is no adequate approximation of either total. By contrast, there are well-established estimates of plant biodiversity, which are likely to prove accurate to within 5–10%. The conclusion is that invertebrate-pathogenic fungi probably account for more than 4% of fungal biodiversity and may account for a significant proportion of the hidden fungal diversity.

The *Clavicipitaceae*: a Significant 'Hot Spot' for Invertebrate Pathogens

Mycologists rely upon *Ainsworth and Bisby's Dictionary of the Fungi* (Kirk *et al.*, 2001) as a rough guide to numbers. However, there is every indication that the estimates in the *Dictionary* are grossly below the actual figures for many major groups. The *Dictionary* records 100 species of *Cordyceps* and ten of *Torrubiella*. However, Kobayasi (1982) listed 282 *Cordyceps* and 59 *Torrubiella*. Similarly, the *Dictionary* records 30 *Hypocrella*, whereas Evans and Hywel-Jones (1990) recorded 38. Significantly, there are many instances where an anamorph is known but no association has yet been made with a teleomorph (Hywel-Jones, 1996, 1997b; Hywel-Jones *et al.*, 1998). In Thailand to date, 286 taxa of invertebrate-pathogenic fungi have been recorded. Of these, 257 taxa are *Clavicipitaceae* with the remainder being either *Entomophthorales* or non-clavicipitaceous *Hypocreales*. Of the *Clavicipitaceae*, 114 taxa (44%) are teleomorphs. Significantly, of the 143 clavicipitaceous anamorphs recorded, only 55 (38%) have been linked with teleomorphs.

In the field there are instances where teleomorphs and anamorphs are regularly seen together. The genus *Hypocrella* often has an *Aschersonia* state present either within the same stroma or within the same population of stromata on a leaf: e.g. *Hypocrella raciborski* Zimm. – *Aschersonia placenta* B. & Br.; *Hypocrella discoidea* (B. & Br.) Sacc. – *Aschersonia samoensis* P. Henn; and *Hypocrella javanica* (Penz. & Sacc.) Petch – *Aschersonia coffeae* P. Henn. Of 12 species of *Hypocrella* from Thailand, ten spontaneously produced an *Aschersonia* state in culture. However, *Hypocrella schizostachyi* P. Henn. and *Hypocrella scutata* (Cooke) Sacc. did not produce an *Aschersonia* state in culture or in the field, calling into question whether these are best placed in this genus. *Hypocrella schizostachyi*, in particular, is of questionable placement, as it produced anamorphs in culture with affinities to *Nectria* (Hywel-Jones and Samuels, 1998). Although members of the genus *Hypocrella sensu stricto*

appear to be the most recently evolved of invertebrate-pathogenic *Clavicipitaceae* (Artjariyasripong *et al.*, 2002), our observation that *H. schizostachyi* produces a 'nectriaceous' anamorph suggests this could be more ancestral within the *Clavicipitaceae*.

Although there does not seem to be a significant temporal component to the presence of teleomorph and anamorph states in *Hypocrella,* this is not the case for *Cordyceps*. Many species of *Cordyceps* recorded from Thailand are found first as the anamorph, while the teleomorph develops over 4–8 weeks. Notable examples of this development include: *Hymenostilbe nutans* Samson & Evans and *Cordyceps nutans* Pat. (Hywel-Jones, 1995b); *Hirsutella brunneapunctata* Hywel-Jones and *Cordyceps brunneapunctata* Hywel-Jones (Hywel-Jones, 1995c).

Are the Origins of the *Clavicipitaceae* Tropical?

Undoubtedly, the diversity of the invertebrate-pathogenic *Clavicipitaceae* is greater in the tropics than in temperate regions (Evans, 1982). Six man-hours of searching in tropical forest in Thailand usually produces 25–30 species. By comparison, the same collecting in temperate forest in Japan produced 10–15 species. Rogerson (1970) reviewed the Hypocrealean fungi and concluded, based on morphology, that the clavicipitaceous genera could stand as a separate order – the *Clavicipitales*. Later molecular phylogenetic studies, however, did not support the clavicipitaceous fungi as a separate order but placed them within the *Hypocreales* (Spatafora and Blackwell, 1993; Rehner and Samuels, 1995). These and further molecular studies (Nikoh and Fukatsu, 2000; Artjariyasripong *et al.*, 2002) supported the view that the *Clavicipitaceae* are monophyletic within the *Hypocreales*, with other hypocrealean families apparently basal to the *Clavicipitaceae*. By examining all the genera considered by Rogerson (1970) and by Kirk *et al.* (2001), the conclusion can be drawn that the majority are wholly tropical in distribution. Within the invertebrate-pathogenic genera the two largest genera, *Cordyceps* and *Torrubiella*, have many tropical species, while smaller genera such as *Atricordyceps, Cordycepioideus, Hyperdermium* and *Hypocrella* are either wholly tropical or tropical/sub-tropical in their distribution (Petch, 1921b; Blackwell and Gilbertson, 1981; Samuels, 1983; Sullivan *et al.*, 2000).

These considerations beg the question: what is the ancestor of the *Clavicipitaceae?* There are invertebrate pathogens within the *Hypocreaceae* and these also are tropical. Notably, in Thailand, *Cosmospora diploa* (Berk. & M.A. Curtis) Rossman & Samuels has been reported from scale insects (*Homoptera*). Petch (1921a) described many species of hypocrealean fungi from 'scale insects' and commented on the extent of the bambusicolous nature of many of these associations. A hypothesis (Hywel-Jones' basis for Artjariyasripong, 1999) was that *Hypocrella* (wholly tropical in distribution)

was ancestral for the *Clavicipitaceae*, with *Torrubiella* and *Cordyceps* evolving after radiation from homopteran scale insects into other insect orders and, ultimately, other invertebrates, such as spiders, as well as into other fungi. Recent studies involving species from all three genera suggest, however, that *Cordyceps* is basal to the clade, with *Hypocrella* being the most recently derived (Artjariyasripong *et al*., 2002). The problem is clearly more complex than hitherto expected. There is natural interest in inter-kingdom host jumping (Nikoh and Fukatsu, 2000) and it is here posited that the scale insects (largely tropical in distribution) hold the key to understanding the evolutionary developments within the *Clavicipitaceae*. Furthermore, given the observations of Petch (1921b) and of work in Thailand (Hywel-Jones, 1997a), a significant role for bambusicolous scale insects and other invertebrates can be considered, especially as there are many plant-pathogenic genera of *Clavicipitaceae* that are found only on tropical bamboo (Rogerson, 1970).

As the DNA of more of these fungi is sequenced, it is expected that many of these questions will be answered in the next few years and many new ones posed. The tropical invertebrate-pathogenic *Clavicipitaceae* offer interesting problems for study. At the morphological level Rogerson (1970) was able to demonstrate that there were enough features of clavicipitalean morphology to separate them from the *Hypocreales* as an order in their own right. Molecular evidence does not support this view. It is suggested here that the switch to invertebrate hosts has forced the *Clavicipitaceae* to evolve morphological characteristics that aid their new 'lifestyle' and make them stand out from their relatives – the *Hypocreales*. Is a new order developing?

The *Clavicipitaceae* as Biocontrol Agents

Most of the tropical invertebrate-pathogenic *Clavicipitaceae* have highly evolved associations with their hosts. Consequently, it appears that the teleomorph has been largely retained in most species. A few species, however, have made the transition to being 'broad-band' pathogens. Consequently, most of the literature on clavicipitaceous invertebrate pathogens is concerned with a few species that are commonly encountered in agricultural ecosystems. Most important among these are:

- *Beauveria bassiana* – pan-global, with a wide host range in the Insecta
- *Metarhizium anisopliae* – pan-global, limited to *Insecta* and with a wide host range
- *Verticillium lecanii* (Zimm.) Viégas – pan-global, a wide host range within and without the Insecta (also infects Araneae and *Fungi*)
- *Nomuraea rileyi* (Farlow) Samson – pan-global, restricted to Lepidoptera within Insecta
- *Hirsutella citriformis* Speare – pan-tropical, restricted to a single order, Homoptera, and most common on Delphacidae within Insecta

All five of the above species have been considered for use as biocontrol agents. Four of the five genera are encountered as the anamorph with teleomorphs either only anecdotally known or very rare. The exception is *Hirsutella*, where strong relationships with *Cordyceps* are known. Although *H. citriformis* has no known teleomorph described, recent cultural and morphological studies with tropical isolates of *H. citriformis* and *H. saussurei* (Cooke) Speare suggest a close relationship between the two. The teleomorph of *H. saussurei* is *Cordyceps humberti* Robin, which has close morphological affinities with *C. unilateralis* (Tull.) Sacc. from ants. Future molecular studies on these should clarify their relationships.

For *Nomuraea*, there is a teleomorph recorded for *N. atypicola* (Yasuda) Samson – *Cordyceps cylindrica* Petch (Evans, 1982; Hywel-Jones and Sivichai, 1995). However, the relationship of *N. atypicola* to *N. rileyi* is in doubt and no teleomorph has yet been reported for *N. rileyi* (O. Boucias, Bangkok, Thailand, 2001, personal communication). More than 2500 man-hours of survey work in natural forest in Thailand has failed to find *N. rileyi* and yet it can be commonly collected in agricultural ecosystems in Thailand. Although *N. rileyi* is now pan-global, it is possible that its centre of origin is not South-East Asia but that it has been imported to Thailand as a pathogen of crop pests over time. The alternative is that it is present in natural forest, but is rare and has yet to be found.

Within *Beauveria*, a teleomorph, *Cordyceps brongniartii* Shimazu, has been described for *B. brongniartii* (Sacc.) Petch (Shimazu *et al.*, 1988) and has recently been reported from Thailand (Hywel-Jones, 2002). *Metarhizium anisopliae* has received much attention as a potential biocontrol agent. Significantly, this also has not had a teleomorph reliably reported. Recently, a *Metarhizium* state was described as the anamorph of *Cordyceps taii* Liang et Liu (Zongqi *et al.*, 1994). *M. anisopliae* has a worldwide distribution and has been recorded from a wide range of hosts in many insect orders. However, the continued reporting of *Metarhizium* species associated with *Cordyceps* from sub-tropical southern China hints at this area being the centre of origin for *Cordyceps* spp. producing a *Metarhizium* state. A *Cordyceps*–*Metarhizium* combination is occasionally found in Thai forest. Further work is needed to determine if China/South-East Asia is the centre of origin for *Metarhizium*-producing *Cordyceps*.

In anamorph genera, where there has been a move out of natural forest and into agricultural ecosystems, it is notable that these contain few species. *Nomuraea* has three species, *Metarhizium c.* five species and *Beauveria* also *c.* five species. Variation is apparently intraspecific. An exception is the genus *Hirsutella* which is linked with many *Cordyceps* species and is an important part of the life cycle. As originally conceived by Patouillard (1892) and recognized by Speare (1920), the genus was a sound one. The genus *Hirsutella* is now in need of revision. Evans (in Hawksworth, 1991) speculated that each species of thrips may have its own unique *Hirsutella* species. Other anamorph

genera linked with *Cordyceps* are equally rich in species despite a poor record of research on them. For example, *Hymenostilbe* (mostly tropical in distribution) is only dealt with in < 20 papers and yet it contains *c.* ten published species with many more awaiting description (Samson and Evans, 1975; Hywel-Jones, 1995a,b,d).

It must be presumed that the few (compared to those that have been recorded from natural forest) agricultural invertebrate pathogens have migrated either from natural forest to the agricultural ecosystem or have been imported from elsewhere by man to Thailand. More than ten species of *Aschersonia* have been recorded from natural forest in Thailand while only one species, *A. placenta*, has been found in agricultural ecosystems. Significantly, the *Hypocrella* state of *A. placenta* has apparently not made the transition from forest to agricultural ecosystem. In conclusion, few invertebrate-pathogenic *Clavicipitaceae* have moved from the forest to agricultural ecosystems. Most fungi being assessed for biocontrol originate from isolates taken from agricultural ecosystems. The potential for new biocontrol agents from natural forest is vast.

Cordyceps from an Ethnomycological Point of View

Although several species of *Cordyceps* are used as herbal medicines, the most famous is the Chinese *Cordyceps, C. sinensis* Berk. (Sacc.). This made the popular press in the mid-1990s with news that it was one of the ingredients in tonics used by Chinese long-distance runners (Pegler *et al.*, 1994; Steinkraus and Whitfield, 1994). A casual search of the Internet reveals thousands of 'hits' for the genus *Cordyceps*. The majority of these refer to the medicinal properties of *C. sinensis*. Apart from *C. sinensis*, which is popular throughout East Asia, other species that have been developed into herbal medicines include: *Paecilomyces tenuipes* (Peck) Samson in Korea (R.A. Samson, Bangkok, Thailand, 1999, personal communication), *Paecilomyces cicadae* (Miquel) Samson in Korea, Japan and China and *Cordyceps militaris* (L.:Fr.) Link in Japan.

Despite the many species of *Cordyceps* (and related anamorphs) recorded from Thailand, there is no example of a Thai *Cordyceps* being used as a herbal medicine. The probable reason is that, while species diversity is high in the tropics, there are few examples where large numbers of stromata can be harvested on a regular and predictable basis, but the increasing interest in the west in 'alternative herbal medicines' from the east is driving research into the therapeutic values of these eastern herbals. This has also resulted in increased interest in invertebrate-pathogenic *Clavicipitaceae* as sources of novel secondary metabolites.

Tropical Invertebrate-pathogenic *Clavicipitaceae* as Sources of Novel Secondary Metabolites

The invertebrate-pathogenic *Clavicipitaceae* are not easy to find. In Thailand, the author could locate *c.* 70–200 specimens (representing 15–30 species) in a 4-day survey and secure *c.* 40–80 isolates representing 10–15 species. A similar survey in temperate Japan produced *c.* 70 isolations (21 species) from 226 specimens (40 species) collected in 7 days.

However, the *Hypocreales* (especially the *Clavicipitaceae*) and *Eurotiales* are two closely related orders that have proved to be important sources of secondary metabolites used in the pharmaceutical industry (Rossman, 1996). Significantly, few species have had their novel secondary metabolites studied. However, the extensive programme of isolating insect fungi from Thai forest has now resulted in a large number of species being available for culture. Seifert *et al.* (2000) concluded that 'the importance of fungal cultures is increasing'.

The pharmaceutical industry still has a mentality of preferring to work with easily acquired, easily handled and easily maintained organisms. In the early days, this was achieved by working with fungi isolated from soil samples. Increasingly, industry goes through trends of interest in different organisms. Recent examples have included marine fungi, endophytes and xylariaceous fungi. It is as if a seam is mined before moving on to a new one. Similarly, there are organism trends, e.g. fungi versus actinomycetes. Recently, novel secondary metabolite discovery has also had to compete with combinatorial chemistry, which has been made possible with the advent of powerful computing systems.

The random discoveries of combinatorial chemists cannot possibly compete in the long run with the organized natural selection of which mankind is also an integral part. The author fully expects that the important 'drug leads' of the future will continue to be found from nature's great diversity. Four billion years of genetic evolution will continue to supply the drugs of the future. Combinatorial chemistry and 40 years of 'genetic modification' will merely help to fine-tune the process. For this to occur, the greatest diversity of genes must occur where there is greatest species diversity, assuming that general genetic diversity equates to metabolic diversity. The tropics provide that diversity. Within the animal kingdom, Insecta also provide high genetic diversity, and within the fungal kingdom it is likely that the tropical invertebrate-pathogenic fungi will provide a great level of diversity (Bills *et al.*, Chapter 11, this volume).

Conclusions

In the future, if we are to obtain a reasonable estimate of global fungal biodiversity, more attention must be paid to the tropical invertebrate-pathogenic

fungi. With their rich diversity, the tropics are expected to provide a large pool of material for future research. The clavicipitaceous fungi have long been considered as a source of biocontrol agents. More recently, they have been considered to be a source of novel metabolites and, if industry is prepared to invest some time, energy and money into a detailed investigation that goes beyond the routine, then it is anybody's guess what metabolites of benefit to mankind might come from tropical invertebrate *Clavicipitaceae*.

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Tropical Mycoses: Hazards to Travellers 10

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Introduction

Fungi are now recognized as an important cause of disease in humans. Some of these fungi lie in wait for tropical hitch-hikers.

The incidence of fungal disease has increased markedly over the last decade. Infections of skin, hair, nail and mucous membranes affect up to 20% of the population. Potentially fatal deep-seated infections have increased, mainly because of the larger numbers of people with a defective immune system (immunocompromised), for example, transplant patients and those with acquired immune defiency syndrome (AIDS). Some fungal infections occur mainly in tropical or sub-tropical parts of the world and, in general, people from temperate zones, particularly Europe, have little immunity to them. Increased leisure and business travel to these exotic tropical locations have resulted in a higher prevalence of unusual fungal diseases.

In general, skin infections are caused mainly by ringworm fungi (dermatophytes) and several types of ringworm (tinea) are specific to tropical areas, often producing different lesions from those seen in temperate zones. Some of these are animal ringworms, for example, from monkeys. In addition to these superficial diseases, there are large numbers of fungi in soils and on plant debris in tropical countries that are capable of infecting tissues beneath the skin, including bone, causing disfiguring and debilitating conditions. These infections include mycetoma, chromoblastomycosis and sporotrichosis, all of which

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tend to affect the limbs. All are caused by the introduction of fungi through unprotected skin into the underlying tissues by thorns or splinters. The last and most serious group of diseases is the deep-seated infections, which are frequently fatal. As the name suggests, these can affect any or many organs of the body, although they usually begin by inhalation of fungal spores into the lungs. A lung infection then develops, which in some cases spreads to other organs. Diseases such as histoplasmosis and coccidioidomycosis are included in this group. Both are restricted to hot climates where the causal fungi are present in the soil, but a surprising number of cases of infection are seen in Europe in people who have visited the disease areas. Immunocompromised people, such as those with AIDS, are especially vulnerable. Histoplasmosis, for example, is acquired from the droppings of birds and bats and so presents a particular threat to those tropical hitch-hikers who also enjoy hanging around in dark caves! The types of fungal infections are reviewed below.

Fungal Diseases of Humans

About 180 species of fungi are recognized as able to cause disease (mycosis) in humans and animals. Most of these are moulds, but there are a number of pathogenic yeasts and many are dimorphic. The dimorphic fungi usually assume the mould form when growing as saprotrophs in nature and the yeast form when causing infection; both morphological forms can be induced in the laboratory by varying the culture conditions. Some fungi, for example, the systemic pathogens *Histoplasma capsulatum* Darling and *Coccidioides immitis* Rixford & Gilchrist, are highly pathogenic and are capable of establishing an infection in all exposed individuals. Others, such as *Candida* and *Aspergillus* species, are opportunist pathogens, which in general cause disease only in immunocompromised hosts. The form and severity of infections often depend on the degree of exposure to the fungus and the extent to which the person is immunocompromised.

Most fungal infections are caused by fungi which grow as saprotrophs in the environment. Some yeasts are commensals of humans and cause endogenous infections when there is some imbalance in the host. Only ringworm infections are truly contagious. Many fungal diseases have a worldwide distribution, but some are endemic to specific geographical regions, usually because the causal agents are saprotrophs that are restricted in their distribution by soil and climatic conditions.

Superficial mycoses

Ringworm

Diseases of the skin, hair and nail are the most common of all fungal infections and have a worldwide distribution. They are caused by a group of closely

related mould fungi called dermatophytes that have the ability to colonize and digest keratin. Ringworm infections occur in both humans and animals. Ringworm infections are also referred to as dermatophytoses.

There are about 20 species of dermatophyte fungi that cause ringworm and they are grouped into three genera, *Trichophyton*, *Microsporum* and *Epidermophyton* (Table 10.1). A number of dermatophytes are primarily animal pathogens that may also infect humans (Table 10.2), and some species are restricted to or are more common in certain parts of the world. Ringworm infections are spread by direct or indirect contact with an infected individual or animal. The infective particle is usually a fragment of keratin containing viable fungus. Indirect transfer may occur via the floors of swimming pools and showers or on brushes, combs, towels and animal-grooming implements. In addition to exposure to the fungus, some abnormality of the epidermis, such as slight peeling or minor trauma, is probably necessary for the establishment of infection.

In the industrialized countries, ringworm of the scalp accounts for only a small proportion of infections, although it is on the increase in Europe. However, the use of communal bathing facilities has resulted in a considerable increase in the incidence of foot ringworm (Fig. 10.1) and associated nail (Fig. 10.2) and groin infections. The predominant cause of these infections is *Trichophyton rubrum* (Castell.) Sabour., which is responsible for *c.* 75% of all ringworm infections diagnosed in temperate zones. In developing countries,

Table 10.1. Common dermatophyte pathogens of humans.

Species	Common site(s) of infections	Main area of distribution
Epidermophyton floccosum (Harz) Langeron & Milchevitch	Groin, feet (nail)	Worldwide
Microsporum audouinii Gruby	Scalp (body)	Africa, America and Europe
M. ferrugineum Ota	Scalp (body)	Africa, Balkans and Asia
Trichophyton mentagrophytes var. *interdigitale* (Robin) Blanchard	Feet (nail, groin)	Worldwide
T. concentricum Blanchard	Body	South Pacific
T. rubrum (Castell.) Sabour.	Feet, nail, groin, body	Worldwide
T. schoenleinii (Lebert) Langeron & Milochevitch	Scalp (body, nail)	Eurasia and North Africa
T. soudanense Joyeux	Scalp (body)	Africa
T. tonsurans Malmsten	Scalp, body (nail)	Europe and America
T. violaceum Sabour.	Scalp, body (nail)	Africa and Eurasia

Parentheses in second column indicate secondary sites of infection.

Table 10.2. Common dermatophyte species of animals.

Species	Animals commonly affected	Main area of distribution
Microsporum canis Bodin	Cat, dog	Worldwide
M. distortum Di Menna & Marples	Cat, dog	Australasia, USA
M. nanum Fuentes	Pig	Worldwide
M. persicolor (Sabour.) Guiart & Grigor.	Bank field voles	Europe
Trichophyton mentagrophytes var. *mentagrophytes* (Robin) Blanchard	Rodents (horse, cat, dog)	Worldwide
T. equinum (Matr. & Dassonville) Gedoelst	Horse	Worldwide
T. erinacei (Smith et Marples) Padhye & J.Carm.	Hedgehog	UK, New Zealand
T. quinckeanum (Zopf) MacLeod & Muende	Mice	Europe, North America
T. simii (Pinoy) Stockdale, Mackenzie et Austwick	Monkey, chicken	India
T. verrucosum Bodin	Cattle	Worldwide

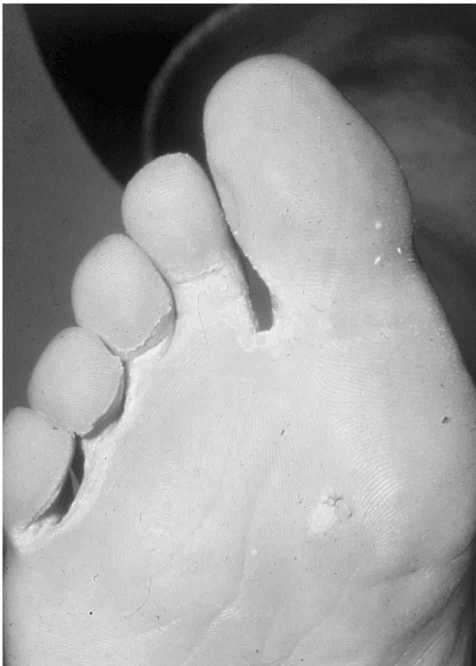

Fig. 10.1. Athlete's foot caused by *Trichophyton rubrum*.

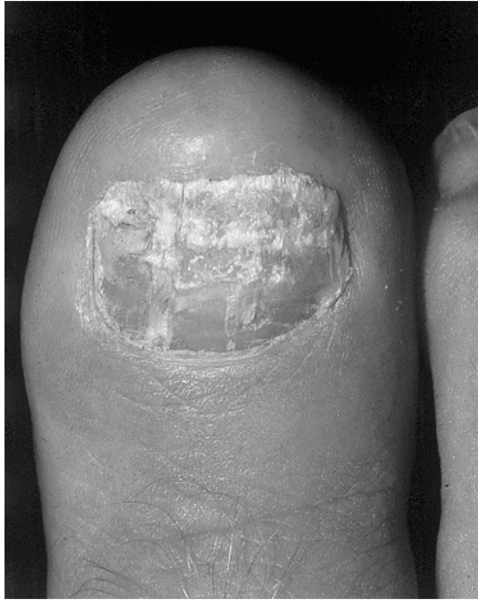

Fig. 10.2. Destruction of nail due to *Trichophyton rubrum* infection.

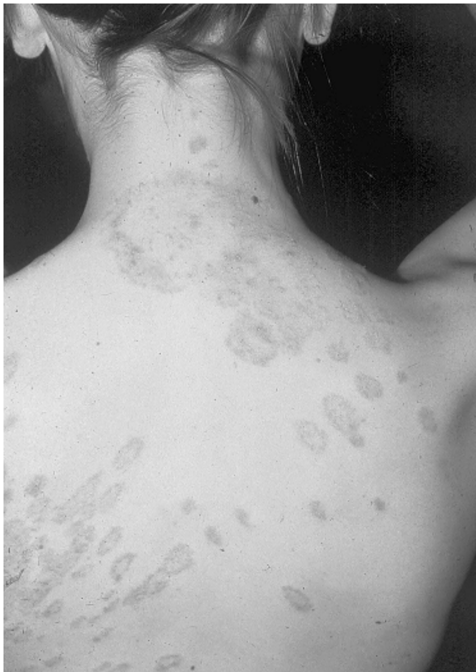

Fig. 10.3. Extensive body lesions due to cattle ringworm (*Trichophyton verrucosum* Bodin).

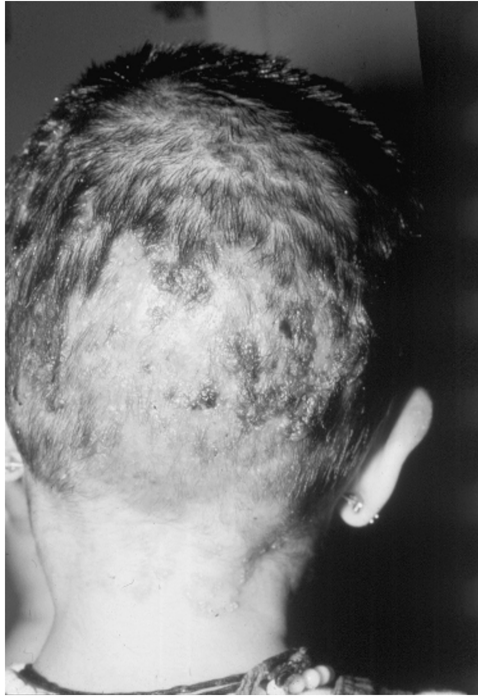

Fig. 10.4. Ringworm infection of the scalp caused by *Trichophyton violaceum*.

particularly in warm climates, body (Fig. 10.3), scalp (Fig. 10.4) and groin infections predominate, with *T. rubrum* and *T. violaceum* Sabour. apud Bodin among the most common causes, and visitors to these regions are exposed to these fungi. There is no evidence of natural immunity to ringworm, but scalp ringworm is predominantly a disease of children and foot ringworm a disease mainly of adult males.

In culture, many dermatophyte species produce two types of asexual spore, macroconidia and microconidia. Classification into the three genera *Trichophyton*, *Microsporum* and *Epidermophyton* is based on the morphology of the macroconidia, although the identification of species is also based on the shape and disposition of the microconidia and the macroscopic appearance of the colony.

Ringworm lesions vary considerably according to the site of the infection and the species of fungus involved. Sometimes there is only dry scaling, but more commonly there is irritation, reddening, swelling and sometimes vesicles. More inflammatory lesions with weeping vesicles, pustules and ulceration are usually caused by animal species of dermatophyte. In skin infections of the body, face and scalp, spreading circular lesions with a raised, inflammatory border are produced. It is the appearance of these lesions that led to the name ringworm. In scalp infections there is scaling and hair loss, the

extent of which depends on the causal fungus. Some species from animals cause a highly inflammatory, raised pustular lesion called a kerion. It is important that scalp ringworm is recognized and treated promptly since it can lead to scarring and permanent hair loss.

Ringworm infections may be reliably diagnosed in the laboratory by direct microscopical examination and culture of skin, crusts, hair and nail. Microscopy of wet-mounts of keratinous material in potassium hydroxide is simple and reliable. Dermatophytes are seen in skin and nail as branching, septate hyphae that often fragment into vegetative spores (arthroconidia) (Fig. 10.5). For culture, small fragments of infected human material are scattered on Sabouraud glucose agar and incubated at 27–30°C for up to 3 weeks.

Topical therapy, with creams and ointments, is satisfactory for treating most dermatophyte infections of skin, but oral antifungals are required to treat infections of the nail and scalp. Topical agents include terbinafine and azole compounds. Oral griseofulvin is useful for scalp, skin and fingernail infections, but it is poor for toenail infections. The newer oral antifungals such as terbinafine and itraconazole are gradually replacing griseofulvin for the treatment of all forms of ringworm, since they give much better cure rates with much shorter periods of treatment.

Yeast infections

Candida: superficial *Candida* infections are very common throughout the world although they are not especially prevalent in warm climates. These mycoses involve the skin, nails and mucous membranes of the mouth and vagina; infection of the mucous membranes is commonly referred to as thrush (Fig. 10.6). One species, *Candida albicans* (Robin) Berkhout, accounts for 80–90% of cases. *C. albicans* and occasionally other *Candida* species are found in small

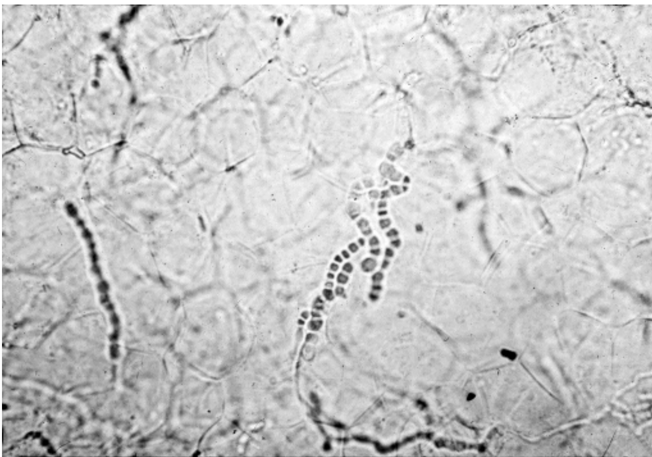

Fig. 10.5. Dermatophyte mycelium and arthroconidia seen in skin (KOH mount).

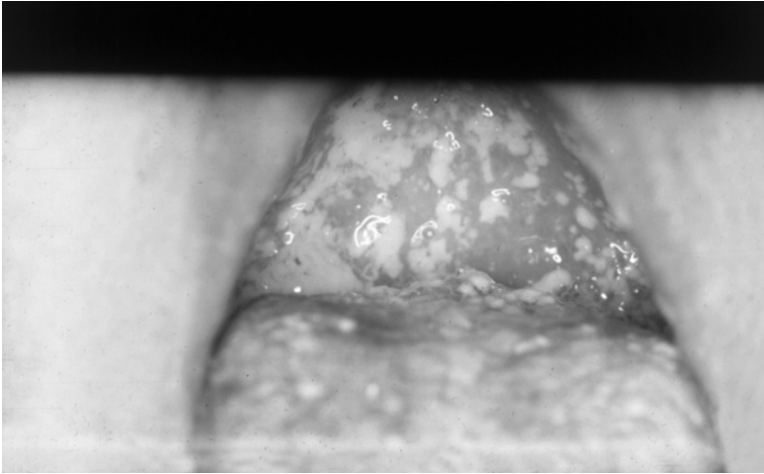

Fig. 10.6. Florid *Candida* infection of the mouth showing typical white plaques.

numbers in the commensal flora (mouth, gastrointestinal tract, vagina and skin) of about 20% of the normal population. The carriage rate tends to increase with age and is higher in the vagina during pregnancy. Chronic, intractable oropharyngeal candidosis is common in AIDS patients (< 100%) and may extend to give infection of the oesophagus. The appearance of this infection is often the indicator of the transition from HIV-positive to full-blown AIDS. However, in developed countries, where the newer, so called 'triple-therapy' is available for treatment of HIV infection, the prevalence is much lower (10%).

Pityriasis versicolor: this is a mild, chronic infection of the stratum corneum that produces a patchy discoloration of the skin and is caused by yeasts of the genus *Malassezia,* species of which are common members of the normal skin flora, and most infections are thought to be endogenous. Development of the disease is probably related to host or environmental factors. It is very common in the tropics and it is most prevalent in young adults. There is no evidence that it is contagious. The organisms are dimorphic and both yeasts and short hyphae are associated with pityriasis versicolor. The lesions of pityriasis versicolor are non-inflammatory; scaling is usually present on the upper trunk or neck and in white-skinned people are brownish in colour, whereas in dark-skinned individuals they appear as light-coloured patches (Fig. 10.7a,b).

Malassezia species require lipids for growth, and media containing Tween and lipid supplements have been developed specifically for growing the organism. *Malassezia* has also been associated with conditions such as dandruff and seborrhoeic dermatitis, where only yeasts that characteristically produce buds on a broad base are seen.

Diagnosis of yeast infections can be confirmed reliably by demonstration

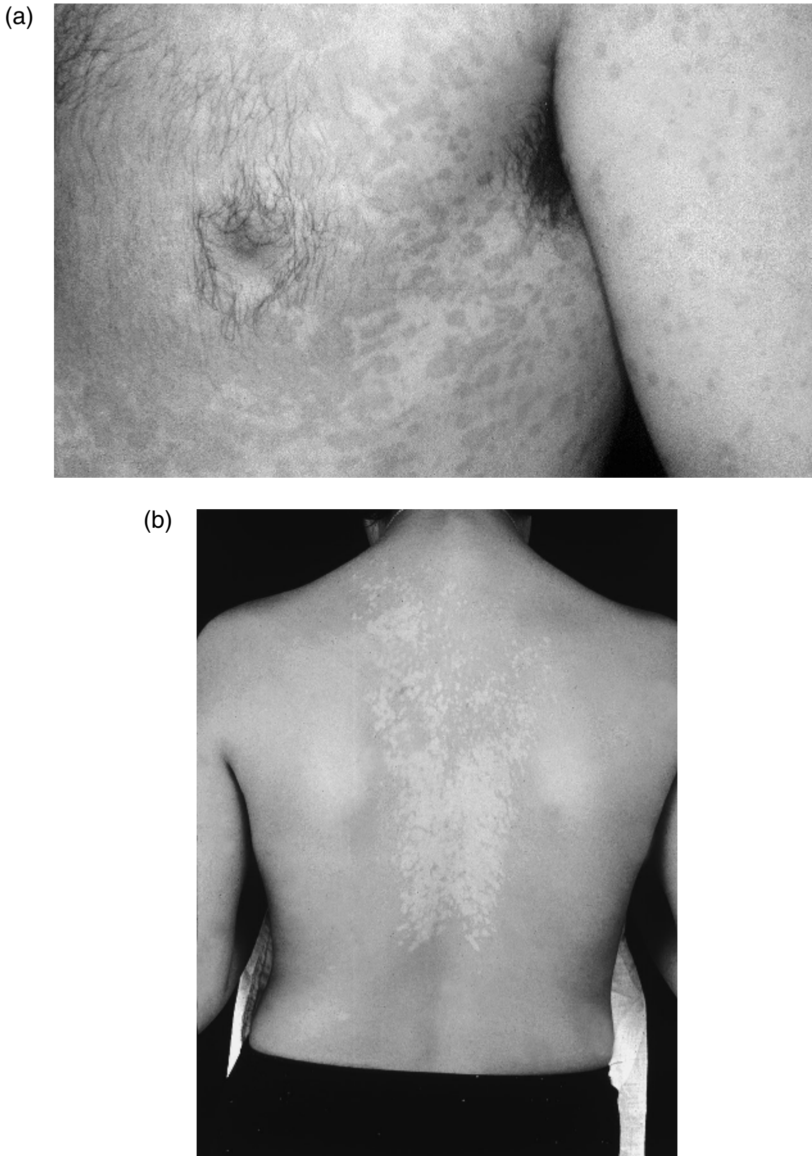

Fig. 10.7. Pityriasis versicolor showing (a) hyper-pigmented and (b) hypo-pigmented lesions. Photographs courtesy of Dr D.T. Roberts.

of the organism in skin scales or mouth/vaginal swab smears by microscopy and culture. For pityriasis versicolor, culture is unnecessary. In the tissues, *Candida* appears as budding yeast cells, often with mycelium, and *Malassezia* as numerous clusters of round yeast cells along with short, stout hyphae.

Candida infections and pityriasis versicolor respond well to treatment with antifungal creams. Oral therapy, with either fluconazole or itraconazole, is sometimes needed for widespread infections. Relapse of pityriasis versicolor is common, particularly in hot climates.

Other superficial infections

Skin and nail: certain moulds (non-dermatophyte) may cause infection of skin and nail. It is important that these are recognized since they are often resistant to the agents used to treat ringworm and superficial candidosis.

In the UK, non-dermatophyte moulds cause about 5% of fungal nail infections. *Scopulariopsis brevicaulis* Bainier, a ubiquitous saprotroph of soil, is the most common, although other saprotrophic moulds, such as *Fusarium, Aspergillus* and *Penicillium* species, are also occasionally implicated. Infection usually follows trauma.

Scytalidium dimidiatum (Penz.) B. Sutton & Dyko, a pathogen of fruit trees and a soil fungus, can cause nail and skin infection among those in tropical areas, and visitors to these areas will be exposed to this fungus. *Scytalidium* infections are diagnosed by microscopy and culture, but they are not amenable to treatment with any of the currently available antifungals.

Tinea nigra: this is a superficial, asymptomatic skin disease characterized by pigmented brown areas, usually on the palms and soles. It is caused by a black mould, *Exophiala werneckii* (Horta) v. Arx, and occurs mainly in the tropics. Tinea nigra is not contagious but is contracted by contact with the fungus in soil. Again, those in the endemic areas will be exposed to this fungus. However, this infection responds well to treatment with antifungal creams.

Mycotic keratitis: fungal infections of the cornea of the eye are usually secondary to injury. They are caused by moulds that occur commonly as saprotrophs in soil or on plants, in particular species of *Aspergillus* and *Fusarium*. These infections are serious and any splinter, thorn or soil in the eye that develops into a lesion requires medical attention immediately. Treatment is with topical antifungal agents, in particular natamycin.

Subcutaneous mycoses

Mycoses of the skin, subcutaneous tissues and bone occur mainly in the tropics and subtropics. They result from the inoculation of saprotrophic fungi from soil or decaying vegetation into the subcutaneous tissue. The principal subcutaneous mycoses are mycetoma, chromoblastomycosis and sporotrichosis. Anyone visiting the endemic areas is exposed to the fungi that cause these diseases.

Mycetoma

This is a chronic infection of the skin, subcutaneous tissues and bone that is

characterized by deep abscesses, with pus draining to the surface through channels in the tissue. It most often affects the foot or the hand. One of a number of different actinomycetes (actinomycetoma) or moulds (eumycetoma) can cause it. The disease is most prevalent in tropical and subtropical regions of Africa, Asia and Central America. Infection results from inoculation of the organism into the subcutaneous tissue from soil or plant sources, usually on thorns or splinters. Consequently, infection occurs most frequently in those who have close contact with soil. A number of organisms have been implicated in this disease, including the moulds *Madurella, Exophiala, Acremonium, Pseudallescheria*, and the actinomycetes *Actinomadura, Nocardia* and *Streptomyces*. The organisms form compacted colonies (grains) < 2 mm across in the tissues, the colour of which depends on the organism responsible; for example, *Madurella* grains are black and *Actinomadura pelletieri* (Laverau) Lechevalier & Lechevalier grains are red.

Mycetoma usually takes the form of localized swollen lesions which are usually found on the limbs. The foot is often involved (Fig. 10.8) and the infection can spread up the long bones of the leg. There is often a long period between the initial infection and formation of the characteristic lesions. Diagnosis is made by examination of pus or biopsy material. The grains are usually visible to the naked eye and their colour may help to identify the organism responsible. Microscopical examination enables differentiation between actinomycetoma and eumycetoma (Fig. 10.9). This is important since actinomycetoma responds well to antibiotics (e.g. rifampicin in combination with sulphonamides or co-trimoxazole), but eumycetoma does not respond to antifungal treatment, and amputation of the foot or limb is usually necessary. However, some of the newer antifungals have yet to be properly evaluated in treating this condition.

Chromoblastomycosis

This disease is a chronic disease of the skin and subcutaneous tissues, characterized by crusted, warty lesions, usually of the limbs (Fig. 10.10). It is mainly encountered in the tropics and can be very disfiguring. The principal causes are the dematiaceous moulds *Fonsecaea pedrosoi* (Brumpt) Negroni, *F. compacta* Carrion, *Phialophora verrucosa* Medlar and *Cladosporium carrionii* Trejos. Like mycetoma, the disease is seen most often in rural areas. Diagnosis is straightforward, as the dark-coloured fungal elements are easy to see on microscopical examination of skin scrapings, crusts and pus, but the fungi are difficult to identify. The disease responds well to antifungal therapy with the newer drugs, such as itraconazole and terbinafine.

Sporotrichosis

This is a chronic, nodular infection of the skin and subcutaneous tissues in which there are abscesses and pus. Unlike the other subcutaneous mycoses,

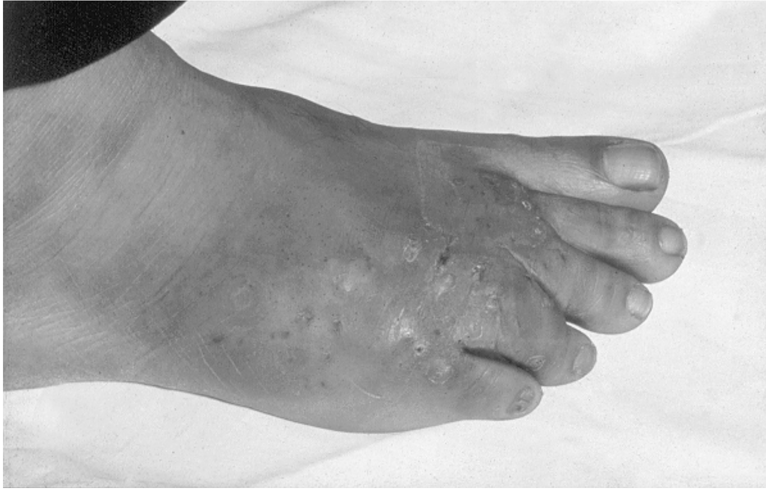

Fig. 10.8. Mycetoma of the foot caused by *Madurella grisea* Mackinnon, Ferrada & Montemayer.

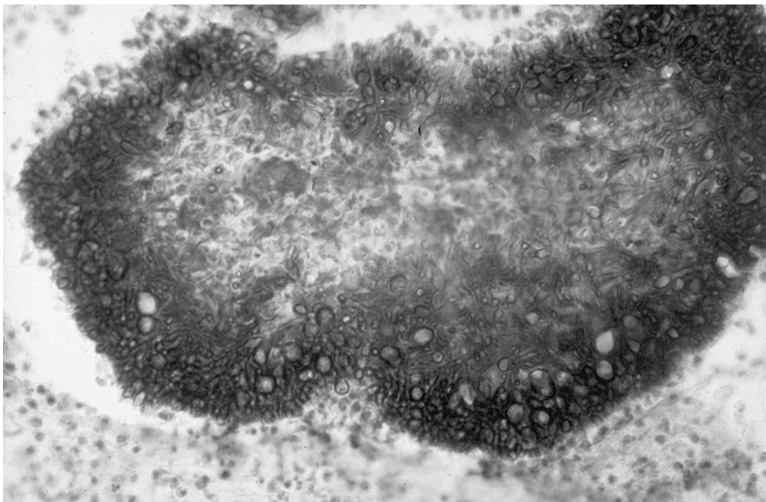

Fig. 10.9. Grain of *Madurella grisea* in tissues of the foot.

it can spread through the lymphatic system (Fig. 10.11). Typically, the primary lesion is on the hand with secondary lesions extending up the arm, although in immunocompromised individuals it can spread to involve the bones, joints, lungs and, in rare cases, the central nervous system. The disease occurs mainly in Central and South America, parts of the USA and Africa, and Australia. The localized form responds to treatment with a number of systemic antifungals but disseminated disease is more difficult to treat.

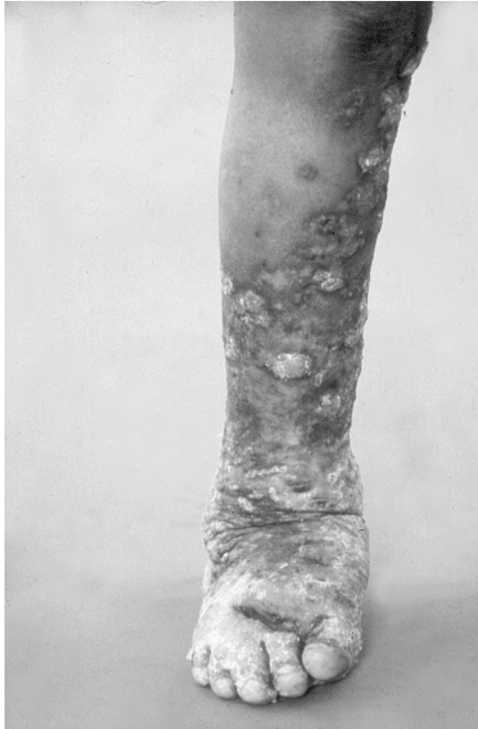

Fig. 10.10. Chromoblastomycosis of the leg due to *Fonsecaea pedrosoi* with raised, warty lesions.

Systemic mycoses

Deep-seated fungal infections generally result from the inhalation of airborne spores produced by the causal moulds, present as saprotrophs on plant material and in soil. Many are caused by dimorphic fungi, which assume a yeast phase in tissues. The principal diseases are coccidioidomycosis (caused by *Coccidioides immitis*), blastomycosis (*Blastomyces dermatitidis* Gilchrist & Stokes), histoplasmosis (*Histoplasma capsulatum*) and paracoccidioidomycosis (*Paracoccidioides brasiliensis* (Splendore) Almeida).

Systemic mycoses caused by opportunistic pathogens, such as *Aspergillus*, *Candida* and *Cryptococcus* species, have a more widespread distribution, and are being seen with increasing frequency in patients compromised by disease or drug treatment. In transplant patients, for example, these fungi are among the most frequent causes of mortality due to infection.

Systemic mycoses occur most frequently in workers in agriculture or the construction industry, especially following disturbance of soils containing the causal fungi. The incidence of systemic infections caused by opportunistic pathogens has also increased with developments in medical and surgical

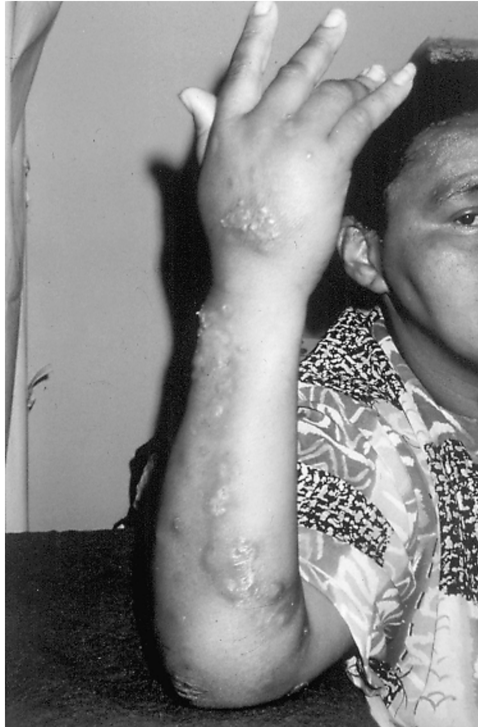

Fig. 10.11. Sporotrichosis of the arm with a chain of ulcerated lesions due to lymphatic spread of the organism (*Sporothrix schenckii* Hektoen & Perkins).

practice and with the increased number of immunocompromised individuals, e.g. neutropenic and AIDS patients.

Cryptococcosis

This is caused by the encapsulated yeast *Cryptococcus neoformans* (Sanfelice) Vuill., and is most frequently manifested as a disease of the central nervous system, although the primary site of infection is the lung. It occurs sporadically throughout the world, but it is most frequently associated with patients suffering from AIDS.

Four serotypes of *C. neoformans* can be differentiated (A, B, C, D) which represent two varieties of the organism, namely, *C. neoformans* var. *neoformans* (A, D) and *C. neoformans* var. *gattii* Vanbr. & Takashio apud DeVroey & Gattii (B, C). The majority of infections are caused by var. *neoformans*, which is found in large quantities in the excreta of wild and domesticated birds throughout the world. *C. neoformans* var. *gattii* causes infection in warmer climates. It is associated with the flowers of the Red River gum tree (*Eucalyptus camaldulensis* Dehnh.) and so infections caused by this variety coincide with the distribution of this tree.

Inhalation of *C. neoformans* may lead to a mild, self-limiting pulmonary infection, which is the most common form. Symptomatic pulmonary infection causes the formation of small nodules that heal leaving scar tissue, or enlarge to form a chronic cryptococcoma. The meningeal form of the disease may also occur in healthy individuals, but is most commonly seen in people with cell-mediated immune defects. It develops as a headache, low-grade fever followed by changes in mental state, weight loss, visual disturbances and ultimately coma. Unless cryptococcal meningitis is treated, it is uniformly fatal. Lesions may occur at other sites, including the skin, mucosa, major internal organs and bone.

Diagnosis of cryptococcosis is relatively straightforward. The yeast can often be seen in direct microscopy of cerebrospinal fluid (CSF), sputum or pus as cells of 4–10 μm diameter. In unstained wet preparations of CSF, the capsule surrounding the yeast may be visualized by the addition of a drop of India ink, which is excluded by the capsule, with the resultant appearance of a halo around the cell (Fig. 10.12).

Although cultures can be 'performed', a much quicker method for the diagnosis of cryptococcal meningitis is the use of a latex agglutination test to determine the presence of capsular polysaccharide material in the CSF. The test is highly sensitive and specific and gives better results than microscopy and culture. Over 90% of infected patients will give a positive reaction with this test, with the highest levels seen in patients with AIDS.

Patients with the mild, self-limiting form of cryptococcosis need no treatment. However, patients with meningitis require intravenous therapy with amphotericin B and flucytosine, and AIDS patients will require lifelong maintenance therapy to prevent relapses.

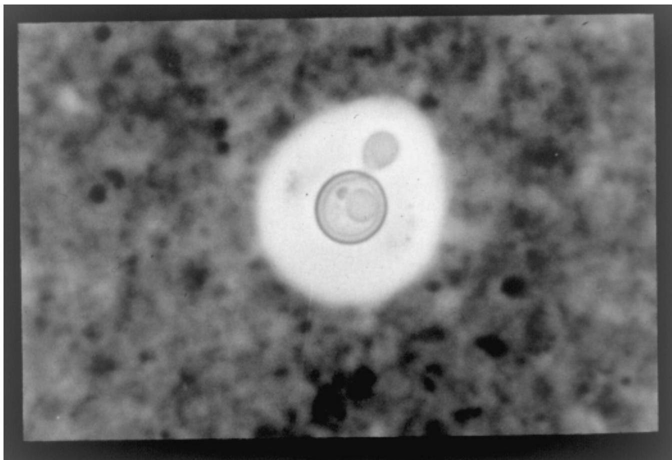

Fig. 10.12. Yeast cells of *Cryptococcus neoformans* in cerebrospinal fluid. Note the large transparent capsule.

Histoplasmosis

The causal organism is *Histoplasma capsulatum.* Histoplasmosis is usually an asymptomatic or mild self-limiting pulmonary infection but there are also chronic and acute disseminated forms of the disease. The fungus occurs in nature in soil enriched with bird or bat droppings in specific endemic areas. However, histoplasmosis has been reported in most countries of the world in those who have travelled to the endemic areas, which include central and eastern North America, particularly the Mississippi and Ohio river valleys, Kentucky, Arkansas and Missouri. Caves in these areas are particularly likely to be a source of infection. In the environment, *H. capsulatum* grows as a mould, but in human tissue it grows as an intracellular yeast.

Manifestations of histoplasmosis vary considerably, depending on the level of exposure and the health of the person exposed. In most cases, infections are asymptomatic, but an acute influenza-like illness may occur if large numbers of the organism are inhaled. Chronic pulmonary histoplasmosis may occur in middle-aged men with pre-existing lung disease. Symptoms include pneumonia, weight loss and night sweats, resulting in fibrosis, tissue destruction and lung cavitation, which may lead to disseminated disease or death due to lung failure in extreme cases. Disseminated histoplasmosis is usually seen at the extremes of age or in those with some impairment of the immune system. Dissemination in immunocompetent people is usually indolent over months or years, with liver, adrenal and mucosal lesions common. In immunocompromised patients disease progression may be much more aggressive and rapid.

Diagnosis of histoplasmosis can be carried out using several methods, but, as none is totally reliable, several are usually undertaken simultaneously. Culture of the organism is the 'gold standard', but as the organism can take several weeks to grow, it does not give quick results. Serological detection of either antibodies or antigen may also be useful. Antibody detection can be carried out using complement fixation and immunodiffusion, but cross-reaction with antibodies to other organisms may occur. A radioimmunoassay to detect antigen has been developed but is not widely available. The treatment of choice for disseminated histoplasmosis in immunocompromised patients is intravenous amphotericin B. Itraconazole may be used in immunocompetent patients and as maintenance therapy in AIDS patients.

A second form of histoplasmosis, called African histoplasmosis, is caused by *H. duboisii* Vanbr. and is restricted to the continent of Africa. It mainly affects the skin and underlying structures, sparing the lungs. The organism is a variety of *H. capsulatum* and it is identical to it in culture but has larger yeast cells (12–15 μm diameter) in the tissues (Fig. 10.13). Treatment is the same as for infections with *H. capsulatum*.

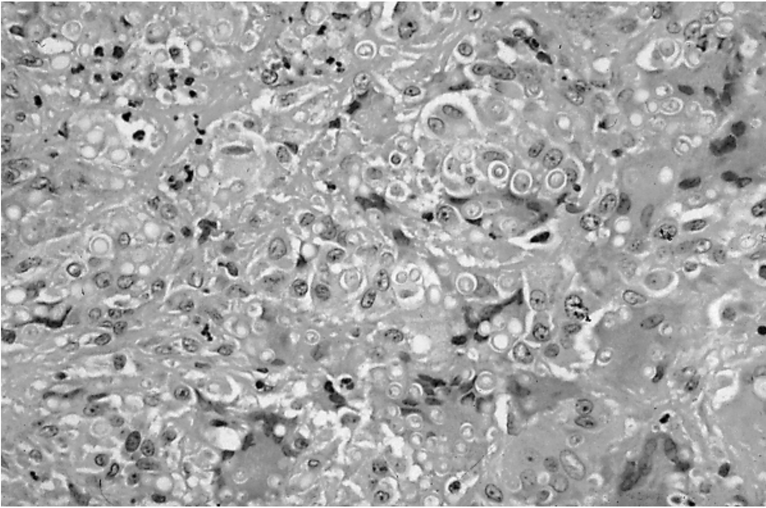

Fig. 10.13. Histology of a lymph node showing large yeast cells of *Histoplasma duboisii*.

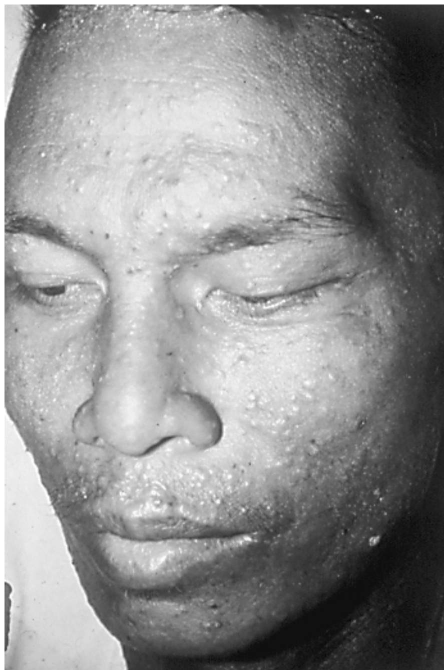

Fig. 10.14. Cutaneous lesions caused by *Penicillium marneffei* in an AIDS patient. Photograph courtesy of Dr A.J. Hamilton.

Penicillium marneffei infections

Penicillium marneffei Segretain has only recently emerged as a significant cause of infection. It infects both immunocompetent and immunocompromised patients in South-East Asia and people who have travelled to the region. It is now the fourth most common cause of death in AIDS patients in this region.

P. marneffei was first identified and cultured from a bamboo rat in South Vietnam. Initially, it appeared to be a mycological curiosity, but the rising number of cases of infection due to this organism in patients with AIDS and also in some healthy individuals changed that. The exact relationship between the fungus, the bamboo rat and humans remains unknown. *P. marneffei* can be isolated from some bamboo rats and their burrows, but is only rarely isolated from soil and so its environmental reservoir is still unknown.

Infection with *P. marneffei* results in fever, chronic cough, anaemia, weight loss and skin lesions which are considered to be characteristic of the disease (Fig. 10.14). In AIDS patients, the onset of symptoms is often more intense, particularly in children. Diagnosis of this disease is often difficult. *P. marneffei* can resemble *H. capsulatum in vivo* as both are intracellular parasites. Culture of the organism is the definitive diagnostic test, but confirmation of the identity of the organism may be problematic. Serology is not yet useful for diagnosis, a combination of diagnostic tests is therefore required. Mild cases may respond to itraconazole, but more severe forms will require amphotericin B. As with cryptococcosis and histoplasmosis, long-term maintenance therapy is required in patients with AIDS to prevent relapse.

Other tropical mycoses

There are several other fungal infections that occur in tropical countries and may pose a threat to travellers to endemic areas. All are caused by dimorphic fungi. Coccidioidomycosis, due to *Coccidioides immitis*, is endemic to the western states of North America, through Central America into Venezuela, Colombia and Argentina. Blastomycosis, caused by *Blastomyces dermatitidis*, is endemic to the Mid-West and south-eastern regions of North America and many countries in Africa. Paracoccidioidomycosis, caused by *Paracoccidioides brasiliensis*, is endemic to all Latin American countries, except Chile and Nicaragua. In common with the previous infections, these diseases have a range of presentations from asymptomatic, acute or chronic to disseminated disease. Diagnosis in most cases is relatively straightforward, but treatment with amphotericin B is necessary for the more serious forms of these diseases.

Summary

In summary, potentially pathogenic fungi capable of causing a whole range of fungal infections, from the trivial to the life-threatening, are found in soil and vegetation in tropical climes. People who visit these areas, particularly those whose travels bring them into close contact with nature, run the risk of developing exotic fungal diseases. It is advisable to be aware of the risks so that the infections can be prevented or dealt with promptly should they occur.

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Recent and Future Discoveries of Pharmacologically Active Metabolites from Tropical Fungi 11

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Introduction

All major commercial medicines and agrochemicals based on fermentation products of fungi are derived from metabolites of strains that originated from temperate regions (Table 11.1). These natural products (NP), semisynthetic derivatives and synthetic analogues include the β-lactams, cyclosporin, mycophenolic acid, ergonovine, ergotamine, lovastatin, pneumocandins and echinocandins, and the strobilurins. The vast numbers of untested taxa, the few but highly successful fungal-derived products, ease of laboratory manipulations and, to a lesser degree, hypotheses about chemically mediated relationships between fungi and other organisms are frequently cited reasons for advocating fungi as a resource for low-molecular-weight organic compounds for drug discovery (Dreyfuss and Chapela, 1994; Gloer, 1997; Pearce, 1997). Since the late 1980s, the unrealized potential of metabolites of tropical fungi as sources of starting molecules for drug development has been emphasized (Nisbet and Porter, 1989; Petrini *et al.*, 1992; Fox, 1993).

Despite an increasing awareness of and discussion regarding the potential for metabolites from tropical fungal species, arguably the numbers of metabolites discovered from tropical fungi, as judged from the patent and NP literature, at best have only equalled those from temperate fungi (Wildman, 1997). Some of the most significant NP discoveries of the 1990s have resulted from temperate fungi, a few of which are mentioned in this chapter. Nevertheless, some important lead compounds have been discovered from tropical fungi, and a selection resulting from projects carried out at Merck Research Laboratories (MRL) are included here. Some misconceptions about

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Table 11.1. Major medicinal and agrochemical products developed, or in development, that are based on fungal metabolites. The common name of the fungal metabolite is given followed by its biosynthetic origin, the fungal taxon that produces the metabolite, and generic and trade names of the commercial products or derivatives.

Metabolite	Biosynthetic family	Fungi	Commercial products[a]
Penicillins	Peptide	*Penicillium*, *Aspergillus* spp.	Penicillin G, ampicillin, carbenicillin, methicillin, amoxicillin
Cephalosporins	Peptide	*Acremonium*, *Emericellopsis* spp.	Cephalosporin N, Cefoxitin, Rocephin, Cefzil, Monocid
Lovastatin, pravastatin	Polyketide	*Aspergillus terreus*, *Penicillium* spp., *Monascus ruber*, *Pleurotus* spp.	Mevacor, Zocor, Pravachol
Cyclosporin A	Peptide	*Tolypocladium* spp., other *Hypocreales*	Sandimmune
Ergotamine	Tryptophan-isoprenoid	*Claviceps* spp.	Ergostat, Cafergot, Bellergal-S, Maxalt
Mycophenolic acid	Polyketide-isoprenoid	*Penicillium* spp.	CellCept (mycophenolate mofetil)
Pneumocandin B_0	Peptide-polyketide	*Glarea lozoyensis*	Cancidas[b] (caspofungin acetate)
Strobilurins	Polyketide-amino acid	*Strobilurus tenacellus* and other basidiomycetes	Allegro, Brio (kresoxim-methyl), Amistar, Heritage (azoxystrobin)

[a]Registered trade names are capitalized. Some of the products are semi-synthetic derivatives, or wholly synthetic derivatives based on a fungal-derived natural product.
[b]In phase III clinical trials.

the priorities and goals of fungal metabolite screening programmes and questions of the metabolic distinctiveness of tropical fungi are also addressed. Recent sweeping changes in pharmaceutical research, molecular biology of biosynthetic pathways, high-throughput screening (HTS), and the Convention on Biological Diversity (CBD) have redirected MRL's approaches to fungal screening. In the light of this experience, recommendations are made on how to ensure that tropical fungi contribute to future discoveries.

An Evolving Role for Natural Product Research in Drug Discovery

The landscape for NP screening within drug discovery has changed dramatically during the last 10 years (MacIlwain, 1998; Archer, 1999; Service, 1999a). Most pharmaceutical companies have established large collections of structurally distinct, small molecules that are used as the starting point for drug lead identification. A corporate compound collection may consist of 100,000–500,000 discrete chemical entities. The initial task of screening hundreds of thousands of compounds is usually performed by HTS laboratories, which are operated by scientists in applied biology and chemistry. They utilize specialized assay technologies, dedicated robotic systems and data-handling software to screen the chemical samples and evaluate the primary screening data within a time frame of weeks to a few months.

Access to chemical diversity may no longer be the rate-limiting step in drug discovery. As a result, NPs have shifted from being a primary chemical source to a complementary source of chemical structural diversity. Combinatorial synthesis, the automated synthesis of structurally related small molecules, can generate millions of new structures in the search for an active drug or probe molecule for research or diagnosis (Ellman *et al.*, 1997; Karet, 2000). Most compounds do not bind or interact with a target, and therefore many researchers in industrial and academic laboratories are mixing the power of combinatorial chemistry with the specificity of targeted, or rational, drug design to create focused libraries of compounds more likely to 'hit' their targets. Still, much structural diversity found in NPs cannot be achieved by synthetic means, and many experts argue that NPs offer a source of often unpredictable chemical structures that can lead to unanticipated and alternative medicinal chemistry programmes (Shu, 1998; Henckel *et al.*, 1999; Service, 1999b; Harvey, 2000; Strohl, 2000).

HTS technology obliges researchers to use natural extract collections dispensed in 'screen-ready' formats in microwell plates. Sample-testing formats have continuously moved towards higher-density and smaller-volume microwell plates (96-, 384- and 1536-well plates), and the pace at which synthetic chemical collections, combinatorial chemistry libraries and NPs are tested in parallel has increased exponentially. To accrue and deliver large numbers of

testable NP extracts for HTS stations, most laboratories have abandoned past approaches to microbial NP testing, in which a dedicated team continually screens fermentations of freshly isolated strains. Instead, large extract collections, often called NP libraries, are assembled either in-house or obtained from a number of speciality companies or research institutes that offer NP extracts or purified NPs. Collections of organism extracts are carefully assembled, with each microbial extract backed by a preserved microbial strain. If an extract needs extensive retesting, the microbe is revived to reproduce the fermentation extract. Plant and animal extracts are backed by well-documented voucher specimens that allow rapid recollection of the organism when more of its tissue is sought for isolation chemistry and biological evaluation.

The extract collection/screening model has several advantages over the traditional continuous isolation/fermentation model and allows for systematic exploration of biological diversity. Organism extracts are accumulated and predispensed over time. They are immediately available for screening and facilitate integration of NPs into the HTS laboratory. Representative organisms can be selected to ensure thorough coverage of chemically important species. In very metabolite-rich taxa, e.g. *Eurotiales*, sampling the entire range of phylogenetic diversity may be desirable. The screening strategy shifts efforts from continuous collection and screening of easy-to-collect speciose organisms (Bills, 1995), towards a focus on difficult-to-obtain organisms, which are stored and retested instead of being grown once and discarded. Fungi are usually grown in a variety of conditions to obtain a full array of metabolites. Since the collections are used repeatedly for many tests, efforts previously devoted to regularly obtaining new sets of organisms for screening are redirected to ensure that each organism is distinct, kept in a stable form, grown to produce a metabolite-rich fermentation, and that its extract uniquely contributes to the overall chemical diversity in the collection (Julian *et al.*, 1998).

Extract collections, however, have their own liabilities, most of which centre on maintenance of the biological and chemical integrity and adequate inventory of extracts. The need to return to collections after years of storage requires meticulous and stable preservation of strains. Chemical stability of stored natural product extracts, how to reconstitute them so that they give results representative of a fresh extract and inventory management are critical considerations. However, the success of the extract library versus the traditional continuous isolation/fermentation model as a discovery tool still awaits validation.

What are Tropical Fungi? Are They Metabolically Distinct from Other Fungi?

Using a geographic definition of tropical, the experience in the modern HTS environment at MRL has been that an extract of a tropical fungus is equally

likely to produce a new lead compound as one of boreal or austral origin. It is generally accepted that in the tropics, especially the humid tropics, certain taxonomic and ecological groups of fungi are more speciose than in temperate habitats. Less sampling effort yields more species than in temperate or boreal regions, and certain taxa appear to be uniquely tropical. At the same time, temperate habitats, e.g. high-elevation forests, are also present in tropical latitudes. The rich diversity of tropical fungi and varied habitats within the tropics are the primary consideration that leads to the incorporation of a tropical component into a metabolite screening programme.

Although fungal NPs display an extraordinary degree of structural diversity, most of the major structural classes share their biosynthetic origins. The genes required for biosynthesis of polyketides, non-ribosomal peptides and terpenoids are now known to be clustered and to occupy a significant proportion of chromosome loci. Other fungal NPs are derived from shikimic acid, carbohydrates and fatty acids. Tropical fungi can produce the same compounds as their temperate counterparts, and undiscovered metabolites are frequently found among both. Examples of similar or identical secondary metabolites produced by taxonomically disparate fungi abound in the literature (Turner and Aldridge, 1983; Anon., 1999).

The redundancy of NP biosynthetic pathways among fungi may be explained by several hypotheses on the evolution of secondary metabolites. One proposal was that NPs evolved from ancestral low-molecular-weight substances that facilitated primordial biochemical reactions (Davies, 1990). If this is true, fungi would share various NP pathways because they share a common ancestry. Another hypothesis for NP evolution is that some secondary metabolic pathways are modified primary pathways. This is strongly supported by the similarities between pathways involved in polyketide and fatty acid biosynthesis. The main differences are that polyketide synthases (PKSs) can incorporate a large variety of acyl starting units and lack the partial or complete reductions of the β-keto groups formed after each chain extension. Furthermore, the primary nucleotide sequences and functional domain organization of known fungal PKS genes are highly conserved (Fig. 11.1). Horizontal gene transfer hypotheses of evolution may explain why some secondary metabolic pathways are redundant among fungi (Walton, 2000). The β-lactam biosynthetic pathways in fungi and bacteria share significant homologies, suggesting that fungi may have acquired the genes from bacteria.

The incongruencies between metabolite redundancy among unrelated species and projections of infinite chemical diversity from taxonomic diversity should be considered when interpreting the articles of the CBD that pertain to sovereign rights over genetic resources and benefit sharing. Metabolite redundancy also affects assignment of intangible intellectual property rights. Breakthrough products like cyclosporin and lovastatin are examples of redundant metabolites. Retrospective data indicate that valuable fungal metabolites are likely to be widespread. It is predicted that cyclosporin-, strobilurin-, and

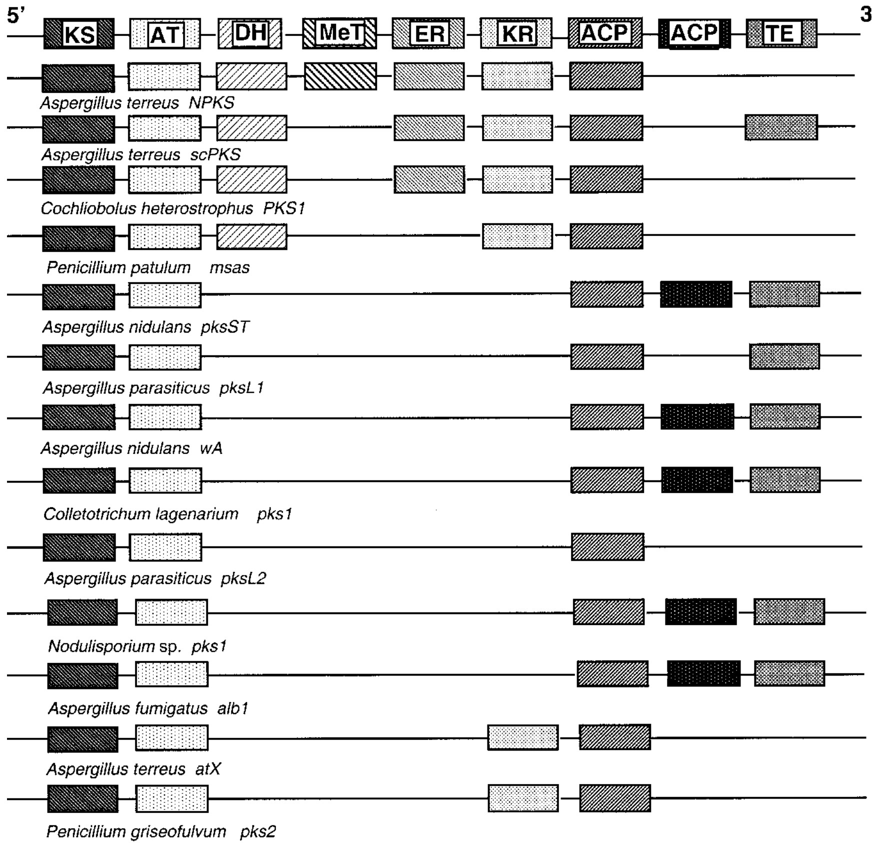


Fig. 11.1. Organization of fungal type I iterative polyketide synthase gene clusters. KS (ketoacyl synthase), AT (acyltransferase), DH (dehydratase), MeT (methyltransferase), ER (enoyl reductase), KR (ketoreductase), ACP (acyl-carrier protein, possibly more than one per gene cluster), TE (thioesterase-cyclase).

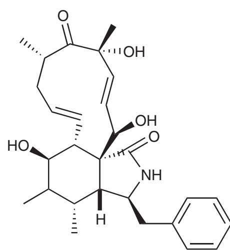
lovastatin-producing fungi could be found in every country of the world (Gunde-Cimerman *et al.*, 1993; Möller *et al.*, 1996; Traber and Dreyfuss, 1996; Anke and Steglich, 1999). Despite the confusing redundancy, the distributions of many metabolite families occur frequently and predictably among certain taxa, and often with striking taxonomic distributions (Turner and Aldridge, 1983; Frisvad *et al.*, 1998). Some fungal metabolites are claimed to be rare or strain-specific because they have been reported once from a single strain. In some instances this may be true, perhaps because of recent recombinations of biosynthetic genes in rapidly evolving populations (Schardl and Wilkinson, 2000), or because a species is rare and geographically isolated. However, in most instances, it is suspected that populations of producing organisms were not systematically compared using the same detection methods. In other

cases, incorrect identifications have prompted misguided comparative searches for metabolites (Frisvad, 1989; Bills *et al.*, 1999). Therefore, lack of data or erroneous data should be considered with respect to apparent discoveries of rare and strain-specific metabolites.

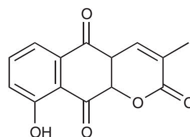
The widespread distribution of metabolites raises questions concerning assignment of economic value to any particular fungal strain or any particular fungal habitat as a source for drug discovery. The first discovery of a compound may be the most valuable, while subsequent discoveries of that compound will probably be of insignificant worth (Simpson and Sedjo, 1996a,b). New uses for known biological products further complicate the question of the value of the producing organisms, since the chemical structures and methods for production are already publicly known and not generally patentable. Nevertheless, the advent of new targets and screening tools makes re-exploration of familiar fungal species as exciting as exploration of new and exotic species. Undoubtedly, the novel application of known metabolites can constitute as important a breakthrough as that of novel chemicals. When expediency in discovery can determine economic value, does the over-regulation of biological resources which probably transcend political boundaries make sense, or would it be more advantageous to test resources as quickly and as often as possible to achieve discovery first, and capture the potential benefit?

Metabolic redundancy can be a problem in screening NPs. Many compounds recur even among seemingly unrelated species from different geographical regions and habitats. Fermentations produce mixtures of known and unknown metabolites. Primary and constitutive metabolites produced during active growth, as well as secondary metabolites, are extracted simultaneously. Although redundancy can be alleviated to some extent by careful strain selection, isolation chemists are still burdened with the task of sorting out known and ‘nuisance’ compounds from unknown components (Corely and Durley, 1994). Fungi, like bacteria and plants, produce their own sets of ‘nuisance’ compounds (Fig. 11.2). Certain metabolites can be troublesome and highly reactive in sensitive biochemical assays because of highly oxidized or reactive chemical groups, such as multiple hydroxyls, quinones, epoxides, or pigmented chromophores. Other characteristics include production in high titres, often causing extracts to behave like potent active samples. Such compounds are frequently produced under varied growth conditions, thus increasing the probability that they may be detected from different fermentations of the same strain and found interfering with assays.

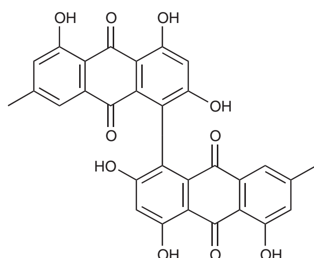
During the period from 1995 to 1997, more than 13,000 fungal isolates were examined for production of bioactive secondary metabolites at MRL’s Centro de Investigación de España (CIBE). An approximate distribution of the geographical origins and types of substrata are listed in Table 11.2. Certain ubiquitous taxa, e.g. *Bionectria ochroleuca* (Schwein.) Schroers & Samuels and *Lasiodiplodia theobromae* (Pat.) Griffiths & Maubl., may have been screened



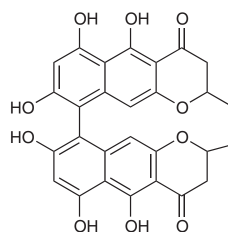
Deacety-cytochalasin C
from *Beltraniella portoricensis*



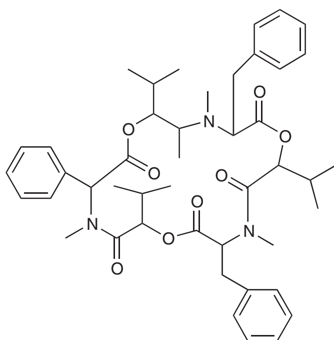
Lambertellin
from *Encoelia heteromera*



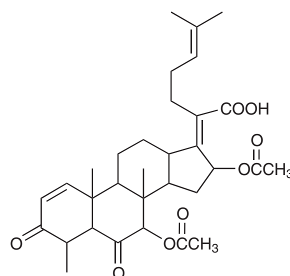
Skyrin
from *Hyperdermium bertonii*



Cephalochromin
from *Nectria vilior*



Beauvericin
from *Paecilomyces tenuipes*



Helvolic acid
from *Metarrhizium anisopliae*

Fig. 11.2. Examples of 'nuisance' metabolites from tropical fungi commonly encountered during natural products screening.

multiple times from among the different substrata. The percentages of active strains refer to those of strains scored as active in a broad array of biological assays targeting therapeutic areas such as antifungal and antibacterial antibiotics, antivirals, insecticides, antihelminthic agents, cancer, diabetes mellitus, inflammation, and endocrinology. Each strain was usually fermented by two to four methods, and at least one and sometimes two solvents were used to extract each fermentation. The data are based on the unrefined primary active extracts, before applying secondary assays for prioritization. If one or multiple extracts from a strain were scored as active in at least one assay, then the strain was considered to be active. Only a small fraction of the active components were purified and fully elucidated to give chemical structures.

Fungi from temperate and tropical regions performed similarly. A χ^2 goodness-of-fit test revealed significant differences ($P < 0.01$) only between the 'hit rates' of endophytes (fungi isolated from living plants) from tropical versus temperate areas. Tropical endophytes appear to provide a higher 'hit rate' than their temperate counterparts. Likewise, the 'hit rate' obtained with the tropical endophytes was significantly higher than with fungi from other tropical substrata according to a χ^2 test. Ascribing meaning to differences in rates of detection of actives in HTS needs cautious interpretation. The perception of higher bioactivity may or may not translate into increased discovery opportunities. Endophyte isolates from humid-tropical plants are taxonomically biased towards the *Xylariales* and *Hypocreales*, two especially metabolite-rich taxa. However, high rates of active extracts are not always

Table 11.2. Performance[a] of strains from tropical and temperate areas in a screening programme for bioactive natural products. Results were obtained at CIBE during the years 1995–1997. Only those types of substrata giving statistically relevant numbers of isolates are considered as separate categories.

	Tropical[b]		Temperate and boreal		Total	
Substratum class	Isolates tested	Per cent active	Isolates tested	Per cent active	Isolates tested	Per cent active
Living plants	3,005	12.2	3,217	14.6	6,222	13.4
Leaf litter	3,463	11.4	511	12.0	3,974	11.9
Dung	396	11.6	387	10.9	783	11.2
Others[c]	209	12.4	1,827	21.5	2,036	13.3
Total	7,073	12.1	5,942	13.3	13,015	12.8

[a]A strain is scored as active if one or more of its extracts are active in at least one biological assay.
[b]Tropical is defined geographically, i.e. fungi isolated from substrata collected between the Tropics of Cancer and Capricorn.
[c]Soils, marine, freshwater, other substrata and macroscopic fungi.

related to pharmaceutically desirable products. A high proportion of the strains could produce reactive and toxic classes of compounds, e.g. trichothecenes and cytochalasins, or high concentrations of primary metabolites, e.g. unsaturated fatty acids, which interfere non-specifically with biochemical assay mixtures to generate false positives. Either way, differences in rates of activity from tropical endophytes did not result in a higher number of novel structures with respect to other fungi. Twenty different compounds (or new biological activities for known compounds) were discovered from all the fungal isolates tested during the period. The ratio of the compounds' geographical distributions was the same as that of the tested isolates, i.e. 11 compounds were from tropical areas and nine from temperate areas. Data reported by Glaxo's NP laboratory during the early 1990s (Wildman, 1997) resulted in a similar ratio of activities and new compounds between tropical and temperate fungi.

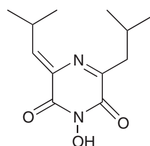
Important Metabolites from Tropical Fungi

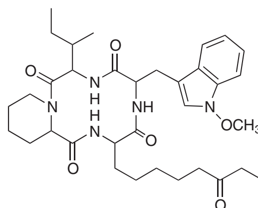
Examples of discoveries from tropical fungi

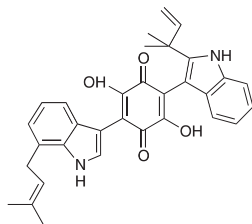
Six case studies from MRL illustrate a range of discovery scenarios involving tropical fungal metabolites. Most of the discoveries were potentially important because new modes of action for drug targets were uncovered or because the fungal metabolite provided the first proof that the target was valid and could be affected by a small organic molecule. For some of the leads, extensive medicinal chemistry investigations were carried out to modify and improve the potency and spectrum of the lead molecule's activity. To sustain delivery of the metabolite to a preclinical investigation, gram or kilogram quantities of purified compound must be produced. The case studies include some of MRL's experiences with metabolite titre improvement and the difficulties in predicting the physiological responses of wild fungal species.

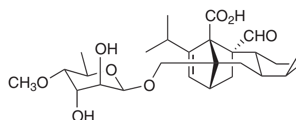
Flutimide, an inhibitor of flu transcriptase

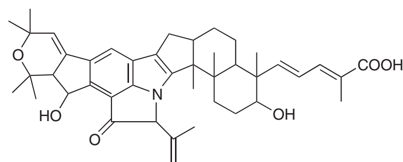
Flutimide (Fig. 11.3) was discovered in the MRL screening programme seeking inhibitors of cap-dependent transcription of influenza viruses (Hensens *et al.*, 1995). Flutimide is a substituted 2,6-diketopiperazine, structurally related to the aspergillic acid family (Turner and Aldridge, 1983). Its mode of action is that of an inhibitor of cap-dependent transcriptase of influenza A and B viruses that specifically targets the endonuclease activity of the transcriptase. Other viral polymerases were not affected by flutimide (Tomassini *et al.*, 1996). Flutimide was the only NP structure isolated after screening several thousand microbial extracts. The compound is produced by *Delitschia confertospora* Peláez, Polishook, Valldosera and Guarro, a new fungal species isolated from dung of a dassie (*Procavia* sp.) collected in Namibia (Peláez *et al.*, 1994).











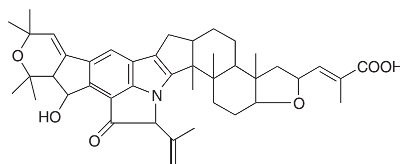


Fig. 11.3. Examples of pharmacologically active metabolites from tropical fungi.

Flutimide was isolated only from a single strain, but an extensive search for additional strains was not carried out after the lead was identified. Shortly thereafter, the molecule was made synthetically to verify the structure and produce sufficient material for preclinical testing (Singh, 1995).

Apicidin, an antiprotozoal agent

A number of human and animal parasitic diseases, including malaria, cryptosporidiosis, toxoplasmosis and coccidiosis, are caused by protozoa of the subphylum *Apicomplexa*. The cyclic tetrapeptide apicidin (Fig. 11.3) was identified from MRL's NP screening programme and has been described as a broad-spectrum agent effective against a range of apicomplexan parasites (Darkin-Rattray *et al.*, 1996; Singh *et al.*, 1996). It acts as a potent inhibitor (IC50 1–2 nM) of the parasite's histone deacetylase. Apicidin exhibits a broad

range of activity *in vitro* (minimum inhibitory concentrations of 4–70 ng ml^{-1}) against the *Apicomplexa*, as well as *in vivo* activity against *Plasmodium berghei* Vinke & Lips. malaria in mice.

Fusarium pallidoroseum (Cooke) Sacc. and an unidentified *Fusarium* species, both isolated from Costa Rican plants, were found to produce apicidin (Cannova *et al.*, 1997). The first strain, *F. pallidoroseum*, produced 50–80 μg g^{-1} in solid substratum fermentation but less in liquid media. Discovery of the second *Fusarium* species accelerated delivery of apicidin for evaluation. In vermiculite-based screening medium, the second strain produced 10-fold higher levels than those by *F. pallidoroseum*. Empirical fermentation studies with the second strain eventually improved titres to over 1 mg ml^{-1} in liquid media in shake flasks and 300–400 μg ml^{-1} in 600-l fermenters (A. Dombrowski *et al.*, unpublished).

It is predicted that apicidin and other tetrapeptides with histone-deacetylase activity, e.g. HC toxin, are likely to be geographically widespread among *Fusarium* species. Apicidin has recently been implicated in a haemorrhagic disease syndrome of livestock in Korea (Park *et al.*, 1999). *Fusarium* isolates recovered from soybeans produced titres of apicidin in the range of 340–680 μg g^{-1} in wheat grain cultures, which is of the same order of magnitude as the titres MPL obtained in our titre improvement project (A. Dombrowski *et al.*, unpublished).

A potent insecticide from a pantropical Hypoxylon

Nodulisporic acids (Fig. 11.3) are novel indole diterpenes, the first compounds of this class reported from the *Xylariales* that exhibit potent insecticidal properties against the larvae of the blowfly, *Lucilia sericata* Meigen (Ondeyka *et al.*, 1997; Ostlind *et al.*, 1997; Hensens *et al.*, 1999). Levels of toxicity are in ranges intermediate between those of two of the most potent NP insecticides, the avermectins and paraherquamide (Ostlind *et al.*, 1997). The mode of action of nodulisporic acid is that of an activator of insect glutamate-gated chloride channels (Smith *et al.*, 2000). Nodulisporic acids are structurally similar to janthitrems, shearinines, paspalines, and lolitrems. However, they differ in having a five-membered substituted ring on the indole moiety, an inversion of the tetramethyl pyran ring system and a five-membered hemiketal ring instead of a six-membered ring.

The compounds were first isolated from a fermentation broth of an endophytic *Nodulisporium* species. This endophyte was cultured from a surface-sterilized dried voucher specimen (woody stem) of *Bontia daphnoides* L. collected from Kuaui Island, Hawaii, during a collaborative project between MRL and the New York Botanical Garden (Hensens *et al.*, 1999). An intensive search was made for other *Nodulisporium* strains and other xylariaceous ascomycetes to discover more potent nodulisporic acid analogues or strains with improved fermentation titres. Twelve more isolates of *Nodulisporium* were found to produce nodulisporic acids (J. Polishook *et al.*, 2001). The strains

were isolated from diverse substrata, not only plants, from six different tropical countries on four continents (Table 11.3).

Alignment and parsimony analysis of the rDNA intertranscribed spacer regions and morphological comparisons indicated that the 13 nodulisporic acid-producing *Nodulisporium* strains were conspecific. The xylariaceous anamorphs were characterized by faster radial growth at 37°C than at 23°C, a dark brown soluble pigment in agar and liquid cultures, a sweet, medicinal odour, and deposits of a melanin-like pigment on the conidiophores. No particular isolate exhibited a striking advantage in nodulisporic-acid titre; titres of nodulisporic acid A ranged from 2 to 10 $\mu g\ ml^{-1}$. Comparisons of ITS sequences with a sequence database from other xylariaceous fungi (Sánchez *et al.*, 2000) failed to link the nodulisporic acid-producing *Nodulisporium* species to a teleomorph species but demonstrated that it is a *Hypoxylon* species, phylogenetically close to *H. fendleri* Berk. ex Cooke. To date, nodulisporic acids may be the only examples of exclusively tropical fungal metabolites that have been observed at MRL.

A non-peptidyl insulin mimetic

Bis-demethyl-asterriquinone B-1 (Fig. 11.3) was discovered as an orally available insulin mimetic agent through a screening assay designed to detect non-peptidyl small molecules that activate the human insulin receptor tyrosine kinase (Zhang *et al.*, 1999). The compound was shown to be selective for the insulin receptor versus other receptor tyrosine kinases, such as the insulin-like growth factor receptor or the epidermal growth factor (EGF) receptor. It mimicked insulin in several biochemical and cellular assays, including stimulation of glucose uptake in whole cells. Moreover, the oral administration of the compound in two mouse models of diabetes resulted in significant lowering of glucose levels and suppression of the elevated plasma insulin levels associated with one of these mouse models. Although quinones are notoriously reactive and generally considered an undesirable molecular platform for medicinal chemistry, the discovery was celebrated as a breakthrough in the field for the development of orally active antidiabetic agents. For the first time, a small, non-peptidyl molecule was demonstrated to mimic the *in vitro* and *in vivo* function of a protein hormone by interacting with and activating its receptor.

The above compound belongs to the family of the asterriquinones, widely known fungal bis-indolyl-quinones, which have been found in diverse ascomycetes from tropical and temperate regions, including *Aspergillus* species (and the teleomorph *Petromyces muricatus* Udagawa, Uchiyama & Kamiya), *Chaetomium* spp., *Chrysosporium merdarium* (Ehrenb.) J.W. Carmichael, *Botryotrichum* species and in *Humicola* species (Sekita, 1983; De Guzman *et al.*, 1994; Mocek *et al.*, 1996; Fredenhagen *et al.*, 1997; Ooike *et al.*, 1997). The asterriquinones have been studied as potential anti-tumour agents because of their DNA-intercalating properties (Yamamoto *et al.*, 1976; Kaji *et*

Table 11.3. Distribution and origin of nodulisporic acid-producing *Nodulisporium* species[a].

Isolate	Source	Location	Continent
MF5954 (ATCC 74245)	Stems, *Bontia daphnoides*	Kuaui Island, Hawaii, USA	Oceania
MF6230	Horse dung	Hiva Oa, Marquesas Islands, French Polynesia	Oceania
MF6263	Bush twig, unknown host	Capurgana, Colombia	South America
MF6262	Bark disc, unknown host	Capurgana, Colombia	South America
MF6324	Leaf litter, unknown host	Lago Sandoval, Peru	South America
MF6245	Fruticose lichen, *Usnea* sp.	Playa La Parguera, Puerto Rico	North America
MF6321	Leaf litter, unknown host	Curepipe, Mauritius	Africa
MF6315	Leaf litter, *Coffea* sp.	La Region de Sept Couleurs, Mauritius	Africa
MF6380	Twig, *Coula edulis*	Acan-Bot Esaveng, Equatorial Guinea	Africa
MF6246	Bark disc, *Griffonia tessmannii*	Bolondo, Bata, Equatorial Guinea	Africa
MF6379	Twig, *Dorstenia elliptica*	Acan-Bot Esaveng, Equatorial Guinea	Africa
MF6378	Twig, *Anacardium occidentale*	Bata, Equatorial Guinea	Africa
MF6377	Twig, *Scaevola plumieri*	Bome, Bata, Equatorial Guinea	Africa

[a]Polishook *et al.*, unpublished data.

al., 1998). They are also HIV-1 protease inhibitors (Fredenhagen *et al.*, 1997) and toxic to insects (De Guzman *et al.*, 1994). The compound found in MRL's screening had been previously obtained by chemical treatment of asterriquinone B-1(Arai *et al.*, 1981), but its insulin-mimetic properties were unknown. Other asterriquinones are ineffective as insulin-mimetic agents. For example, the similar asterriquinone, hinnuliquinone, is about 100 times less active in the assay (Zhang *et al.*, 1999). However, other asterriquinones have been shown to be inhibitors of the EGF receptor tyrosine kinase, which is closely related to the insulin receptor (Fredenhagen *et al.*, 1997).

Bis-demethylasterriquinone was detected in a liquid fermentation of an endophytic fungus isolated from unidentified living leaves collected near Kinshasa, Democratic Republic of the Congo. Sporulation was unachievable in agar culture, but cultivation of the fungus on sterilized wood strips induced formation of a mature ascoma that permitted tentative classification in the ascomycete genus *Pseudomassaria*. The compound was detected only once among more than 5000 fungal isolates tested from tropical and temperate regions.

A new class of antifungal agents: inhibitors of the protein synthesis elongation

The rediscovery of sordarins and related compounds has given rise to a promising new class of agents for treatment of human and plant fungal infections. Analyses of the discovery of sordarin and development of its analogues as antifungal agents for human mycoses illustrate the difficulties in interpretation of the CBD. Sordarin (Fig. 11.3) was first discovered in a soil isolate of *Sordaria araneosa* Cain from Sri Lanka by researchers at Sandoz searching for antifungal agents (Sigg and Stoll, 1969; Hauser and Sigg, 1971). Sandoz investigators patented and published their discovery, but sordarin was overlooked for more than two decades. During the mid-1990s, several laboratories independently reported the discovery of sordarin, zofimarin (Ogita *et al.*, 1987), and other antifungal diterpene glycosides with the sordaricin aglycone (Okada *et al.*, 1994; Coval *et al.*, 1995; Schneider *et al.*, 1995; Daferner *et al.*, 1999). GlaxoWellcome's HTS programme for inhibitors of *Candida albicans* (C.P. Robin) Berkhout protein synthesis resulted in the discovery of GB135402, a new sordarin analogue from *Graphium putredinis* (Corda) S. Hughes from the UK, possessing promising potency and selectivity for fungal versus mammalian protein synthesis (Kinsman *et al.*, 1998). At about the same time, sordarins were rediscovered at MRL through non-mode-of-action screening for antifungal agents (Bills *et al.*, 1998).

The exceptional potency and biological spectrum of sordarin derivatives stimulated investigations to solve the molecular basis for their antifungal activity. Sordarin and its analogues are tetracyclic diterpene glycosides that selectively inhibit fungal protein synthesis by impairing the function of eukaryotic elongation factor 2 (eEF2) (Domínguez *et al.*, 1998; Justice *et al.*,

1998, 1999). Sordarin and its derivatives bind to the eEF2–ribosome–nucleotide complex in sensitive fungi, stabilizing the post-translocational GDP form.

Sordarin and similar compounds occur widely among ascomycetous fungi, especially in the *Sordariales* and *Xylariales* (Daferner *et al.*, 1999). MRL's investigations of sordarin relied on the compound produced by a non-sporulating endophyte from living roots of a mangrove shrub, *Conocarpus erectus* L. from Costa Rica (MF6232) and from *Rosellinia subiculata* (Schwein.) Sacc. collected in New Jersey (Bills *et al.*, 1998). Although studies at Glaxo-Wellcome initially focused on GB135402 from *Graphium putredinis*, further scale-up of sordarin analogues relied on superior titres provided by the original sordarin-producing strain, *Sordaria araneosa* (ATCC 36386) (Hayes *et al.*, 1996). MRL obtained the same species of *Sordaria* (ATCC 36386) and compared it with strains isolated in their laboratory. Fermentations on solid substrata were converted to liquid fermentation media and manipulated to improve the titres. The titres of the sterile strain, MF6232, were more readily improved, obtaining production levels up to 700 μg ml^{-1} in shake flasks. Eventually, MF6232 was scaled up successfully in 800-l and 19,000-l stirred tanks. *Rosellinia subiculata* produced only 80–100 μg ml^{-1} in shake flasks. *Sordaria araneosa*, in shake flasks, initially produced 250 μg ml^{-1} sordarin, but the production was increased nearly threefold by media manipulation (A. Dombrowski *et al.*, unpublished).

Antifungal sphingolipid inhibitors

Sphingolipid synthesis is a vital pathway for cell membrane components. Fungal sphingolipid biosynthesis (Fig. 11.4) has been a target for discovery of new human health antifungal agents for candidiasis, aspergillosis, and other mycoses because certain enzymes in the pathway are unique to fungi (Dickson, 1998). Identification of specific and potent inhibitors of fungal enzymes could be potential starting points for lead development. Over the course of several years, an assay for detection of fungal pathway inhibitors was run at CIBE. Natural product inhibitors for three different enzymes, serine palmitoyltransferase, ceramide synthase, inositol phosphoceramide synthase, and for the fatty acid elongation pathway were discovered from temperate and tropical fungi and actinomycetes (Mandala and Harris, 1999).

Inhibitors derived from tropical fungi and the specific enzymes they affect are shown in Fig. 11.4. Viridiofungin A (Fig. 11.4), B, and C form a novel family of amino alkyl citrates and are potent broad-spectrum antifungals (Mandala *et al.*, 1997b; Onishi *et al.*, 1997). They were isolated from a strain identified as *Trichoderma viride* Pers. recovered from soil collected in Micronesia (Harris *et al.*, 1993). Although viridiofungins are moderate inhibitors of yeast squalene synthase, they do not specifically inhibit fungal ergosterol biosynthesis *in vivo*. Their antifungal mode of action is based on their nanomolar-level inhibition of serine palmitolytransferase (Mandala *et al.*, 1997b). An

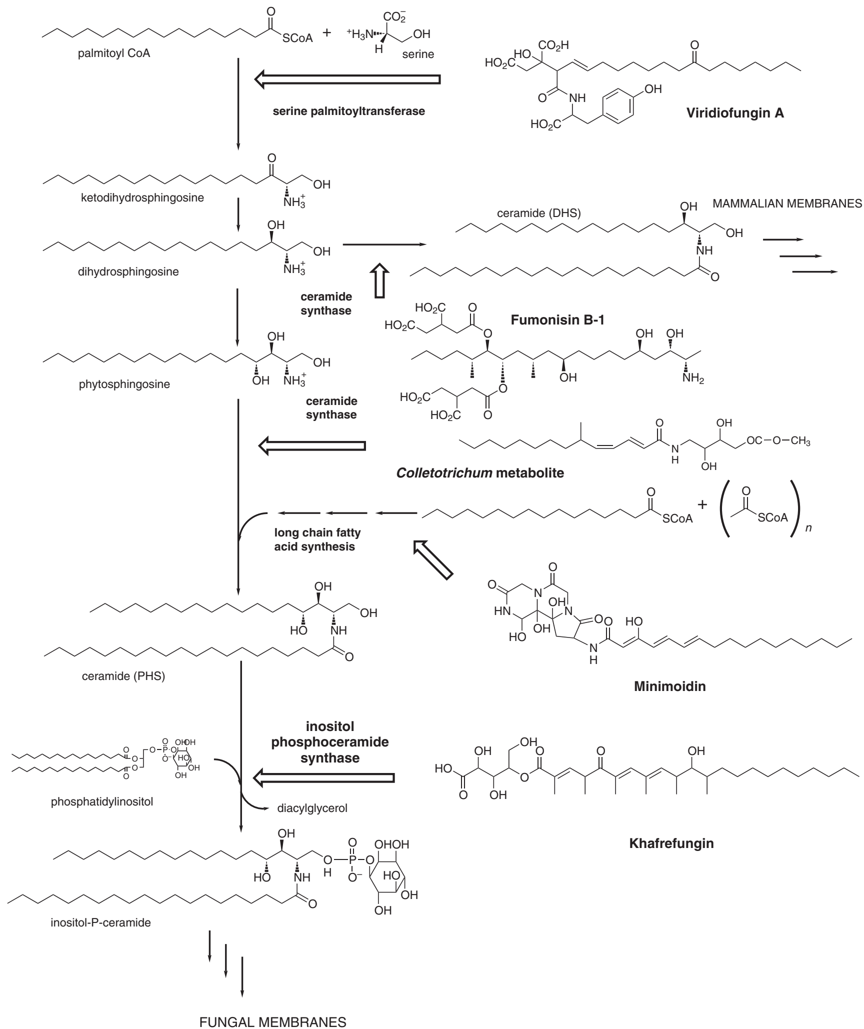


Fig. 11.4. Examples of fungal natural product inhibitors of sphingolipid biosynthesis. Viridiofungin A, the *Colletotrichum* metabolite, minimoidin and khafrefungin originated from tropical fungi. White arrows indicate enzymatic steps inhibited. Adapted from Mandala and Harris (1999).

unnamed sphingosine-like metabolite isolated from a Costa Rican isolate of *Colletotrichum acutatum* J.H. Simmonds (Fig. 11.4) inhibits fungal ceramide synthase (Bolessa *et al.*, 1996). Australifungin is another fungal-specific inhibitor of this step of the pathway, while the mycotoxin fumonisin is a potent inhibitor of mammalian ceramide synthase (Mandala and Harris, 1999). Khafrefungin (Fig. 11.4), a linear polyketide isolated from a non-sporulating endophyte of *Tetragastris panamensis* Kuntze collected in Costa Rica, is a

fungal-specific subnanomolar inhibitor of inositol phosphoceramide synthase (Bills *et al.*, 1997; Mandala *et al.*, 1997a; Mandala and Harris, 1999). Inositol phosphoceramide synthase is essential for fungal growth and is the target of the antifungal fungal metabolite aureobasidin (Zhong *et al.*, 2000). Finally, minimoidin (Fig. 11.4) is a potent inhibitor of sphingolipid synthesis that acts by inhibiting the fatty acid elongation pathway. Minimoidin contains a novel heterocyclic ring system acylated with a β-keto fatty acid (Clapp-Shapiro *et al.*, 1998; Mandala and Harris, 1999). It was discovered from a strain of *Sporormiella minimoides* S.I. Ahmed & Cain isolated from giraffe dung collected in Namibia. Minimoidin has minimum inhibitory concentrations against *Candida* spp., *Cryptococcus neoformans* (San Felice) Vuill. and *Aspergillus fumigatus* Fresen. in the range of 0.125–2 μg ml^{-1} (Clapp-Shapiro *et al.*, 1998), and therefore is the most potent metabolite found among the antifungal sphingolipid pathway inhibitors.

Although numerous potent and specific NP inhibitors of the sphingolipid target were discovered, many of the structures were disadvantaged by poor solubility or stability and limited whole-cell activity, while others were lytic to red blood cells, and were therefore eliminated as candidates for further development.

Fungal Natural Product Discovery in the Genomic Era

Discoveries of new NPs from easily culturable fungi will continue, but expansion of sources of fungal natural products to unculturable and slow-growing fungal species might lead to even more new structural classes. Improved cultivation techniques could take advantage of less-manageable fungi, but the task is likely to become increasingly intractable, and some fungi may never be cultivated. Advances in gene sequencing, molecular biology and genetic engineering make it possible to express genes from unculturable organisms in laboratory fungal strains, therefore providing an alternative route for realizing the genetic potential.

A molecular genetic approach is being tested (Fig. 11.5). This approach is based on the fact that genes involved in fungal and bacterial secondary metabolite biosynthesis are often clustered within 30–60 kb of DNA (Martin, 1992; Kimura and Tsuge, 1993; Keller and Adams, 1995; Brown *et al.*, 1996; Kennedy *et al.*, 1999). Therefore, it is feasible to transfer large pieces of DNA that include most, if not all, of the gene cluster for a particular secondary metabolite from unculturable fungi, as exemplified by cosmid clones (35–45 kb) and bacterial and yeast artificial chromosome clones (> 100 kb).

An assumption underlying this approach is that genes or clusters of genes from fungi can be expressed in laboratory strains, because transcription and translation control sequences from one organism often function among closely related organisms, and sometimes even in distantly related organisms (Punt

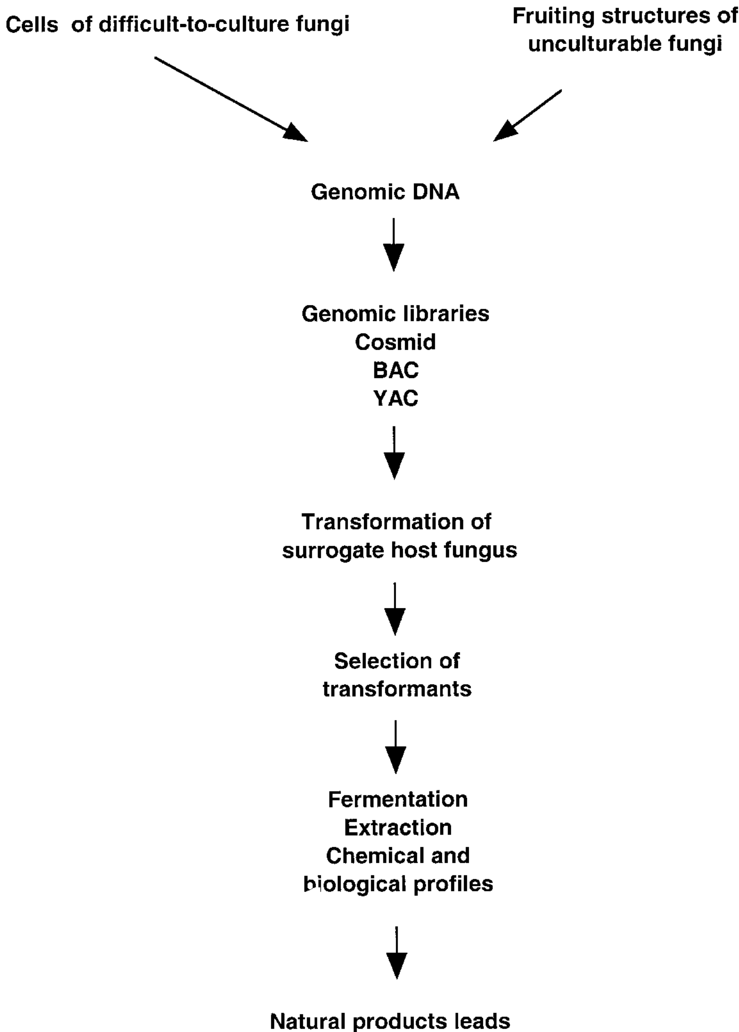


Fig. 11.5. Capture and engineering of secondary metabolite biosynthetic genes from nature and their heterologous expression in a surrogate fungus for discovery of new natural products. BAC, bacterial artificial chromosome; YAC, yeast artificial chromosome.

et al., 1987; Barrett *et al.*, 1990; Mooibroek *et al.*, 1990; Smith *et al.*, 1990; Hondel *et al.*, 1991; Romanos *et al.*, 1992; Kennedy *et al.*, 1999). Although the studies suggest that foreign genes may be expressed in laboratory strains, donor–recipient combinations must be carefully matched; not all transcription control sequences from one fungus function in other fungal hosts. Several strategies, such as mRNA analysis, reporter gene analysis, and

complementation of auxotrophic markers, can be applied to test gene expression. The recipient strains for expressing genes from donor fungi should be easily manipulated genetic model organisms. These host fungi often grow quickly and are easily fermented on a large scale. The availability of multiple-recipient laboratory strains increases the chance for heterologous expression of foreign genes.

Undirected transfer of genetic material from unculturable and slow-growing organisms to a surrogate host will generate large numbers of transgenic strains. Screening huge numbers of extracts of random transformants would be costly and impractical. One of the key obstacles to realizing the approach is how to eliminate non-productive transformants before they are sent for NP screening. Some type of sensitive and high-throughput prescreen(s) is needed to discern novel extracts (Fig. 11.5). At the same time, prescreens should not discard potentially productive candidates. If the prescreen employs biological readouts, they should reflect broad biological signals, such as general stress responses and signal transduction. A possible strategy would be to fuse transcription regulatory sequences of broad interest to sensitive reporters in cell-based prescreens.

Many microbial secondary metabolite-encoding genes have been cloned, sequenced, and characterized, and more will become available as more microbial genomes are determined. Conserved biosynthetic genes can be used as probes to preselect clones that are most likely to contain secondary metabolite-encoding gene(s). This approach will work only if the prescreen is in a high-throughput mode. Significant progress has been made in DNA array technology during the last 5 years, some of which can be practically applied to search for secondary metabolite-encoding genes from a larger collection of cloned DNA. Briefly, a secondary metabolite-encoding gene library can be created by including consensus DNA fragments applied from genes encoding various secondary metabolites, such as polyketides, non-ribosomal peptides and alkaloids. The DNA fragments can be used individually as probes to screen libraries for secondary metabolite gene clones. The DNA fragments can also be placed on membranes or glass to create a secondary metabolite-encoding DNA array.

Another new concept of NP drug discovery, known as the biocombinatorial production of synthetic NPs, is evolving. Several biotechnology companies have been formed based on the promise of this genetic engineering/chemistry hybrid approach for developing novel NPs. Understanding of genetic programming of bacterial PKSs has progressed tremendously, leading to the rational design of novel polyketides (McDaniel *et al.*, 1993, 1994, 1995; Fu *et al.*, 1994; Kim *et al.*, 1995; Tang *et al.*, 2000). As mentioned above, fungal polyketides represent a structurally diverse chemical class, but the genes that encode these compounds are conserved in primary nucleotide sequences and the organization of functional motifs (Fig. 11.1). Most current efforts focus on polyketides of bacterial origin, because the genetic systems for

bacteria are well developed, but the same principles are applicable to fungal NP systems.

In summary, exploitation of the genetic diversity of unculturable fungi by manipulation of 'biocombinatorial' diversity immeasurably expands opportunities for discovering novel or 'unnatural' secondary metabolites. Since recipient strains are fast-growing, 'industrial' organisms, when drug-producing transformants are identified, scale-up fermentation for commercial production can be quickly implemented.

Obstacles to Commercialization of Tropical Fungal Metabolites

Considering the increased awareness of fungal diversity, one might ask why tropical fungi have not made a greater impact in NP discovery. Pharmaceutical companies have established few projects to screen tropical fungi, because demands for large numbers of test species have been tempered by alternative sources of chemical diversity. The number of industrial laboratories engaged in NP research has declined and, of those that remain active, many have filled their extract collections to capacity in order to adapt to the HTS environment of industrialized drug discovery. Mature NP collections may continue to expand, but the need for strains should not increase substantially. The issues faced by those who collect tropical fungi parallel those of researchers exploring tropical plants and animals. Geographic separation of the major discovery and fermentation facilities in industrialized temperate countries in North America, Europe and Asia from forests in the tropics has complicated utilization of tropical fungi as a drug discovery resource. At least two NP laboratories of multinational pharmaceutical companies have been established in tropical countries: the Centre for Natural Product Research represents a major initiative in drug discovery, jointly funded by GlaxoWellcome, the Singapore Economic Development Board and the Institute of Molecular and Cell Biology; similarly Hoechst Marion Roussel has a long-established research laboratory for natural products chemistry in India. However, a lack of adequate research facilities and microbiological/mycological expertise in many biologically rich regions remains a limiting factor. Until biocombinatorial approaches are realized on an industrial scale, the search for secondary metabolites from fungi will continue to require that the organisms be cultured successfully to produce sufficient quantities of compounds to fuel medicinal chemistry and preclinical investigation programmes. Many fungi are not amenable to long-distance transport or commonly used culturing techniques (Bills, 1995). To culture perishable species, transport time between the field and laboratory should be minimal; therefore, a laboratory near the habitats is needed. Facilities need to be extended; skilled personnel are required to work in often suboptimal conditions, knowing how to select organisms,

document their collections, culture them cleanly, and avoid repetitive collection of common species.

Many tropical fungi will never be used for pharmaceutical exploration because of the lack of adequate facilities and personnel in their source countries. Even more crucial is the need for a regulatory environment that encourages collaboration with industry, or at least allows independent contractual agreements. Exploration can be integrated into ongoing research at government or academic microbiology, plant pathology, or other biological research laboratories. Simple agreements in accordance with the CBD can be set rapidly into place if the regulatory environment permits and financial expectations for success are realistic. Such complications increase the costs and delays in acquiring tropical species. Given that temperate and boreal habitats may be equally productive, and, to an unknown degree, metabolically redundant, the difficulties in establishing a tropical collaborative project can favour establishment of explorations of fungi in the temperate zones.

MRL supports the spirit and recommendations of the CBD, but the authors have witnessed some unintended consequences brought about by unrealistic expectations raised by publicity and discussions surrounding the treaty. The time, perseverance and expense required to negotiate contracts to transfer microorganisms severely limits the number of exploration projects undertaken (Gollin, 1999). Fears about hidden past claims to unrealized products favour agreements in which a clean title to microorganisms is acquired directly from the original source. Simple two-party agreements avoid the unneeded expenses of intermediaries and reduce uncertainties about ownership. The authors are concerned that the CBD environment will stifle use of strains accumulated in academic research collections and public culture collections because of complex requirements to track the history of strains and wariness of unexpected claims to potential products dictated in material transfer agreements.

Ten years ago, collecting easy-to-transport-and-culture organisms worldwide, e.g. soil actinomycetes, was a common strategy to feed screening programmes. Today that strategy is impossible. NP laboratories have shifted from worldwide collections to geographically focused collections and to cultivating long-term collaborations with partners at the sources of microbial diversity. An unfortunate consequence of geographically restricted sampling is that valuable revelations about the taxonomic and biogeographical patterns of metabolites, e.g. in the case of the cyclosporins (Traber and Dreyfuss, 1996), zaragozic acids (Bergstrom *et al.*, 1995), sordarins, and nodulisporic acids, will be obscured. Maximizing microbial diversity based on collections from one or a few locations means shifting from dependence on speciose organisms from one substratum, e.g. endophytic fungi or litter fungi, to methodically mining out organisms from a few forests, deserts or estuaries. On the other hand, long-term collaborations and stratified collection strategies meld well with semi-permanent extract collections needed for the HTS environment, because

expensive and difficult-to-obtain fungi are held and reused. It is predicted that the gold rush for tropical microorganisms, if it ever appeared, will dissipate, and that means fewer viable opportunities for tropical fungi to contribute to the drug discovery process.

Conclusions and Outlook

A major drug or agrochemical from a tropical fungus has yet to reach the market. Evidence from academic laboratories that are focusing on isolating unusual organic structures from fungal fermentations has shown that fungi produce large numbers of novel compounds (Gloer, 1997), but the goal of NP research in the pharmaceutical industry is to find new health care products, not novel compounds. Therefore, new uses for known metabolites can be as interesting as novel metabolites. During the 1990s, a more profound understanding of how fungal metabolites are biosynthesized and the commonality among biosynthetic genes has been gained, leading to new ways to recombine and express biosynthetic genes.

Several models have been proposed to utilize organisms from threatened habitats as sources for drug discovery or other types of biotechnological innovations (Baker *et al.*, 1995; Porzecanski *et al.*, 1999). These models assume that, to obtain the benefits of revenue sharing from the discovery of biologically derived, commercial products, biologists in tropical countries must actively participate in the discovery process. However, participation also means risk-sharing. Discovery at the leading edge of NP research is not unlike playing the lottery. Luck and persistence are essential components for winning. The financial risks and capital investment in basic research and development are extremely high for drug discovery, while the investment and risk in selecting a single fungal strain or a substratum from which to isolate fungi is minimal in proportion to the potential rewards. Investigators who believe they have potentially valuable organisms, and who are willing to assume part of the risk, should not wait to be contacted by a commercial group interested in product development because that invitation may never arrive. Scientists who are eager to promote sustainable use of microbial resources must be proactive in formulating strategies for microbial collection and cultivation, identifying and contacting serious collaborators, and finding ways to streamline negotiations for legal exchange of germplasm and intellectual property. Instead of asking how to gain short-term profit from selling biological resources, perhaps the better question is how to make tropical resources competitive with other sources of chemical diversity and maximize exposure to opportunities over the long term, so that those much-needed and successful examples of the value of tropical fungi will be realized.

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