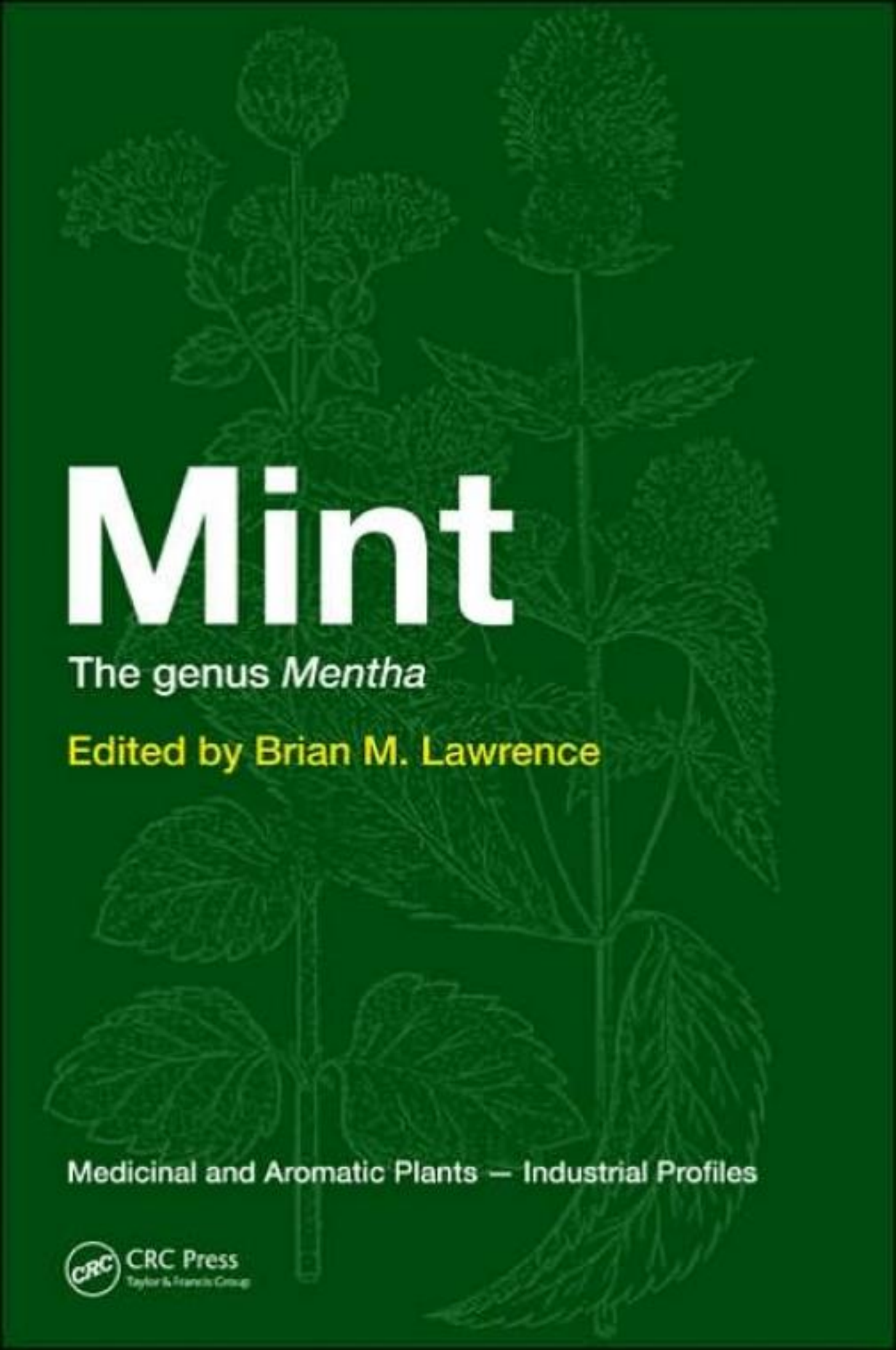


Mint

The genus *Mentha*

Edited by Brian M. Lawrence

Medicinal and Aromatic Plants — Industrial Profiles

CRC Press
Taylor & Francis Group

Mint

The Genus *Mentha*

Medicinal and Aromatic Plants — Industrial Profiles

Individual volumes in this series provide both industry and academia with in-depth coverage of one major genus of industrial importance.

Series Edited by Dr. Roland Hardman

Volume 1
Valerian, edited by Peter J. Houghton
Volume 2
Perilla, edited by He-ci Yu, Kenichi Kosuna and Megumi Haga
Volume 3
Poppy, edited by Jenö Bernáth
Volume 4
Cannabis, edited by David T. Brown
Volume 5
Neem, edited by H.S. Puri
Volume 6
Ergot, edited by Vladimír Křen and Ladislav Cvak
Volume 7
Caraway, edited by Éva Németh
Volume 8
Saffron, edited by Moshe Negbi
Volume 9
Tea Tree, edited by Ian Southwell and Robert Lowe
Volume 10
Basil, edited by Raimo Hiltunen and Yvonne Holm
Volume 11
Fenugreek, edited by Georgios Petropoulos
Volume 12
Ginkgo biloba, edited by Teris A. Van Beek
Volume 13
Black Pepper, edited by P.N. Ravindran
Volume 14
Sage, edited by Spiridon E. Kintzios
Volume 15
Ginseng, edited by W.E. Court
Volume 16
Mistletoe, edited by Arndt Büssing
Volume 17
Tea, edited by Yong-su Zhen
Volume 18
Artemisia, edited by Colin W. Wright
Volume 19
Stevia, edited by A. Douglas Kinghorn
Volume 20
Vetiveria, edited by Massimo Maffei
Volume 21
Narcissus and Daffodil, edited by Gordon R. Hanks
Volume 22
Eucalyptus, edited by John J.W. Coppen
Volume 23
Pueraria, edited by Wing Ming Keung
Volume 24
Thyme, edited by E. Stahl-Biskup and F. Sáez
Volume 25
Oregano, edited by Spiridon E. Kintzios
Volume 26
Citrus, edited by Giovanni Dugo and Angelo Di Giacomo
Volume 27
Geranium and Pelargonium, edited by Maria Lis-Balchin
Volume 28
Magnolia, edited by Satyajit D. Sarker and Yuji Maruyama
Volume 29
Lavender, edited by Maria Lis-Balchin
Volume 30
Cardamom, edited by P.N. Ravindran

Mint

The Genus *Mentha*

Edited by Brian M. Lawrence

Medicinal and Aromatic Plants — Industrial Profiles

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an informa business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Printed in the United States of America on acid-free paper
10 9 8 7 6 5 4 3 2 1

International Standard Book Number-10: 0-8493-0779-1 (Hardcover)
International Standard Book Number-13: 978-0-8493-0779-9 (Hardcover)

Library of Congress Cataloging-in-Publication Data

Lawrence, Brian M.
Mint : the genus mentha / Brian M. Lawrence.
p. cm.
Includes bibliographical references and index.
ISBN-13: 978-0-8493-0779-9 (alk. paper)
ISBN-10: 0-8493-0779-1 (alk. paper)
1. Mints (Plants) I. Title.

SB295.M5L38 2006
633.8'2--dc22 2006014190

Visit the Taylor & Francis Web site at
http://www.taylorandfrancis.com

and the CRC Press Web site at
http://www.crcpress.com

Preface to the Series

There is increasing interest in industry, academia, and the health sciences in medicinal and aromatic plants. In passing from plant production to the eventual product used by the public, many sciences are involved. This series brings together information that is currently scattered through an ever-increasing number of journals. Each volume gives an in-depth look at one plant genus, about which an area specialist has assembled information ranging from the production of the plant to market trends and quality control.

Many industries are involved, such as forestry, agriculture, chemicals, food, flavor, beverage, pharamaceutical, cosmetics, and fragrance. The plant raw materials are roots, rhizomes, bulbs, leaves, stems, barks, wood, flowers, fruits, and seeds. These yield gums, resins, essential (volatile) oils, fixed oils, waxes, juices, extracts, and spices for medicinal and aromatic purposes. All these commodities are traded worldwide. A dealer's market report for an item may say "drought in the country of origin has forced up prices".

Natural products do not mean safe products, and account of this has to be taken by the above industries, which are subject to regulation. For example, a number of plants that are approved for use in medicine must not be used in cosmetic products.

The assessment of "safe to use" starts with the harvested plant material, which has to comply with an official monograph. This may require absence of, or prescribed limits of, radioactive material, heavy metals, aflatoxin, pesticide residue, as well as the required level of active principle. This analytical control is costly and tends to exclude small batches of plant material. Large-scale, contracted, mechanized cultivation with designated seed or plantlets is now preferable.

Today, plant selection is not only for the yield of active principle, but for the plant's ability to overcome disease, climatic stress, and the hazards caused by mankind. Such methods as *in vitro* fertilization, meristem cultures, and somatic embryogenesis are used. The transfer of sections of DNA is giving rise to controversy in the case of some end uses of the plant material.

Some suppliers of plant raw material are now able to certify that they are supplying organically farmed medicinal plants, herbs, and spices. The Economic Union directive CVO/EU No. 2092/91 details the specifications for the *obligatory* quality controls to be carried out at all stages of production and processing of organic products.

Fascinating plant folklore and ethnopharmacology lead to medicinal potential. Examples are the muscle relaxants based on the arrow poison, curare, from species of *Chondrodendron*, and the antimalarials derived from species of *Cinchona* and *Artemisia*. The methods of detection of pharmacological activity have become increasingly reliable and specific, frequently involving enzymes in bioassays and avoiding the use of laboratory animals. By using bioassay-linked fractionation of crude plant juices or extracts, compounds can be specifically targeted which, for example, inhibit blood platelet aggregation, or have antitumor, or antiviral, or any other required activity. With the assistance of robotic devices, all the members of a genus may be readily screened. However, the plant material must be fully authenticated by a specialist.

The medicinal traditions of ancient civilizations such as those of China and India have a large armamentarium of plants in their pharmacopoeias that are used throughout Southeast Asia. A similar situation exists in Africa and South America. Thus, a very high percentage of the world's population relies on medicinal and aromatic plants for their medicine. Western medicine is also responding. Already in Germany all medical practitioners have to pass an

examination in phytotherapy before being allowed to practice. It is noticeable that medical, pharmacy, and health-related schools throughout Europe and the United States are increasingly offering training in phytotherapy.

Multinational pharmaceutical companies have become less enamored of the single compound, magic-bullet cure. The high costs of such ventures and the endless competition from "me-too" compounds from rival companies often discourage the attempt. Independent phytomedicine companies have been very strong in Germay. However, by the end of 1995, 11 (almost all) had been acquired by the multinational pharmaceutical firms, acknowledging the lay public's growing demand for phytomedicines in the Western world.

The business of dietary supplements in the Western world has expanded from the health store to the pharmacy. Alternative medicine includes plant-based products. Appropriate measures to ensure their quality, safety and efficacy either already exist or are being answered by greated legislative control by such bodies as the U.S. Food and Drug Administration and the recently created European Agency for the Evaluation of Medicinal Products based in London.

In the United States, the Dietary Supplement and Health Education Act of 1994 recognized the class of phytotherapeutic agents derived from medicinal and aromatic plants. Furthermore, under public pressure, the U.S. Congress set up an Office of Alternative Medicine, which in 1994 assisted the filing of several Investigational New Drug (IND) applications, required for clinical trials of some Chinese herbal preparations. The significance of these applications was that each Chinese preparation involved several plants and yet was handled as a Single IND. A demonstration of the contribution to officialy of each ingredient of *each* plant was not required. This was a major step toward more sensible regulations in regard to phytomedicines.

My thanks are due to the staff of CRC Press who have made this series possible and especially to the volume editors and their chapter contributors for the authoriative information.

Dr. Roland Hardman

Preface

All species and natural hybrids of *Mentha* are essential oil bearing. Today, taxa of *Mentha*, either native or naturalized, are ubiquitous and found on all continents except Antarctica. Because of the aromatic properties of these taxa, some have been used traditionally for more than 2000 years.

Mentha is the most important genus in the Labiatae (Lamiaceae) family because it contains a number of taxa the essential oils of which have achieved high economic value. These oils are cornmint (the source of natural menthol), peppermint, Scotch spearmint, and Native spearmint. The amount of the oils produced annually is in excess of 23,000 metric tonnes with a value exceeding $400 million. This makes them the most economically important essential oils produced. Mint oils are produced in different parts of the world. For example, the lion's share of peppermint oil, Native spearmint, and Scotch spearmint oil is produced in North America, whereas almost all the cornmint oil and natural menthol are produced in China and India.

The correct taxonomic source of cornmint oil is *Mentha canadensis* L. not the synonyms *M. arvensis* L., *M. arvensis* f. *piperascens* Malinv.ex Holmes, *M. haplocalyx* Briq., etc. Similarly, the correct taxonomic source of Scotch spearmint oil is *M.* ×*gracilis* Sole and not *M. cardiaca* Gerarde ex Baker. Finally, the correct taxonomic source of Mentha citrata oil is *M. aquatica* L. var. *citrata* (Ehrh.) Fresen. not *M. citrata* Ehrh. Therefore, the correct taxonomic nomenclature has been used throughout this treatise.

For an essential oil to be natural and genuine, it must be isolated from a whole plant or plant part of known taxonomic origin by physical means only. The physical processes used to isolate essential oils are as follows:

1. Hydrodistillation (water distillation) in which the plant material (herbage in the case of *Mentha* species) is immersed in water, the heterogeneous mixture is boiled, and the oil is condensed and separated from the steam and oil vapor mixture.
2. Water and steam distillation in which water is boiled in a container with the herbage held above the boiling water on a grid so that the wet steam can release the oil in vapor form, which is condensed and separated as with water distillation.
3. Normal steam distillation in which the steam is produced in a satellite steam generator (boiler) and passed through the bottom of a container of herbage to yield a steam and oil vapor mixture, which is condensed and separated.
4. Hydrodiffusion is a modification of the steam distillation process in which the steam is passed through the top of a container of herbage, thus allowing the steam to percolate down through the plant material by gravity, after which the oil vapor and steam are condensed and separated.
5. Expression (also known as cold processing) in which the oil-bearing part of the plant is compressed to remove the oil. This process is mainly used to isolate oils from the peels of *Citrus* taxa. An essential oil cannot be obtained by supercritical CO_2 extraction, although it is sometimes erroneously referred to that way in the literature, because extraction processes produce extracts irrespective of the solvent type or volatility.

A short summary of the chapters that appear in the text follows:

The *Mentha* genus is complex as more than 3000 epithets of *Mentha* have been published, although a redefinition of the genus has revealed that it contains 18 species and 11 hybrids (Tucker and Naczi).

Anatomically *Mentha* taxa contain both capitate and peltate glandular trichomes like other members of the Labiatae family. Studies on biosynthesis and biotechnology have led to a better understanding of the physiology, molecular biology, and tissue culture potential for the production of oil of the important *Mentha* taxa (Maffei et al.).

The four economically important *Mentha* taxa are cultivated extensively (a) in the United States (peppermint, Scotch, and Native spearmint); (b) in India (cornmint, peppermint, Scotch spearmint, and Native spearmint); and (c) in China (cornmint and Native and Scotch spearmint). Their cultivation practices are dissimilar as are the control measures used to combat disease and insect infestation (Morris, USA; Kumar et al., India; and Liu and Lawrence, China).

The isolation of mint oils from mint herbage is more complex than described earlier. As a result, the theoretical aspects of distillation must be applied if efficient cost-effective oil isolation is to be realized (Denny and Lawrence).

The number and type of components that have been isolated from the commercially important oils (also including pennyroyal and Mentha citrata) and their quantitative differences found in the same taxa grown in different environments are of importance to the mint oil user. The enantiomeric distribution of the main constituents can also assist in the choice of oil supplier (Lawrence).

The oils produced from the 18 *Mentha* species and 11 hybrids, either cultivated or collected from their naturalized habitats, reveal a wide compositional diversity within the genus. In addition, similarities and differences between oil compositions found either within or between a species or hybrid shed light on the widespread occurrence of infraspecific chemical differences and the fact that composition cannot be used solely to characterize a species or hybrid (Lawrence).

The annual production statistics for peppermint, Native, and Scotch spearmint oils produced in the United States reveal the changes in production regions over the years. Also, the quality control aspects including gas chromatographic profiles will assist the users in characterizing their oils of choice (Sheldon).

Menthol is an extremely important flavor chemical that can be produced by freeze crystallization from cornmint oil. This same enantiomerically pure compound can also be produced synthetically either from pulegone, piperitone, δ-3-carene, thymol, and *N*,*N*-diethylgeranylamine (Hopp and Lawrence).

The important mint oils in commerce are sometimes found stretched, diluted, or adulterated with single compounds, similar oils, or fractions thereof. The resultant mixture is no longer a pure genuine oil. The use of blends of oils of different geographical origin with a label of the most expensive origin or the standardization of an oil to ameliorate year-to-year oil composition variation is fairly widespread (Lawrence).

As mint oils and their major isolates have a wide range of use, an assessment of their safety, biological, and toxicological properties reveals that much work has been completed on menthol and pulegone. The methods of determining these properties provide an insight into the conclusions drawn from such studies (Hayes et al.).

The antibacterial and antifungal properties of mint oils and some of their isolates have been the subject of study over the years because of their use as preservatives. As expected, the majority of the work has been directed toward controlling the microbes that cause food spoilage (Deans).

Mint oils and menthol are widely used in the flavor industry where they are integral components of the flavor systems used in confection, alcoholic beverage, and tobacco industries. They are also widely used in the fragrance industry in personal care and oral care products and, to a lesser extent, in fine fragrances. An almost equivalent use of these materials can be found in the pharmaceutical industry, particularly in nonprescription products. A more recent use of the commercially important mint oils and the lesser-known mint oils is in aromatherapy (Tucker).

Acknowledgments

The editor would like to thank Professor K.H.C. Baser (Anadolou University, Turkey) for providing data, a summary of which was used in his manuscript "Essential Oils of *Mentha* species from Northern Turkey" [*J. Essent. Oil Res*., 11, 579–588 (1999)]. Thanks are also extended to Professor E.H. Graven (University of Fort Hare), for providing compositional data on South African peppermint oil; J. Thacker (Prairie Aromatic Natural Oils, Ltd., Canada) for statistical data on Scotch spearmint oil production in Canada; and M. Monroe (Quest International, U.K.) for providing data on mint oils.

The editor would like to acknowledge the skillful assistance of Ken Baker (R.J. Reynolds Tobacco Co.) who painstakingly computerized and clarified the photos and diagrams used in the various chapters. The following people are thanked for supplying copies of some of the photos used:

Chapter 6. Figure 6.1, Ramon Bordas (Distilaciones Bordas Chinchurreta SA, Spain); Figure 6.4, Ian Blair (A.M. Todd Co., USA); Figure 6.4 Richard Carrington (I.P. Callison & Sons, USA); Figure 6.5 and Figure 6.6, Bob Lundy (Newhouse Manufacturing Co., USA). Chapter 5, Figure 5.1 and Figure 5.2, David Moyler (Fuerst Day Lawson, U.K.).

Finally, the editor is grateful for assistance in typing the chapters Ann G. Niten (Typing Service) and for the excellent proofreading and editing skills of Judith A. Lawrence (*Journal of Essential Oil Research*).

Editor

Brian M. Lawrence was born in London, England, in 1939. In 1960, he was awarded a Fulbright scholarship to study chemistry in the United States. In 1965, he went to Toronto, Ontario, to undertake basic research on spices and herbs at Stange Canada Ltd. In 1978, he obtained a doctorate in pharmacognosy at the State University in Groningen, the Netherlands. In 1978, he returned to the United States to work for various divisions of R.J. Reynolds Tobacco Co. In 1979, he assumed the position of director, Research and Development, for the Avoca Division. From 1984 to 1990, he was manager of the Flavor Division for R.J. Reynolds Tobacco Co. In 1990, he became principal scientist or manager, Flavor Division. In 2000, he was promoted to the post of senior principal scientist or manager, Flavor Division, a position he held until he retired in 2003. He then started a consultancy business, which continues to this day.

Dr. Lawrence has published in excess of 80 original articles and reports. Since 1976, he has been the scientific editor of *Perfumer and Flavorist*. In this position, he has written more than 180 reviews titled "Progress in Essential Oils." He has published seven books and eighteen book chapters on essential oils and acted as senior editor for the proceedings of the 10th International Congress of Essential Oils, Flavors and Fragrances. In 1989, he started the *Journal of Essential Oil Research*, an international peer-reviewed journal of which he remains editor-in-chief.

In 1984, Lawrence was the first recipient of the "Distinguished Service Medal" of IFEAT. In 2004, he received the "Medal of Honor" from the University of Messina for his studies on essential oils.

Contributors

Cinzia M. Bertea
Department of Plant Biology
University of Torino
Torino, Italy

Stanley G. Deans
South Ayrshire,
United Kingdom

E.F.K. Denny
Denny McKenzie Associates
Lilydale, Tasmania, Australia

Johnnie R. Hayes
Kernersville, North Carolina

Rudolph Hopp
Holzminden, Germany

S. Sushil Kumar
National Center for Plant Genome Research
Jawaharlal Nehru University
New Delhi, India

Brian M. Lawrence
Journal of Essential Oil Research
Winston-Salem, North Carolina

Wennuan Liu
Wake Forest University School of Medicine
Center for Human Genomics
Winston-Salem, North Carolina

Massimo Maffei
Department of Plant Biology
University of Torino
Torino, Italy

Mark A. Morris
A.M. Todd Company
Eugene, Oregon

Marco Mucciarelli
Department of Veterinary
Morphophysiology
University of Torino
Torino, Italy

Robert F.C. Naczi
Department of Agriculture
and Natural Resources
Delaware State University
Dover, Delaware

Shashi Pandey-Rai
National Center for Plant
Genome Research
Jawaharlal Nehru University
New Delhi, India

S.K. Rai
National Center for Plant Genome
Research
Jawaharlal Nehru University
New Delhi, India

Ross M. Sheldon
A.M. Todd Company
Kalamazoo, Michigan

Mari S. Stavanja
R.J. Reynolds Tobacco Company
Bowman Gray Technical Center
Winston-Salem, North Carolina

Arthur O. Tucker
Department of Agriculture
and Natural Resources
Delaware State University
Dover, Delaware

Table of Contents

1 *Mentha*: An Overview of Its Classification and Relationships

Arthur O. Tucker and Robert F.C. Naczi

CONTENTS

1.1 INTRODUCTION

The taxonomy of the genus *Mentha* has been in a state of flux, with more than 3000 names published since 1753. On the basis of a phylogenetic analysis of morphology, chromosome numbers, and major essential oil constituents, the genus *Mentha* is redefined to include 18 species and 11 named hybrids, placed in four sections. *Mentha cunninghamii* is excluded and may be more closely paired with the genus *Micromeria* (*s.l.*). An enumeration of the taxa, with more important synonymy, follows a key to the species.

Mentha is a member of the Lamiaceae (Labiatae), in the Order Lamiales, which includes many other families, such as Verbenaceae, Scrophulariaceae, and Acanthaceae. The delimitation of the family has recently been redefined, with many genera formerly in Verbenaceae being reassigned to the Lamiaceae (Cantino et al., 1992). As a result, the circumscription of the Lamiaceae has been changed to include eight subfamilies: Ajugoideae, Chloranthoideae, Lamioideae, Nepetoideae, Pogostemonoideae, Scutellarioideae, Teucrioideae, and Viticoideae. Nevertheless, over 47% of the Lamiaceae fall within the subfamily Nepetoideae, as do most of the more familiar genera, many of them economically important. The subfamily Nepetoideae is characterized as more or less bilabiate, five-lobed corolla (sometimes four-lobed by fusion); two or four stamens inserted on the corolla tube; an ovary of four one-seeded mericarps separated by a gynobasic style; a bifid style with one lobe reduced; fruit of four one-seeded nutlets (sometimes reduced by abortion); nutlets without endosperm; a quadrangular stem with opposite or rarely verticillate leaves, covered with subsessile glands; hexacolpate, trinucleate pollen; essential oils and rosmarinic acid present.

Mentha is presently classified within the tribe Mentheae, which also includes the economically important genera *Agastache*, *Calamintha*, *Cedronella*, *Hedeoma*, *Hyssopus*, *Monarda*, *Nepeta*, *Origanum*, *Rosmarinus*, *Salvia*, *Satureja*, and *Thymus*. The genus *Mentha* is based on

a combination of characters rather than any one, unique character: (1) four ± equal stamens; (2) naked filaments; (3) anthers with parallel distinct thecae; (4) ± actinomorphic calyx with four to five lobes; (5) slightly two-lipped corolla; and (6) subellipsoidal nutlets with rounded apex. On the basis of rbcL sequences, *M. longifolia* and "*M. rotundifolia*" (frequently misapplied, probably *M. suaveolens*) are most closely paired with *Monarda fistulosa*, *Monarda didyma*, and *Monarda menthaefolia* (Kaufmann and Wink, 1994), but this close relationship is not supported by the Internal Transcribed Spacer (ITS) sequence of nuclear ribosomal DNA of "*Mentha rotundifolia* Huds." (again, probably *M. suaveolens*) to 16 species of *Monarda* (Prather et al., 2002). On a morphological basis, many Australasian species of *Mentha* have been previously classified as species of *Micromeria* (see later).

1.2 TAXONOMIC HISTORY OF *MENTHA*

Disregarding the "microspecies" of Gandoger (e.g., 1889), whose names were declared invalidly published (Greuter, 2000), more than 3000 names, from species to *formae*, have been published for the genus *Mentha* since 1753 (the starting date of modern nomenclature). Possibly ca. 95% of these names are synonyms or illegitimate, with most of the remaining ones being infraspecific taxa. The systematics of the genus *Mentha* section *Mentha* are especially difficult because of the ease of hybridization, favored by gynodioecy, which is further complicated by polymorphism, cultivation, polyploidy, and vegetative propagation. This genus also exhibits cytomixis, resulting in somatic chromosome numbers in multiples of $x = 12$, and, coupled with unexpected "hybrid compounds" (see the discussion under *M.* ×*piperita* L.), transgressive segregation, a process normally restricted to the F_2 or backcross progeny, can be observed in the F_1 progeny (Tucker et al., 1980; Tucker and Fairbrothers, 1981; Tyagi, 2003). Within section *Mentha*, the five basic Eurasian and African species (*M. arvensis* L., *M. aquatica* L., *M. spicata* L., *M. longifolia* (L.) L., and *M. suaveolens* Ehrh.) have produced eleven naturally occuring, named hybrids. Because of the heterozygosity of these nominally accepted species, coupled with cytomixis, abundant forms can be generated by simple selfing, and any hybrid progeny will also be correspondingly variable. These simple genetic segregants, not necessarily correlated with any geographical or ecological distribution, have prompted some taxonomists to publish paroxysms of species and subspecific taxa. Thus, for example, Topitz (1911, 1913, 1914a, 1914b, 1915, 1916) published 434 new taxa for Middle Europe, while Trautman (1925) later published an additional 113 taxa for Hungary alone, and Prodan (1925) published an additional 67 new taxa for Romania and Yugoslavia.

This genus also holds many examples of questionable taxonomic practices. Braun (1886) published 108 new names with no clear indication of their rank (most British taxonomists have treated these as species, but German and French taxonomists have treated them as subspecific taxa). Sennen (1927) published 63 *nomina nuda* for Spain. Fraser (1927, 1929) published 39 new taxa for the British Isles, redefining species without a consultation of the types or a full literature survey.

With respect to infrageneric classification, Bentham (1848) was the first to consider the amount of genetic variability in the genus within a worldwide context and publish taxa at the level of section, followed by Pérard (1876) and Briquet (1889). The latest comprehensive classification of the genus *Mentha* was published over a century ago by Briquet (1896), who defined 17 species and 33 subspecies within two genera (*Mentha* and *Preslia*), two subgenera, five sections, and seven subsections. Following late 20th century concepts of nomenclature, Harley and Brighton (1977) defined five sections. No revision or monograph has been

published for the genus, but the unpublished Ph.D. thesis of Metcalf (1936) should be cited as one interpretation.

1.3 MATERIALS AND METHODS

The 27 morphological, karyological, and chemical characters selected were those traditionally used to define species in the genus *Mentha*, i.e., they have maximum interspecific variability but minimum infraspecific variability. Phylogenetic analysis of these 27 characters was carried out using maximum parsimony (heuristic search) provided by PAUP with default options on all except for the branch-swapping option algorithm of nearest-neighbor interchange (NNI) and steepest descent and subsequent bootstrap analysis. All characters were treated as unordered and of equal weight. The neighbor-joining algorithm of the same 27 characters was also used (Swofford, 2001). The use of these analyses and morphological characters is discussed by McDade (1997) and Wiens (2000).

Preliminary heuristic searches with morphological characters, chromosome numbers, and essential oils were performed to determine the best outgroup with species of *Lycopus*, *Micromeria* (*s.l.* and *s.s.*), *Monarda*, *Perilla*, *Salvia*, *Satureja*, *Thymus*, *Thymbra*, and/or *Ziziphora*. Ultimately, *Micromeria brownei* (Swartz) Benth. was selected as the appropriate outgroup. Further supporting the choice of this genus is that *M. juliana* (L.) Benth. falls within the large sister clade to "*Mentha rotundifolia* (L.) Hudson" and *Thymbra spicata* L. on the basis of cpDNA (Wagstaff et al., 1995). *Mentha suaveolens* also has a close affinity with *Clinopodium vulgare* L. based on three plastid DNA regions (Paton et al., 2005). In addition, *Micromeria brownei* has been classified as *Clinopodium brownei* (Swartz) Kuntze; this is supported by analysis of the cpDNA of North American *Satureja* (Cantino and Wagstaff, 1998; Harley and Paucar, 2000). The plants chosen for the analysis fell within var. *pilosiuscula* Gray from Florida, although this species ranges from Argentina to Florida.

The morphology, chromosome numbers, and predominant essential oil chemistry (when known) of the genus *Mentha* and the outgroup, *Micromeria brownei* (Swartz) Benth. var. *pilosiuscula* Gray, are summarized later. The numbers in parentheses after each of the 27 characters refer to the position in the data matrix, which is summarized in Appendix I.

1.3.1 Habit

While most species are either uniformly prostrate or upright, some species, such as *M. pulegium*, assume an upright stance only at the time of flowering.

1. Habit: uniformly prostrate (0), upright when flowering (1), upright during actively growing period of lifespan (2)

1.3.2 Stolon

Most species of the genus *Mentha* produce long, thin rhizomes, commonly called stolons. Jackson (1928) defines a stolon as "a sucker, runner, or any basal branch, which is disposed to root," while a rhizome is "the rootstock or dorsiventral stem, or root-like appearance, prostrate or under ground, sending off rootlets, the apex progressively sending up stems or leaves." *M. arvensis* typically produces only thick subterranean stolons, whereas *M. aquatica* typically produces only thin epigeic ("aerial" or above ground) stolons, but other species and the hybrids may vary, both in thickness and emergence. All have reduced or scale leaves. Thus, the flexible definitions of Jackson are probably the most applicable, and both terms may be used interchangeably.

2. Stolon: absent (0), subterranean (1), epigeic (2)

1.3.3 Leaf

Leaf characteristics are variable, even on the same plant during ontogenetic development, so only leaves at midstem should be considered. In order to better define the leaf shape, the leaf length, and width, the average ratio ($N = 3$) was calculated on each specimen examined. A notable feature of some of the Australasian species is the presence of a thickened leaf margin, actually a nerve that outlines the entire leaf. On drying, the smaller of such leaves appear coriaceous in texture. The hairs of *Mentha* are normally uniseriate (unbranched, multicellular), but *M. suaveolens* has dendroid (sometimes incorrectly termed stellate) and uniseriate hairs, a trait that also infrequently appears in *M. spicata*.

3. Leaf shape: ovate to suborbicular ($l/w = 1.0$ to 2.0) (0), oblong-lanceolate to lanceolate ($l/w = 2.1$ to 4.3) (1), linear-oblanceolate ($l/w = 6.0$ to 10.0) (2)
4. Leaf base: cordate to obtuse (0), obtuse to cuneate (1), cuneate to attenuate (2)
5. Leaf margin: entire (0), serrate (1), crenate (2)
6. Leaf texture: thickened leaf edge, $\pm$ revolute (0); thin leaf edge, flat (1)
7. Leaf surface: smooth (0), rugose (1)
8. Petiole: sessile (0), petiolate (1)
9. Hairs: uniseriate (0), dendroid (1)

1.3.4 Inflorescence

As first noted by Malinvaud (1903), the inflorescence shape of *Mentha* is a useful diagnostic character. The inflorescence of the genus *Mentha* is a modified axillary cyme resulting from reduction (verticillate inflorescence) in which the leaves could be termed bracts, often grouped into a spike-like (*M. longifolia*, *M. suaveolens*, *M. spicata*) or head-like (*M. aquatica*, *M. dahurica*) thyrse with the leaves reduced to bracteoles. Bracteoles are normally simple but are digitately lobed in *M. cervina*. The flower number or axil has been used as a diagnostic character for identification in the Australasian species, and the inflorescence of *M. cunninghamii* is normally reduced to only two flowers per node.

10. Inflorescence: verticillate (0), spicate (1), capitate (2)
11. Bract or bracteole shape: simple (0), digitately lobed (1)
12. Bract size: uniformly equal to leaves (0); ranging from equal to leaves to smaller than leaves, decreasing in size toward apex (1); uniformly smaller than leaves (2)
13. Flower number or axil: 2 (0), 4 to 8 (1), 9 to many (2)

1.3.5 Calyx

As first noted by Malinvaud (1903), the calyx of *Mentha*, as in many members of the family Lamiaceae, is a useful character for identification; the overall shape, the lobe (“tooth”) shape, and the internal pubescence are especially diagnostic. While most species of *Mentha* have five calyx lobes, *M. cervina* has four, and *M. dahurica* varies between four and five. Most species of *Mentha* have calyx lobes within an acute apex, but *M. cervina* and *M. gattefossei* have a whitish apical spine on each lobe.

14. Calyx symmetry: zygomorphic (0), $\pm$ actinomorphic (1)
15. Calyx throat, annulus of hairs: absent (0), present (1)
16. Calyx shape: infundibular (0), tubular (1), tubular-campanulate (2), campanulate (3)
17. Calyx lobe size: unequal (0), $+/-$ equal (1)

18. Calyx lobe number: five (0), four (1)
19. Calyx lobe shape: deltoid-ovate to broadly triangular (0), narrowly triangular to lanceolate (1), subulate-lanceolate to subulate (2), narrowly triangular to subulate (3), tapering to an apical spine (4)
20. Calyx nerve number: 13 to 15 (0), 10 to 12 (1)

1.3.6 Corolla

The corolla in *Mentha* is not as diagnostic as the calyx, but the overall shape and throat pubescence are additional characters.

21. Corolla symmetry: zygomorphic (0), ± actinomorphic (1)
22. Corolla throat, annulus of hairs: absent (0), present (1)
23. Style branches: equal (0), subequal (1)
24. Stamen habit: divergent (0), parallel (1)

1.3.7 Chromosome Number

The species of section *Mentha* typically have the chromosome number $x = 12$, but the other species vary widely.

25. Monoploid (basic) number: $x = 9$ (0), $x = 10$ (1), $x = 12$ (2), $x = 18$ (3), $x = 25$ (4)
26. Ploidy level: $2x$ (0), $4x$ (1), $6x$ (2), $8x$ (3), $10x$ (4)

1.3.8 Essential Oil Chemistry

The genetics of the essential oil pathways and the enzymes involved are well known in mentha. Typically, the predominant essential oil pattern consists of acyclic monoterpenes (e.g., linalool), 2-oxygenated monoterpenes (e.g., carvone), and 3-oxygentated monoterpenes (e.g., menthol). Although populations within a species may have only one or all three pathways, these are normally mutually exclusive within any one plant.

27. Predominant components: only 3-oxygenated monoterpenoid (0), acyclic to 3-oxygenated monoterpenoid (1)

1.4 RESULTS AND CONCLUSIONS

1.4.1 Maximum Parsimony and Neighbor-Joining Analyses

Four most parsimonious trees were found in the analysis with length = 89, CI = 0.517, and RI = 0.060. The strict consensus tree is shown in Figure 1.1. Only bootstrap values greater than 50% after 1000 replicates are reported here.

Cladistics (sensu Hennig) assumes a monophyletic origin of taxa. Thus, the 11 named, recent hybrids enumerated here were eliminated from this analysis. However, *M. canadenis* ($2n = 96$) and *M. spicata* ($2n = 48$), long assumed to be monophyletic, are almost certainly ancient amphidiploids, a hypothesis strongly supported by resynthesis (Harley and Brighton, 1977; Kokkini and Pappageorgiou, 1982; Tucker and Chambers, 2002). Maximum parsimony was repeated without *M. canadensis* and *M. spicata*, but the resolution decreased (i.e., more polychotomies appeared in the strict consensus) and bootstrap support still had only three branches with >50% support.

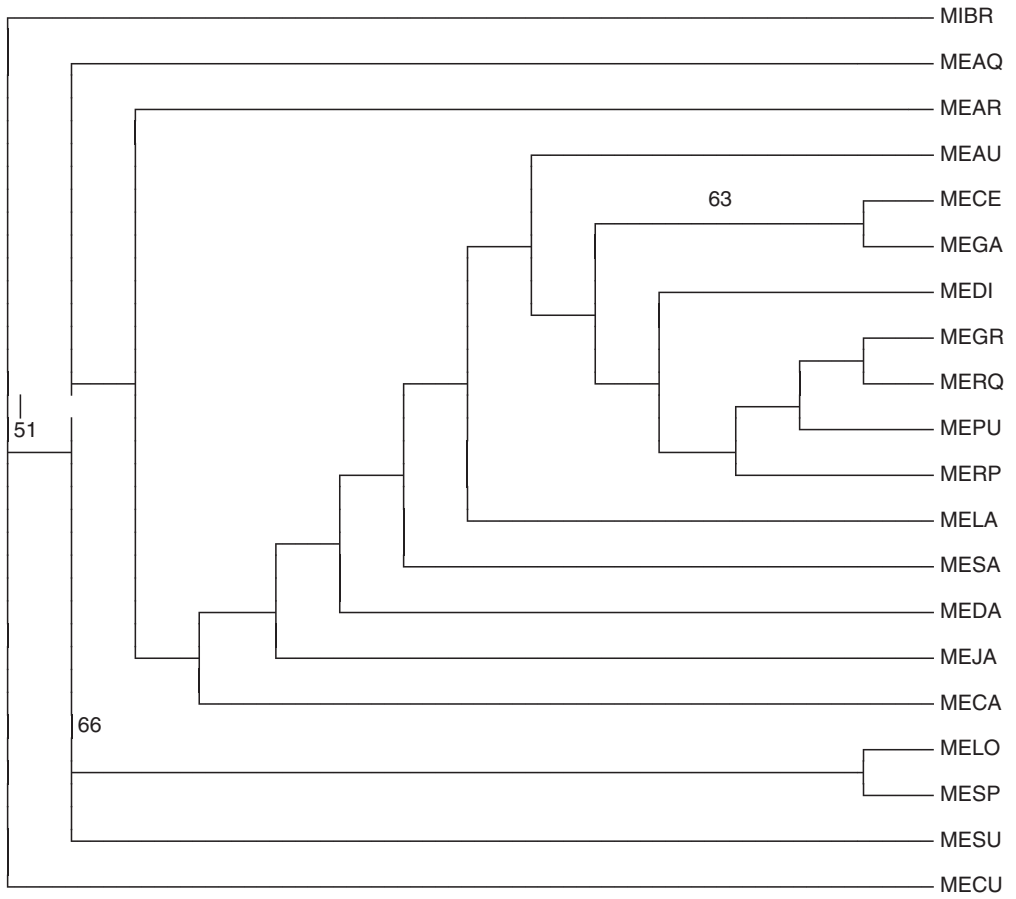


FIGURE 1.1 The strict consensus tree of four trees found in analysis of length = 89, CI = 0.517, RI = 0.060. Bootstrap values greater than 50% are indicated. Taxon key. MIBR = *Micromeria brownei* var. *pilosiuscula*, MEAQ = *Mentha aquatica*, MEAR = *M. arvensis*, MEAU = *M. australis*, MECE = *M. cervina*, MEGA = *M. gattefossei*, MEDI = *M. diemenica*, MEGR = *M. grandiflora*, MERQ = *M. requienii*, MEPU = *M. pulegium*, MERP = *M. repens*, MELA = *M. laxiflora*, MESA = *M. sautureioides*, MEDA = *M. dahurica*, MEJA = *M. japonica*, MECA = *M. canadensis*, MELO = *M. longifolia*, MESP = *M. spicata*, MESU = *M. suaveolens*, MECU = *M. cunninghamii*.

We cannot assume that all chromosome numbers in *Mentha* given earlier, $2n = 20$ to 24, are entirely due to endoduplication; interspecific hybridization may be even more involved in the origin of these species, and some parental species may be extinct (Ikeda, 1961). For example, Ikeda and Ono (1969), on the basis of genome analysis, have postulated that *M. arvensis* ($2n = 72$) may also be an ancient hybrid with *M. japonica* ($2n = 48$), but this hypothesis has never been tested. Thus, cladistic analysis may not be the best choice, even with *à posteriori* adjustments to the known hybrids (Funk, 1981; Wagner, 1983; Wanntorp, 1983; Ashlock, 1984; Cronquist, 1987; McDade, 1990, 1992, 1997, 2000; Arnold, 1997). The dendrogram shown in Figure 1.2 was obtained by the neighbor-joining algorithm and more closely reflects the hybrid origin of the known ancient amphidiploids.

In all analyses that were attempted on PAUP, *M. cunninghamii* was consistently outside of the ingroup. Recognition of *M. cunninhgamii* as a species of *Micromeria*, rather than *Mentha*, is not new; Bentham (1835) described this species as *M. cunninghamii*. New Zealand has nearly 433 genera, of which 10% are endemic. Interchange between Australia and New Zealand has

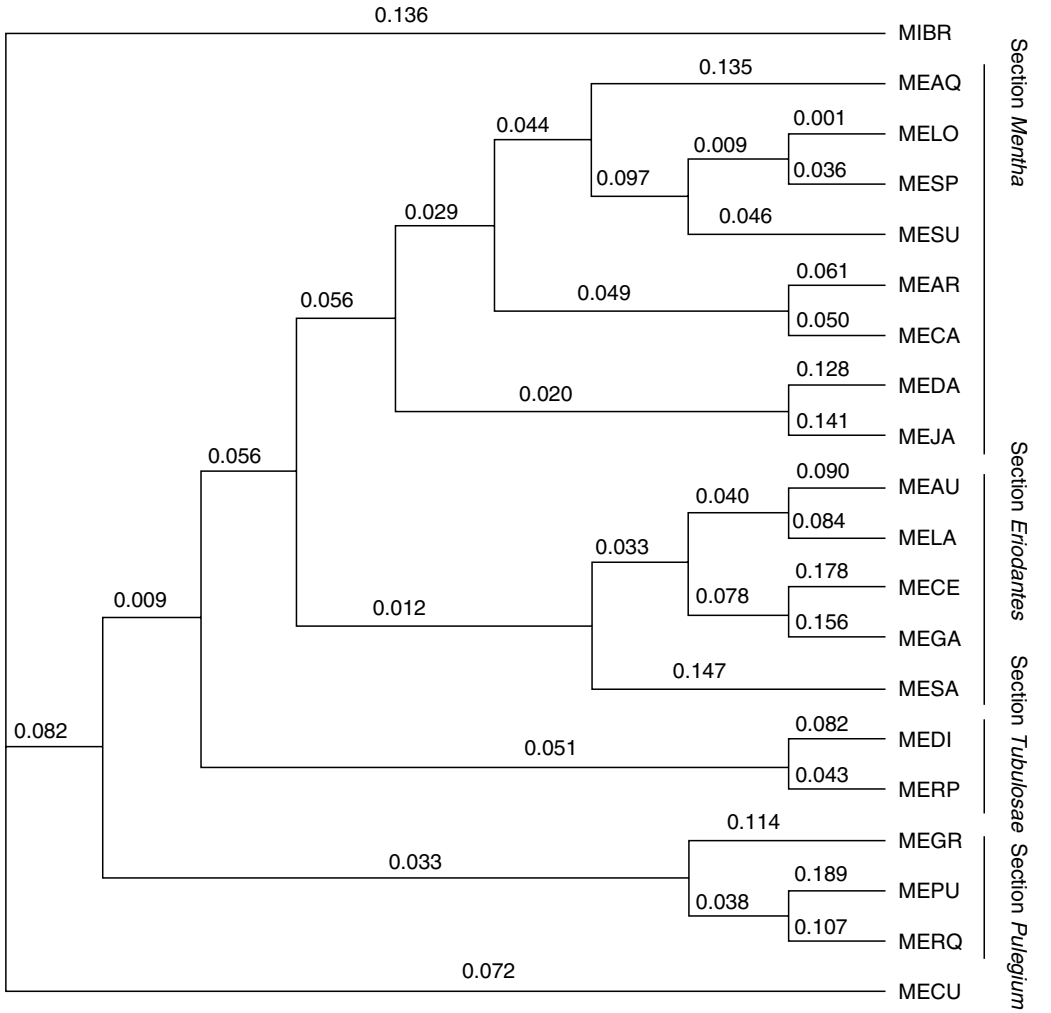


FIGURE 1.2 Neighbor-joining (NJ) phylogram obtained by analysis of 27 characters listed in the text. NJ distances are shown above the branches. Taxon key. MIBR = *Micromeria brownei* var. *pilosiuscula*, MEAQ = *Mentha aquatica*, MEAR = *M. arvensis*, MEAU = *M. australis*, MECE = *M. cervina*, MEGA = *M. gattefossei*, MEDI = *M. diemenica*, MEGR = *M. grandiflora*, MERQ = *M. requienii*, MEPU = *M. pulegium*, MERP = *M. repens*, MELA = *M. laxiflora*, MESA = *M. sautureioides*, MEDA = *M. dahurica*, MEJA = *M. japonica*, MECA = *M. canadensis*, MELO = *M. longifolia*, MESP = *M. spicata*, MESU = *M. suaveolens*, MECU = *M. cunninghamii*.

occurred, though; close to 200 species are common to both regions, but very few are angiosperms. About 320 species of vascular plants also overlap between New Zealand and South America, the result of a migration via Antarctica in the Cretaceous to Lower Tertiary. Affinities of the flora of New Zealand also exist with that of Malaysia and Polynesia (Laing and Blackwell, 1964; Tobler et al., 1970; Mildenhall, 1980; Allan, 1982; Rogers and Walker, 2002). New Zealand has only four species in the Lamiaceae, all endemic: *Mentha cunninghamii*, *Scutellaria novae-zelandiae* J.D. Hook., *Teucridium parviflorum* J.D. Hook., and *Vitex lucens* Kirk (Allan, 1982). *Tetracondra*, endemic to New Zealand and sometimes placed in the Lamiaceae, is derived from a South American ancestor in the Paleocene (Wagstaff et al.,

2000). Affinities of the *Scutellaria* and *Vitex* species could be in either Asia or South America, although the latter is more likely in view of the paleobotanical history of New Zealand.

The similarity of *Mentha cunninghamii* to *Micromeria brownei* should not be surprising, since *Micromeria brownei* is currently found in areas from Argentina to Florida. Of particular note, *Micromeria bahamensis* Shinners and *Micromeria domingensis* Shinners, both insular relatives of *Micromeria brownei* and probably dating from the mid-Eocene to Pleistocene, superficially resemble *Mentha cunninghamii* (Shinners, 1962; Keng, 1977; Li and Hedge, 1994; Múlgura, 1999; Xifreda, 1999; Graham, 2003). Selection of the large, white, solitary, subactinomorphic flowers of *Mentha cunninghamii* from an ancestor in common with *Micromeria brownei* would undoubtedly reflect the pollinators and other ecological factors in New Zealand (Lloyd and Barrett, 1996; Wilson and Thomson, 1996; Galen, 1999). Approximately 61% of the flowering plants in New Zealand bear white flowers, and the most common pollinators are diurnal calyptrate Diptera (flies from the families Muscidae and Tachinidae), diurnal Coleoptera (beetles from the families Helodidae, Melyridae, Mordellidae, and Curculionidae), and a few diurnal short-tongued Colletidae (bees). Other pollinators include small-tongued bees, flower-haunting weevils, and both diurnal and nocturnal moths. New Zealand lacks indigenous long-tongued bees and has only 16 species of butterflies (Godley, 1979). However, the proper classification of *Mentha cunninghamii* must await a future analysis of the entire, worldwide distribution of this complex of over 90 species, which also includes some species variously classified as not only *Micromeria* and *Clinopodium* but also *Satureja* and *Calamintha* (Valverde, 1993; Harley and Paucar, 2000). For example, Doroszenko (1987) has proposed the unpublished genus *Hesperothymus* to encompass the species related to *Micromeria brownei*. The current distribution of this proposed genus is South America and South Africa, which agrees with the paleobotany of these regions and their affinity with New Zealand.

The most robust clade is the grouping of *M. longifolia*, *M. spicata*, and *M. suaveolens*. *Mentha spicata* has been shown to be an ancient amphidiploid of *M. longifolia* × *M. suaveolens* (Harley and Brighton, 1977; Kokkini and Pappageorgiou, 1982; Gobert et al., 2002), and many variants with $2n = 48$ have been identified as either *M. spicata* or *M. longifolia*, depending on the amount of weight given on chromosome number versus morphology.

Another robust clade is the pairing of *M. cervina* from the western Mediterranean with *M. gattefossei* from Morocco. Except for the number of calyx lobes, these two species share many features (Harley, 1972; Bunsawat et al., 2004). Hence, this analysis does not support the recognition of a separate genus, *Preslia cervina*, solely on the basis of the number of calyx lobes.

Both maximum parsimony and the neighbor-joining algorithm grouped endemics from the Mediterranean (*M. cervina*, *M. gattefossei*, and *M. requienii*) with the endemics from Australia (*M. australis*, *M. diemenica*, *M. grandiflora*, *M. laxiflora*, *M. repens*, and *M. satureioides*). Superficially, the close affinity of the Mediterranean and Australian endemics is not easy to explain except on the basis of similar climates today. Considering all 18 species of *Mentha*, Thorne (1972) classified this type of distribution as African–Eurasian–Australasian. Thorne noted 96 genera, such as *Pelargonium*, with this distribution. If current diversity is correlated with origin, then southwest or central Asia is a Laurasian core area for the Lamiaceae with three directions of radiation: (1) along the Mediterranean; (2) to southern Africa via the East African mountains; and (3) to the northeast of Asia, then to western North America, and toward Southeast Asia and Australasia (Hedge, 1992). The time when *Mentha* was involved in this radiation of the Lamiaceae is unknown, but a *Mentha*-like fossil is known from the Eocene in North America; *Menthites eocenicus* Cockerell is possibly one of the oldest known fossils in the Lamiaceae (Cockerell, 1926).

How this distribution was effected is also open to question, since the extant species of *Mentha* have seed dispersal via wind (anemochory) or water (hydrochory), but the rhizomes of the aquatic species and their hybrids are readily spread by water (Bouman and Meeuse, 1992).

Angiosperms did not originate in Australia, and the earliest angiosperms in Australia migrated from a source in northern Gondwana or southern Laurasia during the Barremian–Aptian (Lower Cretaceous). A later invasion of angiosperms occurred during the middle Albian–Cenomanian (mid-Cretaceous) from nonmagnoliid lineages that had evolved in northern Gondwana during the Aptian. The distribution of taxa between Eurasia (which included flora from not only Laurasia but also Gondwana as a result of the collision of the Indian subcontinent during the Eocene) and Australia has been explained as long-distance dispersal down the mountains of Malesia (glacial emergent "microcontinents"). The timing and direction of this species migration are open to speculation, as the Sunda Shelf, now mostly underwater, was subject to massive geological changes and periodic extinctions from the late Cretaceous to the Pleistocene, but periodic contact between Australia and the Sunda Shelf has been documented during this time. Fossils of the Lamiaceae are uncommon in Australasia. However, looking at the well-represented fossils of species of the Fagales, it can be seen that they are documented to have spread from a secondary core in southeast Asia to Australia during the late Cretaceous to early Tertiary (Raven and Axelrod, 1974; Beck, 1976; Windley, 1977; Friis et al., 1987; Truswell et al., 1987; White, 1990; Hill, 1994; Crisp et al., 1999; Hill et al., 1999; Qian, 2002). Endemic species of the Mediterranean, such as *M. requienii* from Corsica, probably originated in the mid-Tertiary (Verlaque et al., 1991, 1995, 1997; Médail and Verlaque, 1997). Thus, considering the current worldwide distribution of *Mentha*, the endemic species of *Mentha* from Australia and the Mediterranean probably arose during the Tertiary from widely distributed species in southwest or central Asia (N.B. the current native range of *M. pulegium* is from Ireland to Kazakhstan, but it has been introduced worldwide and even become invasive in some habitats).

This analysis of morphological characters, chromosome numbers, and essential oil chemistry supports the recognition of four sections from the neighbor-joining phylogram (Figure 1.2). This classification also indicates predictions to be tested. For example, the geographical distribution of the insular endemics (*M. japonica*, *M. repens*, and *M. requienii*) correlates with their nearest geographical neighbors (*M. dahurica*, *M. diemenica*, and *M. pulegium*, respectively), and this provides predictions for the ease of hybridization. Thus, *M. japonica* has been documented to be capable of hybridization with *M. canadensis* (Ikeda et al., 1970; Murray, private communication, 1971) and *M. aquatica* (Ikeda and Ono, 1969), and, as predicted from the neighbor-joining phylogram, *M. japonica* may also be capable of hybridization with *M. dahurica*.

1.4.2 Infrageneric Classification of *Mentha*

This classification expands that of Briquet (1896), who published two subgenera, *Pulegium* (Mill.) Lam. & DC. and *Menthastrum* Coss. & Germ., in the genus *Mentha*. However, this classification redefines Section *Pulegium* to include three species, and also contrary to Briquet, does not recognize the genus *Preslia*. This does not support Miller (1768), who included both *M. pulegium* and *M. cervina* in the genus *Pulegium*. Based upon a strict consensus of ten equally parsimonious trees based on combined analysis of cpDNA *rpl16* intron and *trnL-trmF* region sequences, Bunsawat et al. (2004) showed that *M. australis*, *M. satureioides*, *M. diemenica*, and *M. cunninghamii* form one clade. Previously published infrageneric classifications are indicated in Table 1.1.

TABLE 1.1
Infrageneric Classification of the 18 Species of *Mentha* in Previous Publications. Pérard (1876) Concentrated upon the Species of Central Europe, So Only the Subgenera Are Included Here. Authorities Are Omitted for Brevity, but the Pertinent Ones Are Given in the Text

Species	Classification	References
1. *M. aquatica*	Section *Terminales*	Bentham (1848)
	Subgenus *Trichomentha*	Pérard (1876)
	Subgenus *Menthastrum*	
	Section *Capitatae*	Briquet (1986)
	Section *Mentha*	Harley and Brighton (1977)
2. *M. arvensis*	Section *Arvenses*	Bentham (1848)
	Subgenus *Trichomentha*	Pérard (1876)
	Subgenus *Menthastrum*	
	Section *Verticillatae*	
	Subsection *Arvenses*	Briquet (1986)
	Section *Mentha*	Harley and Brighton (1977)
3. *M. australis*	Section *Arvenses*	Bentham (1848)
	Subgenus *Menthopsis*	Pérard (1876)
	Subgenus Menthastrum	
	Section Verticillatae	
	Subsection Tubulosae	Briquet (1986)
4. *M. canadensis*	Section *Arvenses*	Bentham (1848)
	Subgenus *Trichomenta*	Pérard (1876)
	Subgenus *Menthastrum*	
	Section *Verticillatae*	
	Subsection *Arvenses*	Briquet (1986)
5. *M. cervina*	*Preslia cervina*	Bentham (1848)
	Section *Preslia*	Harley and Brighton (1977)
6. *M. dahurica*	Section *Arvenses*	Bentham (1848)
7. *M. diemenica*	Section *Arvenses*	Bentham (1848)
	Subgenus *Menthopsis*	Pérard (1876)
	Subgenus *Menthastrum*	
	Section *Verticillatae*	
	Subsection *Tubulosae*	Briquet (1986)
8. *M. gattefossei*	Section *Gattefossei*	Harley and Brighton (1977)
9. *M. grandiflora*	Subgenus *Menthastrum*	
	Section *Verticillatae*	
	Subsection *Grandiflorae*	Briquet (1986)
10. *M. japonica*	Not included in Bentham (1848), Pérard (1876), Briquet (1896), or Harley and Brighton (1977)	
11. *M. laxiflora*	Section *Arvenses*	Bentham (1848)
	Subgenus Menthastrum	
	Section Verticillatae	
	Subsection Laxiflorae	Briquet (1986)
12. *M. longifolia*	Section *Terminales*	Bentham (1848)
	Subgenus *Mentha*	Pérard (1876)
	Subgenus *Menthastrum*	
	Section *Spicatae*	Briquet (1986)
	Section *Mentha*	Harley and Brighton (1977)

TABLE 1.1 (continued)
Infrageneric Classification of the 18 Species of *Mentha* in Previous Publications. Pérard (1876) Concentrated upon the Species of Central Europe, So Only the Subgenera Are Included Here. Authorities Are Omitted for Brevity, but the Pertinent Ones Are Given in the Text

Species	Classification	References
13. *M. pulegium*	Section *Pulegia*	Bentham (1848)
	Subgenus *Pulegium*	
	Section *Euplegia*	Briquet (1986)
	Section *Pulegium*	Harley and Brighton (1977)
14. *M. repens*	Section *Eriodontes*	Bentham (1848)
	Subgenus *Menthopsis*	Pérard (1876)
	Subgenus *Menthastrum*	
	Section *Verticillatae*	
	Subsection *Eriodontes*	Briquet (1986)
15. *M. requienii*	Section *Pulegia*	Bentham (1848)
	Subgenus *Pulegium*	
	Section *Audibertiae*	Briquet (1896)
	Section *Audibertia*	Harley and Brighton (1977)
16. *M. satureioides*	Section *Eriodontes*	Bentham (1848)
	Subgenus *Menthopsis*	Pérard (1876)
	Subgenus *Menthastrum*	
	Section *Verticillatae*	
	Subsection *Eriodontes*	Briquet (1986)
	Section *Eriodontes*	Harley and Brighton (1977)
17. *M. spicata*	Section *Terminales*	Bentham (1848)
	Subgenus *Mentha*	
	Section *Silvestres*	Pérard (1876)
	Subgenus *Menthastrum*	
	Section *Spicatae*	Briquet (1986)
	Section *Mentha*	Harley and Brighton (1977)
18. *M. suaveolens*	Section *Terminales*	Bentham (1848)
	Subgenus *Mentha*	
	Section *Rotundifolae*	Pérard (1876)
	Subgenus *Menthastrum*	
	Section *Rotundifoliae*	Briquet (1986)
	Section *Mentha*	Harley and Brighton (1977)

1.4.3 *Mentha* L., *Sp. pl.* 576. 1753; *Gen. Pl.* ed. 5. 250. 1754. Lectotype Species: *M. spicata* (Britton and Brown, 1913)

- *Pulegium* Mill., *Gard. Dict*. ed. 8. 1768. Type species: *Pulegium vulgare* Mill.
- *Preslia* Opiz, *Naturalientausch* **8**: 86. 1824; *Flora* **7**: 322. Type species: *Preslia. glabriflora* Opiz.
- *Audibertia* Benth., *Bot. Reg. Sub.* t. 1282. 1829. Type species: *A. pusilla* Benth.
- *Menthella* Pérard, *Bull. Soc. Bot. France* **17**: 205. 1870. Type species: *Menthella requienii* (Benth.) Pérard (*Mentha requienii* Benth.).

1.4.3.1 Section *Pulegium* (Mill.) Lam. & DC., *Fl. Franç.* 537 1805, emend. Tucker. Type Species: *M. pulegium* L.

Habit uniformly prostate to upright when flowering; stolons absent; leaves ovate to suborbicular, obtuse to cuneate leaf, entire to serrate, thin or revolute, smooth, petiolate; hairs acicular; inflorescence verticillate; bracts similar to leaves, not reduced upward; flowers four to many per axil; calyx subactinomorphic to zygomorphic, throat with a ring of hairs, tubular; five calyx lobes unequal to subequal, subulate-lanceolate to subulate; calyx 10 to 15 nerved; corolla subactinomorphic, throat glabrous to pubescent; style branches subequal to equal; stamens divergent; $x = 9, 10$; essential oils predominantly 3-oxygenated monoterpenoids.

- *M. grandiflora* Benth.
- *M. pulegium* L.
- *M. requienii* Benth.

1.4.3.2 Section *Tubulosae* (Briq.) Tucker. Type Species: *M. diemenica* Spreng

Subsection *Tubulosae* Briq., *Nat. Pflanzenfam.* **4**(3a): 319. 1896.

Habit upright when flowering; stolons absent; leaves ovate to lanceolate, obtuse to cuneate, entire, revolute, smooth, petiolate; hairs acicular; inflorescence verticillate; bracts uniformly equal to leaves and simple; flowers two to eight per axil; calyx subactinomorphic, throat glabrous, tubular to campanulate; five calyx lobes subequal, narrowly triangular to deltoid; calyx 10 to 15 nerved; corolla subactinomorphic, throat glabrous; style branches equal; stamens divergent; $x = 60$; essential oils predominantly 3-oxygenated monoterpenoids.

- *M. diemenica* Spreng.
- *M. repens* (Hook. f.) Briq.

1.4.3.3 Section *Eriodontes* Benth. in DC., *Prodr.* 12: 174. 1848, emend. Tucker. Type Species: *M. satureioides* R.Br.

Habit upright when flowering; subterranean or aerial stolons; leaves oblong-lanceolate to linear-oblanceolate, cordate to attenuate, entire to serrate, flat to revolute, smooth, sessile to petiolate; hairs acicular; inflorescence verticillate; bracts equal to leaves to smaller than leaves, simple to digitately lobed; flowers four to many per axil; calyx zygomorphic to subactinomorphic, throat glabrous to pubescent, tubular to tubular-campanulate; four to five calyx lobes unequal to subequal; calyx lobes narrowly triangular to tapering with an apical spine; calyx 10 to 15 nerved; corolla subactinomorphic, throat glabrous to pubescent; style branches equal; stamens divergent; $x = 12, 13$; essential oils predominantly 3-oxygenated monoterpenoids.

- *M. australis* R.Br.
- *M. cervina* L.
- *M. gattefossei* Maire.
- *M. laxiflora* Benth.
- *M. satureioides* R.Br.

1.4.3.4 Section *Mentha*. Type Species: *M. spicata* L.

Habit uniformly upright; stolons subterranean or aerial; leaves ovate to lanceolate, cordate to cuneate, entire to serrate or crenate, flat to revolute, smooth to rugose, sessile or petiolate; hairs acicular or stellate; inflorescence various; bracts simple, equal to leaves to smaller than

leaves, decreasing toward apex, or uniformaly smaller than leaves; flowers four to many per axil; calyx subactinomorphic, throat glabrous or pubescent, tubular to campanulate; calyx lobes subequal—four to five, deltoid-ovate to subulate, never ciliate, throat glabrous or pubescent; calyx 10 to 12 nerved; corolla subactinomorphic, throat glabrous or pubescent; style branches equal; stamens divergent to parallel; $x = 12$; essential oil chemistry varied.

- *M. aquatica* L.
- *M. arvensis* L.
- *M. canadensis* L.
- *M. dahurica Fisch.* ex Benth.
- *M. japonica* (Miq.) Makino
- *M. longifolia* (L.) L.
- *M. spicata* L.
- *M. suaveolens* Ehrh.

1.5 KEY TO THE SPECIES OF *MENTHA*

1. Calyx with 4 lobes 2
   1a. Calyx with 5 lobes 3
2. Bracts of inflorescence digitately lobed, calyx lobes narrowed to a narrow spine, leaves sessile 5. *M. cervina*
   2a. Bracts of inflorescence simple, calyx lobes deltoid-ovate to broadly triangular, leaves petiolate 6. *M. dahurica*
3. Calyx interior with hairy annulus 4
   3a. Calyx interior glabrous or with only a few scattered hairs 14
4. Stems filiform, leaves 2 to 7 mm, four to eight flowers or leaf axil on prostrate stems, not differentiated into an inflorescence 15. *M. requienii*
   4a. Stems not filiform, leaves normally larger than 7 mm, many flowers/axil usually in a defined inflorescence 5
5. Calyx zygomorphic 6
6. Leaves ovate to suborbicular, entire, obtuse to cuneate, petiolate, corolla interior with hairy annulus 13. *M. pulegium*
   6a. Leaves linear-oblanceolate, serrate, cuneate to attenuate, sessile, corolla interior glabrous 8. *M. gattefossei*
   6b. Calyx ± actinomorphic 7
7. Two to eight flowers/axil 8
   7a. Nine to many flowers/axil 11
8. Leaves serrate with thin edge 9. *M. grandiflora*
   8a. Leaves entire with thickened edge, ± revolute 9
9. Leaves ovate to suborbicular 14. *M. repens*
   9a. Leaves oblong-lanceolate to lanceolate 10
10. Leaves obtuse to cuneate, calyx with 13 to 15 nerves, corolla interior essentially glabrous 7. *M. diemenica*
    10a. Leaves cuneate to attenuate, calyx with ten nerves, corolla interior with hairy annulus 16. *M. satureioides*
11. Inflorescence verticillate 12
    11a. Inflorescence capitate13
12. Leaves entire with thickened edge, ± revolute, cuneate to attenuate; calyx lobes narrowly triangular to lanceolate 3. *M. australis*
    12a. Leaves serrate with thin edge, cordate to obtuse; calyx lobes narrowly triangular to subulate 11. *M. laxiflora*

13. Leaves oblong-lanceolate to lanceolate, entire to serrate, corolla interior with hairy annulus 6. *M. dahurica*
    13a. Leaves ovate to suborbicular, crenate, corolla interior essentially glabrous 1. *M. aquatica*
14. Inflorescence verticillate 15
    14a. Inflorescence capitate or spicate 17
15. Leaves entire with thickened edge, ± revolute, corolla interior with hairy annulus, calyx lobes narrowly triangular to lanceolate, calyx with 13 to 15 nerves 3. *M. australis*
    15a. Leaves serrate with thin edge, corolla interior essentially glabrous, calyx lobes deltoid-ovate to broadly triangular, calyx with 10 nerves 16
16. Leaves linear-oblanceolate and gradually decreasing in size toward the apex of the blooming stem, usually pennyroyal- or peppermint-odored 4. *M. canadensis*
    16a. Leaves ovate to suborbicular and not decreasing in size toward the apex of the blooming stem, usually fruit-lavender-odored, rarely pennyroyal-odored 2. *M. arvensis*
17. Inflorescence capitate 18
    17a. Inflorescence spicate 19
18. Leaves linear-oblanceolate, entire with thickened edge, ± revolute, calyx lobes deltoid-ovate to broadly triangular, pennyroyal-odored 10. *M. japonica*
    18a. Leaves ovate to suborbicular, crenate with thin edge, calyx lobes narrowly triangular to subulate, musty or lavender-odored 1. *M. aquatica*
19. Leaves rugose, ovate to suborbicular 20
    19a. Leaves not rugose, oblong-lanceolate 21
20. Leaves pubescent with scattered dendroid hairs on abaxial surface, fruit-odored 18. *M. suaveolens*
    20a. Leaves glabrous; spearmint-, peppermint-, or infrequently fruit-odored 17. *M. spicata*
21. Hairs all uniseriate, leaves widest near the middle; fertile anthers 0.28 to 0.38 mm 12. *M. longifolia*
    21a. Scattered dendroid hairs on abaxial surface of leaves, leaves widest near base; fertile anthers 0.38 to 0.52 mm 17. *M. spicata*

1.6 ENUMERATION OF SPECIES

The prominent synonyms cited in floristic treatments published within the last 50 years are listed here. With over 3000 names published within this genus, not all synonyms can be listed in this abbreviated account. Decades of research remain to be completed on typification of all the published names (a thankless task), and, presently, only the names of ca. 1800 have been adequately researched.

1.6.1 *M. aquatica* L., *Sp. pl.* 576. 1753. Type: Clifford Herbarium (Lectotype, BM!), $2n = 96$.

1.6.1.1 var. *aquatica*, Water Mint

- *Mentha hirsuta* Huds., *Fl. Angl.* **223**. 1762.
- *Mentha palustris* Mill., *Gard. Dict.* ed. 8. n. 12. 1768.
- *Mentha capitata* Gilib., *Fl. Lit. Inch.* **1**: 721. 1782.
- *Mentha dubia* Chaix ex Vill., *Hist. pl. Dauphiné* **2**: 358. 1787.
- *Mentha aquatica* L. var. *pedunculata* Pers., *Syn. pl.* **2**: 119. 1806.

- *Mentha ortmanniana* Opiz, *Naturalientausch* **11**: 437. 1826.
- *Mentha purpurea* Host, *Fl. Austriac*. **2**: 141. 1831.
- *Mentha aquatica* L. var. *stricta* K. Koch, *Linnaea* **21**: 651. 1849.
- *Mentha pyrifolia* Heinr. Braun, Verh. K.K. *Zool. -Bot. Ges. Wien*. **40**: 420. 1890.
- *Mentha aquatica* L. var. *ortmanniana* Henir. Braun ex Briq., *Lab. Alp. Mar*. **82**. 1891.
- *Mentha dumetorum* Schultes var. *natalensis* Briq., *Bull. Herb. Boissier* **2**: 702. 1894.
- *Mentha aquatica* L. var. *calaminthifolia* Vis. ex Sagorski & Oswald, Mitt. Thüring. *Bot. Vereins* **26**: t. 4. 1910.
- *Mentha pyrifolia* (Heinr. Braun) *Jáv*., *Magyar Fl*. **940**. 1925.
- *Mentha perhirsuta* (Borbás) Trautman in *Jáv*., *Magyar Fl*. **946**. 1925.
- *Mentha aquatica* L. var. *cordata* Prodan, *Bull. Inform. Grăd. Bot. Univ. Cluj* **5**: 114. 1925.
- *Mentha aquatica* L. f. *ortmaniana* (Opiz) Heinr. Braun ex Jančić, Glasn. Prir. Mus. u Beogradu, Ser. B, *Biol. Nauke* **39**: 30. 1984.

Distribution: throughout Europe except extreme north
Habitat: wet edges of ponds, lakes, and canals

1.6.1.2 var. *citrata* (Ehrh.) Fresen., *Syll. Pl. Nov.* 2: 234. 1828. Type: H.H. [Hortus Herrenhusanus, The Royal Gardens of Herrenhausen, Hannover, Germany], *Ehrhart* s.n. (Lectotype, GOET! Syntypes, GOET!), Orange Mint, Bergamot Mint

- *Mentha citrata* Ehrh., *Beitr. Naturk*. **7**: 150. 1792.
- *Mentha adspersa* Moench, *Methodus* **379**. 1794.
- *Mentha aquatica* L. f. *citriodora* G. Mey., Chloris Han. **290**. 1836.
- *Mentha* ×*piperita* L. subsp. *citrata* (Ehrh.) Briq., *Bull. Soc. Bot. Genève* **5**: 62. 1889.
- *Mentha* ×*piperita* L. var. *citrata* (Ehrh.) Rouy, *Fl. France* **11**: 378. 1909.
- *Mentha piperita* L. nm. *citrata* (Ehrh.) Boivin, *Naturaliste Canad*. **93**: 1061. 1966.

Distribution: Examining the genetics and chromosome numbers of multiple collections from North America and Central Europe, Murray and Lincoln (1970) found most to be a high linalool/linalyl acetate, male sterile forms of *M. aquatica* and not hybrids (although the male sterility promotes hybridization with other species, as in *M.* ×*piperita* "Lavanduliodora" from Italy and some forms of *M.* ×*piperita* from England), and this is further supported by flavonoid data (Voirin et al., 1999).
Habitat: as for var. *aquatica*, often cultivated

1.6.2 *M. arvensis* L., *Sp. pl.* 577. 1753. Type: Linnaean Herbarium (Lectotype, S!), Cornmint, $2n = 72$

1.6.2.1 subsp. *arvensis*

Distribution: southern and western Europe
Habitat: moist fields and roadsides

1.6.2.2 subsp. *parietariefolia* (Becker) Briq., *Bull. Soc. Bot. Genève* 5: 43. 1889. Type: Frankfurt, Germany (Holotype, FR!)

- *Mentha gentilis* L., *Sp. pl*. 1753.
- *Mentha austriaca* Jacq., *Fl. Austriac*. **5**: 14. t. 430. 1778.
- *Mentha praecox* Sole, Menth. Brit. **31**. t. 13. 1798.
- *Mentha variegata* Sole, *Menth. Brit*. 43. t. 19. 1798.

- *Mentha procumbens* Thuill., *Fl. Env. Paris* ed. 2. 288. 1799.
- *Mentha lapponica* Wahlenb., *Fl. Lapp*. **161**. t. 10. 1812.
- *Mentha arvensis* L. var. *parietariaefolia* Becker, *Fl. Frankfurt* **225**. 1827.
- *Mentha arvensis* L. var. *parietariaefolia* (Becker ex Strail) Reichb., *Icon. Bot. pl. Crit*. **10**: 25. t. 970. 1832.
- *Mentha parietariaefolia* Becker ex Steud., *Nomencl. Bot*. ed. 2. 2: 127. 1841.
- *Mentha arvensis* L. var. *parietariaefolia* (Becker) Wirtg., *Herb. Menth. Rhen*. **15**. 1855.
- *Mentha parietariaefolia* (Becker) Boreau, *Fl. centre France* ed. 3. 2: 515. 1857.
- *Mentha parietariaefolia* (Becker) Heinr. Braun, *Verh. KK. Zool. -Bot. Ges. Wien* **36**: 226. 1886.
- *M. arvensis* L. subsp. *austriaca* (Jacq.) Briq., *Lab. Alp. Mar*. **88**. 1891.
- *Mentha arvensis* L. var. *parietariaefolia* (Becker) Fiori & Paol., *Fl. Italia* **3**: 81. 1903.
- *Mentha arvensis* L. subsp. *parietariifolia* (Becker) Vollmann, Mitt. Bayer. *Bot. Ges*. **2**: 207. 1909.
- *Mentha arvensis* L. var. *parietariaefolia* (Becker) Fraser, *Bot. Soc. Exch. Club Brit. Isles* **8**: 245. 1927.

Distribution: northern and eastern Europe
Habitat: moist fields and roadsides

1.6.3 *M. australis* R.Br., *Prodr.* 505. 1810. Type: Cataract River, Port Dalrymple [Tasmania, Australia], *R. Brown* #2349 (Holotype, BM!; Isotypes, BM! K!), $2n = 72$

Micromeria australis (R.Br.) Benth. *Labiat. Gen. Spec*. **380**. 1834.

Distribution: Australia (except West Australia), Tasmania
Habitat: near watercourses and waterholes, damp gullies

1.6.4 *M. canadensis* L., *Sp. pl.* 577. 1753. Type: Kalm Specimen in Linnaean Herbarium 730.18 (Lectotype, LINN!), American cornmint, Japanese Peppermint, *hakka*, $2n = 96$

- *Mentha borealis* Michx., *Fl. Bor.-Am*. **2**: 2. 1803.
- *Mentha canadensis* L. var. *villosa* Benth., *Labiat. Gen. Spec*. **181**. 1833.
- *Mentha canadensis* L. var. *glabrata* Benth., *Labiat. Gen. Spec*. **181**. 1833.
- *Mentha cinerea* Raf., *Herb. Raf*. **68**. 1833; *New Fl*. **4**: 94. 1838; *Autik. Bot*. **112**. 1840.
- *Mentha canadensis* L. var. *glabrior* Hook., *Fl. Bor.-Am*. **2**: 111. 1838.
- *Mentha trachiloma* Raf., *New Fl*. **4**: 984. 1838.
- *Mentha ciliata* Raf., *New Fl*. **4**: 94. 1838; *Autik. Bot*. **113**. 1840.
- *Mentha agrestis* Raf., *Autik. Bot*. **113**. 1840.
- *Mentha terebinthinacea* Willd. ex Steud., *Nomencl. Bot*. ed. 2. 3: 128. 1841.
- *Mentha arvensis* L. f. *piperascens* Malinv. ex Holmes, *Pharm. J. Lond*. **13**: 382. 1882.
- *Mentha haplocalyx* Briq., *Bull. Bot. Soc. Genève* **5**: 39. 1889.
- *Mentha haplocalyx* Briq. subsp. *pavoniana* Briq., *Bull. Soc. Bot. Genève* **5**: 40. 1889.
- *Mentha haplocalyx* Briq. subsp. *austera* Briq., *Bull. Soc. Bot. Genève* **5**: 41. 1889.
- *Mentha hakka* Siebold, *Verh. Batav. Genootsch*. **22**: 32. 1890.
- *Mentha arvensis* L. var. *canadensis* (L.) Kuntze, *Revis. Gen. pl*. **2**: 524. 1891.
- *Mentha arvensis* L. var. *borealis* (Michx.) Kuntze, *Revis. Gen. pl*. **2**: 524. 1891.
- *Mentha disperma* Sessé & Moc., *Fl. Mexic*. ed. 2. 135. 1894.

- *Mentha arvensis* L. var. *canadensis* (L.) Briq., *Bull. Herb. Boissier* **2**: 707. 1894.
- *Mentha arvensis* L. var. *haplocalyx* (Briq.) Briq., *Bull. Herb. Boissier* **2**: 707. 1894.
- *Mentha arvensis* L. var. *zollingeri* Briq., *Bull. Herb. Boissier* **2**: 707. 1894.
- *Mentha arvensis* L. var. *pavoniana* Briq., *Bull. Herb. Boissier* **2**: 708. 1894.
- *Mentha arvensis* L. var. *schmitzii* Briq., *Bull. Herb. Boissier* **2**: 708. 1894.
- *Mentha arvensis* L. var. *sachalinensis* Briq., *Bull. Herb. Boissier* **2**: 708. 1894.
- *Mentha arvensis* L. var. *penardi* Briq., *Bull. Herb. Boissier* **3**: 215. 1895.
- *Mentha arvensis* L. subsp. *haplocalyx* Briq., *Nat. Pflanzenfam*. **4**(3a): 319. 1896.
- *Mentha rubella* Rydb., *Mem. NY Bot. Gard*. **1**: 337. 1900.
- *Mentha arvensis* L. var. *lanata* Piper, *Bull. Torrey Bot. Club* **29**: 222. 1902.
- *Mentha canadensis* L. var. *lanata* (Piper) Piper, *Contrib. U.S. Nat. Herb*. **11**: 492. 1906.
- *Mentha canadensis* L. var. *borealis* (Michx.) Piper, *Contrib. U.S. Nat. Herb*. **11**: 492. 1906.
- *Mentha penardi* (Briq.) Rydb., *Bull. Torrey Bot. Club*. **33**: 150. 1906.
- *Mentha arvensis* L. var. *glabrata* (Benth.) Fernald, *Rhodora* **10**: 86. 1908.
- *Mentha lanata* (Piper) Rydb., *Bull. Torrey Bot. Club* **36**: 687. 1909.
- *Mentha glabrior* (Hook.) Rydb., *Bull. Torrey Bot. Club* **36**: 686. 1909.
- *Mentha occidentalis* Rydb., *Bull. Torrey Bot. Club* **36**: 687. 1909.
- *Mentha canadensis* L. var. *lanata* (Piper) J.K. Henry, *Fl. s. Brit. Columbia* **260**. 1915.
- *Mentha haplocalyx* Briq. var. *barbata* Nakai, *Bot. Mag. Tokyo* **35**: 178. 1921.
- *Mentha sachalinensis* (Briq.) Kudo, *J. Coll. Sci. Imp. Univ. Tokyo* **43**: 47. 1921.
- *Mentha nipponensis* Kudo, *Mem. Fac. Agric. Taihoku Imp. Univ*. **2**(2): 88. 1929.
- *Mentha haplocalyx* Briq. var. *nipponensis* Matsum. & Kudo ex Kudo, *Mem. Fac. Agric. Taihoku Imp. Univ*. **2**(2): 88. 1929.
- *Mentha arvensis* L. var. *villosa* (Benth.) S.R. Stewart, *Rhodora* **46**: 333. 1944.
- *Mentha haplocalyx* Briq. var. *piperascens* (Malinv.) Wu and Li, *Acta Phytotax. Sin*. **12**: 225. 1974.
- *Mentha arvensis* L. subsp. *borealis* Taylor & MacBryde, *Canad. J. Bot*. **56**: 186. 1978.

Distribution: North America and eastern Asia; ancient amphidiploid of *M. arvensis* × *M. longifolia* (Tucker and Chambers, 2002)
Habitat: streambanks, lake shores, moist fields and roadsides, high menthol forms cultivated

1.6.5 *M. cervina* L., *Sp. Pl.* 578. 1753. Type: Burser Specimen in Burser Herbarium (Lectotype, UPS! Syntypes, BM! LINN!), Hart's Pennyroyal, $2n = 26$

- *Pulegium cervinum* (L.) Mill., *Gard. Dict*. ed. 8. n. 3. 1768.
- *Mentha punctata* Moench, Suppl. *Methods* **146**. 1802.
- *Mentha multifida* Stokes, *Bot. Mat. Med*. **3**: 318. 1812.
- *Preslia glabriflora* Opiz, *Naturalientausch* **8**: 88. 1824; *Flora* **7**: 323. 1824.
- *Preslia villiflora* Opiz, *Naturalientausch* **8**: 88. 1824; *Flora* **7**: 323. 1824.
- *Preslia cervina* (L.) Fresen., *Syll. Pl. Nov*. **2**: 238. 1828.

Distribution: western Mediterranean, Spain, Portugal, and Mediterranean France
Habitat: from damp land to water (60 cm deep), normally overwintering under water in its native habitat, spring growth erect with stems rich in aerenchyma

1.6.6 *M. dahurica* Fisch. ex Benth., *Labiat. Gen. Spec.* 181. 1833. Type: Dauria, Siberia (Holotype, Unknown, Not LE), $2n = 72$

Mentha aquatica L. subsp. *sibirica Briq.*, *Nat. Pflanzenfam.* **4**(3a): 319. 1896.

Distribution: eastern Siberia to north China and Hokkaido, Japan
Habitat: water meadows, more rarely dry valley meadows, shores of rivers and lakes, thickets, wood margins

1.6.7 *M. diemenica* Spreng., *Syst. Veg.* 2: 724. 1825. Type: "Terra Diemen" [Tasmania, Australia] (Holotype, B-Willd.?), $2n = 120$

- *Mentha gracilis* R.Br., *Prodr.* **505**. 1810.
- *Micromeria gracilis* (R.Br.) Benth., *Labiat. Gen. Spec.* **380**. 1834.

Distribution: widespread from South Australia to Tasmania and New South Wales, Australia
Habitat: unknown

1.6.8 *M. gattefossei* Maire, *Bull. Soc. Hist. Nat. Afrique N.* 13: 42. 1922. Type: Morocco (Holotype, AL; Isotype, G!), $2n = (\pm 32, 40)\ 48$

Distribution: Grand and Moyen Atlas Mountains, Morocco, between 1500 and 2200 m
Habitat: chiefly at the edge of dayas (pools drying in summer), less commonly in damp pastures and meadows

1.6.9 *M. grandiflora* Benth. in J. Mitch., *J. Exped. Trop. Australia* 362. 1848. Type: "Camp 29" [Depôt Camp, Overlooking the Maranoa Near Mitchell, Queensland, Australia], *T.L. Mitchell* #584 (Holotype, K!), $2n = ?$

Distribution: subtropical eastern Australia from inland Queensland on Dividing Range ($\pm$ 300 to 600 m) to northern New South Wales
Habitat: sandy soil

1.6.10 *M. japonica* (Miq.) Makino, *Bot. Mag.* (*Tokyo*) 20: 1. 1906. Type: in Declivitate Rimosis Montes Fuzi Sama ins. Nippon [Mt. Fuji, Japan], *Pierot* #657 (Holotype, L.!), *hime-hakka*, $2n = 48, (50)$

Micromeria japonica Miq., *Ann. Mus. Bot. Lugduno-Batavi* **2**: 106. 1865.
Satureja japonica Matsum. & Kudo, *Bot. Mag. (Tokyo)* **26**: 299. 1912.

Distribution: Hokkaido and Honshu, Japan
Habitat: wet places in lowlands to mountains

1.6.11 *M. laxiflora* Benth. in DC., *Prodr.* 12: 174. 1848. Type: New Holland [Port Philip, Victoria, Australia], *Gunn* s.n. (Holotype, K!), $2n = ?$

Distribution: Victoria and southern parts of New South Wales, especially Montane
Habitat: forests, usually in gullies, in regions above 508 mm annual rainfall

1.6.12 *M. longifolia* (L.) L., *Fl. monsp.* 19. 1756. Type: Burser Herbarium (Lectotype, UPS!) $2n = 24$, (48)

As discussed in Tucker et al. (1980), Linnaeus may not have intended to publish this as a species in his *Flora Monspeliensis* of 1756, but "intent" is not covered in the *International Code of Botanical Nomenclature* (Greuter, 2000). We can also argue that Linnaeus' intent apparently was to raise this taxon as a species, as he did so in 1763 with the publication of *M. sylvestris*.

This species has the most extensive geographical range and, correspondingly, the most variation. Hence, some late-20th-century taxonomists, such as Borissova (1954) and Jamzad (1987), have published even more new species in this complex without examination of the ponderous amount of prior publications and types; *M. asiatica* Borissova, for example, is conspecific with *M. longifolia* by AFLP markers (Gobert et al., 2002). *Mentha longifolia* has been included in the floras of Great Britain and North America, but almost all specimens from these areas are actually pubescent *M. spicata* with $2n = 48$ or *M.* ×*villosonervata* with $2n = 36$. This treatment essentially follows Briquet (1896) with updates; the 22 subspecies are listed here geographically, from western Europe to India to South Africa (albeit, a temporary solution until the full variation can be examined in detail with plants grown in a common environment).

1.6.12.1 subsp. *longifolia*, Horse Mint

- *Mentha spicata* L. var. *longifolia* L., *Sp. pl.* **576**. 1753.
- *Mentha longifolia* (L.) Huds., *Fl. Angl.* **221**. 1762.
- *Mentha sylvestris* L., *Sp. pl.* ed. 2. 804. 1763.
- *Mentha chalepensa* Mill., *Gard. Dict.* ed. 8. n. 11. 1768.
- *Mentha mollissima* Borkh. ex P. Gaertn., B. Mey. & Scherb., Oekon. Fl. Wetterau **2**: 348. 1800.
- *Mentha incana* Willd., *Enum. pl.* **609**. 1809.

Distribution: Europe
Habitat: wet alpine meadows and stream banks above 305 m

1.6.12.2 subsp. *dumortieri* (Déséglise & Th. Durand) Briq., *Nat. Pflanzenfam.* 4(3a): 322. 1896. Type: Mazures, Province de Liège, Belgium, *Durand* (Holotype, G)

- *Mentha dumortieri* Déséglise & Th. Durand, *Bull. Soc. R. Bot. Belgique* **17**: 334. 1879.
- *Mentha silvestris* L. subsp. *dumortieri* (Déséglise & Th. Durand) Briq., *Bull. Soc. Bot. Genève* **5**: 91. 1889.
- *Mentha longifolia* Huds. var. *dumortieri* Top., *Beih. Bot. Centralbl.* **30**(2): 163. 1913.

Distribution: Belgium
Habitat: unknown

1.6.12.3 subsp. *lavandulacea* (Willd.) Briq., *Nat. Pflanzenfam.* 4(3a): 321. 1896. Type: "Hort. Bot. Berol., Habitat in Hispania" (Holotype, B!)

Mentha lavandulacea Willd., *Enum. pl.* **609**. 1809.

Distribution: Spain
Habitat: unknown

1.6.12.4 subsp. *erminea* Briq., *Nat. Pflanzenfam.* 4(3a): 322. 1896. Type: Platania, Crete, *Reverchon* (Holotype, G)

Mentha longifolia Huds. var. *erminea* Briq., *Bull. Herb. Boissier* **2**: 700. 1894.

Distribution: Crete, central and southern Greece, Turkey
Habitat: marshes

1.6.12.5 subsp. *cyprica* (Heinr. Braun) Harley in Meikle, *Fl. Cyprus* 2: 1260. 1985. In der Schluchten um das Kloster Trooditissa [in Ravines around Trooditissa Monastery, 5 km. Northwest of Platres, Cyprus], *Kotschy* 23 (Lectotype, W!)

- *Mentha cyprica* Heinr. Braun, *Verh. KK. Zool. -Bot. Ges. Wien* **39**: 217. 1889.
- *Mentha longifolia* Huds. var. *cyprica* (Heinr. Braun) Briq., *Bull. Herb. Boissier* **2**: 696. 1894.
- *Mentha cyprica* Heinr. Braun var. *galatae* Heinr. Braun ex Briq., *Bull. Herb. Boissier*. **696**. 1894.
- *Mentha longifolia* Huds. var. *galatae* (Heinr. Braun) Briq., *Bull. Herb. Boissier* **2**: 696. 1894.

Distribution: Cyprus mountains, 61 to 1646 m, generally above 610 m
Habitat: moist ground by streams and springs

1.6.12.6 subsp. *grisella* Briq., *Nat. Pflanzenfam.* 4(3a): 322. 1896. Type: Unknown (Holotype, G!)

- *Mentha grisella* Briq. subsp. *grisella* Briq., *Bull. Soc. Bot. Genève* **5**: 94. 1889.
- *Mentha longifolia* (L.) Huds. var. *grisella* (Briq.) Fiori & Paol., *Fl. Italia* **3**: 76. 1903.

Distribution: Hungary, Rumania, Macedonia, Greece, Asia minor
Habitat: unknown

1.6.12.7 subsp. *diabolina* Briq., Nat. Pflanzenfam. 4(3a): 322. 1896. Type Langenthal, Rumania, *Barth* (Holotype, G!)

Mentha longifolia Huds. var. *diabolina* Briq., *Bull. Herb. Boissier* **2**: 698. 1894.

Distribution: eastern Europe, Asia
Habitat: moist areas

1.6.12.8 subsp. *mollis* (Rochel) Briq., *Nat. Pflanzenfam.* 4(3a): 322. 1896. Type: in Banatu, *Rochel* (Holotype, GJO?)

- *Mentha sylvestris* L. var. *mollis* Rochel, *Linnaea* **12**: 585. 1839.
- *Mentha longifolia* Huds. var. *mollis* (Rochel) Briq., *Bull. Herb. Boissier* **4**: 692. 1896.
- *Mentha longifolia* (L.) Huds. var. *mollis* (Rochel) Hayek, *Repert. Spec. Nov. Regni Veg*. **30**: 395. 1927.

Distribution: Romania, Yugoslavia
Habitat: unknown

1.6.12.9 subsp. *minutiflora* (Borbás) Briq., *Nat. Pflanzenfam.* 4(3a): 322. 1896. Type: prope Iraz in com. Bihar [Hungary], *Borbás* (Holotype, G)

- *Mentha minutiflora* Borbás, *Bot. Centralbl.* **25**: 239. 1886. Borbás in A. Kern., *Sched. Fl. Exs. Austro-Hung.* **5**: 55. 1888.
- *Mentha grisella* Briq. subsp. *minutiflora* (Borbás) Briq., *Bull. Soc. Bot. Genève* **5**: 95. 1889.
- *Mentha longifolia* Huds. var. *minutiflora* (Borbás) Briq., *Bull. Herb. Boissier* **4**: 688. 1896.

Distribution: Hungary, Macedonia, Crete
Habiatat: unknown

1.6.12.10 subsp. *typhoides* (Briq.) Harley, Notes Royal Botanical Garden Edinburgh 38: 38. 1980. Type: In Syria ad rivulos montis Masmenen-Dagh prope Cesaream, *Balansa* (Holotype, G-DEL)

- *Mentha kotschyana* Boiss. apud Kotschy, Iter. Cilicicum n. **307**. 1853.
- *Mentha sylvestris* L. var. *glabrata* Boiss., *Fl. Orient.* **4**: 544. 1879.
- *Mentha silvestris* L. subsp. *kotschyana* (Boiss.) Briq., *Bull. Soc. Bot. Genève* **5**: 87. 1889.
- *Mentha silvestris* L. subsp. *typhoides* Briq., *Bull. Soc. Bot. Genève* **5**: 90. 1889.
- *Mentha longifolia* Huds. var. *typhoides* (Briq.) Briq., *Bull. Herb. Boissier* **4**: 685. 1896.
- *Mentha longifolia* Huds. var. *kotschyana* (Boiss.) Briq., *Bull. Herb. Boissier* **4**: 688. 1896.
- *Mentha longifolia* Huds. subsp. *kotschyana* (Boiss.) Briq., *Nat. Pflanzenfam.* **4**(3a): 321. 1896.

Distribution: Aegean, N.W. Iran, N. Iraq, Turkey, Syria, Lebanon, Israel, Egypt
Habitat: marshy fields, by streams and rivers

1.6.12.11 subsp. *caucasica* Briq., *Nat. Pflanzenfam.* 4(3a): 322. 1896. Type: Caucasi Regio Inferior, *Brotherus* (Holotype, G)

Mentha grisella Briq. subsp. *caucasica* Briq., *Bull. Soc. Bot. Genève* **5**: 93. 1889.

Distribution: Caucasus
Habitat: unknown

1.6.12.12 subsp. *calliantha* (Stapf) Briq., *Nat. Pflanzenfam.* 4(3a): 321. 1986. Type: "in agro Ecbatanensi" Near Hamadan, Iran, *Pichler* s.n. (Holotype, WU!; Isotypes, K, G, WU!)

- *Mentha calliantha* Stapf, Akad. Wiss. Wien, *Math.-Naturwiss. Kl. Denkschr.* **1**: 36. 1885.
- *Mentha silvestris* L. subsp. *calliantha* Briq., *Bull. Soc. Bot. Genève* **5**: 86. 1889.
- *Mentha longifolia* Huds. var. *calliantha* (Stapf) Briq., *Bull. Herb. Boissier* **4**: 687. 1896.

Distribution: N.W. Iran, E. Anatolia
Habitat: marshy ground, streamsides

1.6.12.13 subsp. *noëana* (Boiss.) Briq., *Nat. Pflanzenfam.* 4(3a): 322. 1896. Type: Iraq Near Baghdad, *Noë* #1013 (Holotype, G)

- *Mentha noëana* Boiss., *Fl. Orient.* **4**: 543. 1879.
- *Mentha sylvestris* L. var. *petiolata* Boiss., *Fl. Orient.* **4**: 543. 1879.
- *Mentha royleana* Bent. subsp. *noëana* (Boiss.) Briq., *Bull. Soc. Bot. Genève* **5**: 80. 1889.

Distribution: W. Iran, Iraq, S.E. Anatolia
Habitat: marshy grounds, streamsides

1.6.12.14 subsp. *modesta* Briq., *Nat. Pflanzenfam.* 4(3a): 322. 1896. Type: Unknown (Holotype, G)

- *Mentha royleana* Benth. subsp. *modesta* Briq., *Bull. Soc. Bot. Genève* **5**: 79. 1889.
- *Mentha longifolia* Huds. subsp. *modesta* Trautmann & Urum., *Bot. Közlem.* **31**: 253. 1935.

Distribution: Asia minor, Iran, Tibet
Habitat: unknown

1.6.12.15 subsp. *royleana* (Benth.) Briq., *Nat. Pflanzenfam.* 4(3a): 322. 1896. Type: Unknown (Holotype, K–W?)

Mentha royleana (Wallich), Numer List n. 1537. 1829; Benth. in Wallich, *Pl. Asiat. Rar.* **1**: 30. 1829–1830.
Mentha sylvestris L. var. *royleana* (Wallich) J.D. Hook., *Fl. Brit. India* **4**: 647. 1885.

Distribution: Asia minor, Iran, Afghanistan, Turkestan, Sibiria, Tibet
Habitat: river plains

1.6.12.16 subsp. *hymalaiensis* Briq., *Nat. Pflanzenfam.* 4(3a): 322. 1896. Type: Unknown, *Aitchison* #620 (Holotype, G)

Mentha royleana Benth. subsp. *hymalaïensis* Briq., *Bull. Soc. Bot. Genève* **5**: 79. 1889.

Distribution: Himalayas, Afghanistan
Habitat: unknown

1.6.12.17 subsp. *syriaca* (Déseglise) Briq., *Nat. Pflanzenfam.* 4(3a): 322. 1896. Type: Liban: Saïda, au Bord du Risseau entre le Moulin de Mjédel et Aïa-el-Hennàye; Bord de l'Aqueduc du Moulin de Cheick-Ali, *Gaillardot* (Holotype, G)

- *Mentha syriaca* Déseglise, *Bull. Soc. Études Sci. Angers* **8–9**: 242. 1880.
- *Mentha grisella* Briq. subsp. *syriaca* (Déseglise) Briq., *Bull. Soc. Bot. Genève* **5**: 96. 1889.
- *Mentha longifolia* Huds. var. *syriaca* (Déseglise) Briq., *Bull. Herb. Boissier* **4**: 689. 1896

Distribution: Syria
Habitat: wadis

1.6.12.18 subsp. *pellita* (Déséglise) Briq., *Nat. Pflanzenfam.* 4(3a): 322. 1896. Type: Bois de Pins de Magdoùché S.S.E. Saïda [Syria], *Gaillardot* (Holotype, G)

- *Mentha pellita* Déséglise, *Bull. Soc. Études Sci. Angers* **8–9**: 245. 1880.
- *Mentha silvestris* L. subsp. *pellita* (Déséglise) Briq., *Bull. Soc. Bot. Genève* **5**: 92. 1889.
- *Mentha longifolia* Huds. var. *pellita* (Déséglise) Briq., *Bull. Herb. Boissier* **4**: 690. 1896.

Distribution: Syria, Ethiopia
Habitat: wadis

1.6.12.19 subsp. *schimperi* Briq., *Nat. Pflanzenfam.* 4(3a): 321. 1896. Type: "Vallis Sel" Yemen, *Schimper* #815 (Holotype, G-DEL)

- *Mentha silvestris* L. subsp. *schimperi* Briq., *Bull. Soc. Bot. Genève* **5**: 85. 1889.
- *Mentha longifolia* Huds. var. *schimperi* Briq., *Bull. Herb. Boissier* **4**: 686. 1896.

Distribution: Ethiopia, Sinai Peninsula, Yemen
Habitat: wadis

1.6.12.20 subsp. *capensis* (Thunb.) Briq., *Nat. Pflanzenfam.* 4(3a): 321. 1896. Type: Cape, Lions Head, South Africa (Holotype, UPS!), *balderjan, ballerja, balterja,* Wild Mint, *kruisement, koena*

- *Mentha capensis* Thunb., *Prodr. Fl. Cap*. **95**. 1800.
- *Mentha salicina* Burch. ex Benth., *Labiat. Gen. Spec*. **170**. 1833.
- *Mentha lavandulacea* Willd. var. *latifolia* Benth. in DC., *Prodr*. **12**: 165. 1848.
- *Mentha capensis* Thunb. subsp. *bouvieri* Briq., *Bull. Soc. Bot. Genève* **5**: 76. 1889.
- *Mentha longifolia* Huds. var. *obscuriceps* Briq., *Bull. Herb. Boissier* **2**: 695. 1894.
- *Mentha longifolia* Huds. var. *doratophylla* Briq., *Bull. Herb. Booissier* **2**: 695. 1894.
- *Mentha longifolia* Huds. subsp. *bouvieri* Briq., *Nat. Pflanzenfam*. **4**(3a): 321. 1896.
- *Mentha longifolia* Huds. var. *bouvieri* (Briq.) Briq., *Bull. Herb. Boissier* **4**: 687. 1896.
- *Mentha longifolia* Huds. var. *capensis* (Thunb.) Briq., *Bull. Herb. Boissier* **4**: 687. 1896.
- *Mentha longifolia* Huds. var. *salicina* (Burch.) Briq., *Nat. Pflanzenfam*. **4**(3a): 321. 1896.
- *Mentha longifolia* Huds. var. *cooperi* Cook, *Fl. Cap*. **5**(1): 304. 1910.

Distribution: South Africa, Namibia, Zimbabwe, Lesotho
Habitat: in watercourses and moist places

1.6.12.21 subsp. *polyadena* Briq., *Nat. Pflanzenfam.* 4(3a): 321. 1896. Type: Transvaal, South Africa, *Otto Lincke* (Holotype, G), *koena*

Mentha silvestris L. subsp. *polyadena* Briq., *Bull. Soc. Bot. Genève* **5**: 84. 1889.

Distribution: South Africa, Lesotho
Habitat: along watercourses, on river banks, and in moist places

1.6.12.22 subsp. *wissii* (Launert) Codd, *Bothalia* 2: 170. 1983. Type: Im Sumpfgebiet des Oberen Numastals 2100 m, Otjiseva, SW Africa [Otjiseva River, Omaruru District, Brandberg, Namibia], *Wiss* #1418 (Holotype, FR!; Isotypes, M, PRE)

Mentha wissii Launert, Mitt. Bot., *Staatssaml. München* **2**: 311. 1957.

Distribution: Namibia, South Africa
Habitat: in watercourses and moist places

1.6.13 M. *pulegium* L. *Sp. pl.* 577. 1753. Type: Magnol Specimen in Linnaean Herbarium 730.19 (Lectotype, LINN!), European Pennyroyal, $2n = 20$, (30, 40)

1.6.13.1 var. *pulegium*

- *Pulegium vulgare* Mill., *Gard. Dict*. ed. 8. n. 1. 1768.
- *Pulegium erectum* Mill., *Gard. Dict*. ed. 8. n. 2. 1768.

- *Mentha aromatica* Salisb., *Prod. Stirp. Chap. Allerton* **80**. 1796.
- *Pulegium aromaticum* S.F. *Gray*, *Nat. Arr. Brit. pl*. **2**: 367. 1821.
- *Pulegium pubescens* Opiz ex Boenn., *Prod. Fl. Monast. Westphal*. **174**. 1824.
- *Pulegium heterophyllum* Opiz ex Boenn., *Prod. Fl. Monast. Westphal*. **174**. 1824.
- *Pulegium tomentellum* Presl., *Fl. Sicul*. **36**. 1826.
- *Mentha daghestanica* Borissova, *Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk SSR* **16**: 285. 1954.
- *Pulegium daghestanicum* (Borissova) Holub, *Folia Geobot. Phytotax*. **12**(4): 429. 1977.

Distribution: southern, western, and central Europe, northwards to Ireland and central Poland and extending to western and southern Ukraine Habitat: wet places, roadsides, pond banks

1.6.13.2 var. *micrantha* (Fisch.) Benth. in DC., *Prod.* 12: 175. 1848. Type: River Don, Russia (Holotype, K!)

- *Mentha micrantha* Fisch. ex Benth. in DC., *Prodr*. **12**: 175. 1848.
- *Pulegium micranthum* Claus, Beitr., *Pflanzenk. Russ. Reiches*. **8**: 244. 1850.

Distribution: southeast Russia, W. Kazakhstan
Habitat: sinkholes in steppes

1.6.14 *M. repens* (J.D. Hook.) *Briq.* in Engl. & Prantl, *Nat. Pflanzenfam.* 4(3a): 319. 1896. Type: Woolnorth, Tasmania, Australia (Holotype, K!), $2n = ?$

- *Micromeria repens* J.D. Hook., *London J. Bot*. **6**: 274. 1847.
- *Mentha serpyllifolia* Benth. in DC., *Prodr*. **12**: 174. 1848.

Distribution: Tasmania, Australia
Habitat: moist soil

1. 6.15 *M. requienii* Benth., *Labiat. Gen. Spec.* 182. 1833. Type: Lac de Crena [Corsica], *Requien* s.n. (Holotype, K!), Corsican Mint, $2n = 18$

- *Thymus parviflorus* Req., *Ann. Sci. Nat. (Paris)* **5**: 386. 1825.
- *Thymus corsicus* Pers. in Moris, *Stirp. Sard. Elench. Fasc*. **1**: 37. 1827.
- *Audibertia pusilla* Benth., *Bot. Reg. sub.* t. 1282. 1829.
- *Menthella requienii* (Benth.) Pérard, *Bull. Soc. Bot. France* **17**: 205. 1870.
- *Thymus micranthus* Salzm. & Pouzolz. ex Pérard, *Bull. Soc. Bot. France* **17**: 206. 1870.
- *Audibertia parviflora* Nym., *Consp. Fl. Eur*. **597**. 1881.

Distribution: Tyrrhenian Isles (Corsica, Sardinia, Montecristo, Caprera), ±500 to 1500 m
Habitat: margins of still and running water, turf, and other damp places, often in shade

1.6.16 *M. satureioides* R.Br., *Prodr.* 505. 1810. Type: Port Jackson [Sydney, Parramatta, New South Wales, Australia], *R. Brown* #2350 (Holotype, BM! Isotypes, BM! K!), $2n =$ ca. 144

Micromeria satureioides (R.Br.) Benth., *Labiat. Gen. Spec*. **380**. 1834.

Distribution: throughout Australia except in extreme north
Habitat: usually loamy soils

1.6.17 *M. spicata* L., *Sp. pl.* 576. 1753. Type: Clifford Herbarium (Lectotype, BM!), Spearmint, $2n = 48$

1.6.17.1 subsp. *spicata*

- *Mentha spicata* L. var. *viridis* L., *Sp. pl.* **576**. 1753.
- *Mentha viridis* (L.) L., *Sp. pl.* ed. 2. **804**. 1763.
- *Mentha romana* Garsault, *Fig. pl. Méd.* t. 378. 1764.
- *Mentha glabra* Mill., *Gard. Dict.* ed. 8. n. 2. 1768.
- *Mentha candicans* Mill., *Gard. Dict.* ed. 8. n. 3. 1768.
- *Mentha rubra* Mill., *Gard. Dict.* ed. 8. n. 9. 1768 (*nomen confusum*).
- *Mentha sylvestris* L. var. *glabra* W. Koch, *Syn. Fl. Germ. Helv.* **550**. 1837.

Distribution: this species has been shown to be an ancient amphidiploid of *M. longifolia* × *M. suaveolens* by resynthesis (Harley and Brighton, 1977; Kokkini and Papageorgiou, 1982) and AFLP markers (Gobert et al., 2002). The rugose-leaved plant, commonly sold as "Kentucky Colonel" in the U.S. or *M. cordifolia* Auct., has been designated as a rugose *varietas* by Harley (1963), but this name has not been published (and until all infraspecific epithets are examined in this species, publication is probably best avoided).
Habitat: streambanks, lake shores, moist fields and roadsides, often cultivated

1.6.17.2 var. *crispata* (Schrad.) Schinz & Thellung, *Fl. Schweiz* ed. 3. 2: 292. 1914, Crisped Spearmint. Type: "Hortic. Goett." [Botanischer Garten, Göttingen, Germany] and "Hortic. Bot. Berol." (Holotype, B!)

- *Mentha crispata* Schrad. ex Willd., *Enum. pl.* **608**. 1809.
- *Mentha crispa* L., *Sp. pl.* **576**. 1753.
- *Mentha cordifolia* Opiz ex Fresen., *Syll. Pl. Nov.* **2**: 232. 1828.
- *Mentha viridis* L. var. *crispa* Benth., *Labiat. Gen. Spec.* **174**. 1833.
- *Mentha viridis* L. f. *crispata* (Schrad.) Wirtg., *Herb. Menth. Rhen.* **12**. 1855.
- *Mentha viridis* L. var. *cordifolia* (Opiz) Pérard, *Bull. Soc. Bot. France* **17**: 337. 1870.
- *Mentha viridis* L. var. *crispata* (Schard.) Pérard, *Bull. Soc. Bot. France* **17**: 337. 1870.
- *Mentha spicata* L. var. *cordifolia* (Opiz), Schinz & Thellung, *Fl. Schweiz* ed. 3. 2: 292. 1914.
- *Mentha* ×*cordifolia* (Opiz) Fraser, *Bot. Soc. Exch. Club Br. Isles* **8**: 214. 1927.

Distribution and habitat: associated with cultivation

1.6.17.3 subsp. *condensata* (Briq.) Greuter & Burdet, *Willdenowia* 14: 301. 1985. Type: Laconia, Greece (Holotype, G)

- *Mentha tomentosa* Dum. d'Urv. subsp. *condensata* Briq., *Bull. Soc. Bot Genève* **5**: 97. 1889.
- *Mentha microphylla* K. Koch, *Linnaea* **21**: 650. 1849.
- *Mentha sieberi* K. Koch, *Linnaea* **21**: 649. 1849.
- *Mentha sylvestris* L. var. *stenostachya* Boiss., *Fl. Orient.* **4**: 543. 1879.
- *Mentha tomentosa* Dum. d'Urv., *Mém. Soc. Linn. Paris* **1**: 323. 1822; *Enum. pl. Ponti-Eux.* **67**. 1822.
- *Mentha illyrica* Borbás & Heinr. Braun, *Verh. KK. Zool. -Bot. Ges. Wien* **40**: 390. 1890.

- *Mentha illyrica* (Borbás & Heinr. Braun) Trautmann in Jáv., *Magyar Fl.* **931**. 1925.
- *Mentha microphylla* K. Koch var. *illyrica* (Borbás & Heinr. Braun) Hayek, *Repert. Spec. Nov. Regni Veg.* **30**: 398. 1927.
- *Mentha sofiana* Trautmann, *Bot. Közlem.* **29**: 111, 118. 1932.
- *Mentha spicata* L. subsp. *tomentosa* (Briq.) Harley, Notes Royal Botanical Gardens, Edinburgh **38**: 38. 1980.

Distribution: S. Italy, Sicily, Balkan Peninsula, and Aegean region
Habitat: as for subsp. *spicata*

1.6.17.4 var. *undulata* (Willd.) Lebeau, *Soc. Éch. Pl. Vasc. Eur. Occid. Bassin Médit. III Bull.* 17: 68. 1979. Type: Horti Regii Botanici Berolinensis, *Willdenow* #10813 (Holotype, B-Willd.!)

- *Mentha undulata* Willd., *Enum. Pl.* **608**. 1809.
- *Mentha sylvestris* L. var. *undulata* W. Koch in Sturm, *Deutschl. Fl.* **14**(62): t. 4. 1833; W. Koch, *Syn. Fl. Germ. Helv.* **550**. 1837.
- *Mentha longifolia* Huds. var. *undulta* W. Koch ex Briq., *Lab. Alp. Mar.* **48**. 1891.
- *Mentha longifolia* (L.) Huds. var. *undulata* (Willd.) Fiori & Paol., *Fl. Italia* **3**: 74. 1903.

Distribution and habitat: associated with cultivation

1.6.18 *M. suaveolens* Ehrh., *Beitr. Naturk.* 7: 149. 1792. Type: H.G. [Hortus Göttingensis, Botanischer Garten, Göttingen, Germany], *Ehrhart* s.n. (Lectotype, GOET!), $2n = 24$

- *Mentha rotundifolia* Auct., non L.
- *Mentha macrostachya* Ten., *Fl. Napol.* **1**: xxxiii. 1815.
- *Mentha rotundifolia* (L.) Huds. var. *lachnaiochroa* Malinv. ex Briq., *Lab. Alp. Mar.* **28**. 1891.

1.6.18.1 subsp. *suaveolens*, Pineapple Mint

Distribution and habitat: the type is the cultivated variegated form (Harley, 1972); the wild green form (name uncertain) is found in western Europe from Denmark to eastern Europe and North Africa and the Canaries, along streambanks, lakeshores, moist fields, and roadsides.

1.6.18.2 subsp. *insularis* (Req.) Greuter, *Soc. Éch. Pl. Vasc. Eur. Occid. Bassin Médit. III Bull.* 14: 58. 1972. Type: Unknown (Type, K)

- *Mentha insularis* Req. ex Coss. & Godr., *Fl. France* **2**: 649. 1852.
- *Mentha rotundifolia* Huds. var. *insularis* (Briq.) Briq., *Bull. Herb. Boissier* **2**: 692. 1894.
- *Mentha rotundifolia* (L.) Huds. subsp. *insularis* (Req.) Arcang., *Comp. Fl. Ital.* ed. 2. 420. 1894.
- *Mentha insularis* Req. var. *reverchoni* (Briq.) Rouy, *Fl. France* **11**: 362. 1909.
- *Mentha suaveolens* Ehrh. var. *reverchonii* (Rouy) Greuter, *Soc. Éch. Pl. Vasc. Occid. Bassin Médit. III Bull.* **14**: 58. 1972.

Distribution: islands of the western Mediterranean above 305 m
Habitat: unknown

1.6.18.3 subsp. *timija* (Coss. ex Briq.) Harley ex Harley & Brighton, *Bot. J. Linn. Soc.* 74: 81. 1977. Type: Morocco (Lectotype, G!)

- *Mentha timija* Coss., *Bull. Soc. Bot. France* **22**: 65. 1876; Coss. ex Briq., *Bull. Herb. Boissier* **4**: 679. 1896.
- *Mentha suaveolens* Ehrh. subsp. *timija* (Briq.) Greuter & Burdet, *Willdenowia* **14**: 301. 1985.

Distribution: Morocco
Habitat: unknown

1.6.19 Hybrids of Mentha

Hybridization of species of section *Mentha* is common, but identification may be difficult. Schultz (1854) was probably the first to recognize the complex hybrid origins of many mint hybrids. Amateur taxonomists of the genus have treated sterility as the *sine qua non* criterion of hybrid origin, forgetting that sterility is under genetic control (as well as chromosomal imbalance), and that all the hybrids exhibit varying degrees of fertility that is environmentally influenced. Malinvaud (1901) was the first to enumerate the indications of hybridity, and his characters still bear repeating:

1 On observe, dans les *Eumenthae*, trois modes très distincts de l'inflorescence sur lesquels àété fondée la subdivision Linnéene en *Spicatae*, *Capitatae*, *Verticillatae*. Or, l'observation apprend et l'expérimentaiton confirme que dans les Menthes légitimes le caractère de l'inflorescence est invariable. Donc toute inflorescence mixte, c'est-à-dire offrant sur le même individu une combinaison ou un mélange des modes ci-dessus, sera un signe d'hybridité, lequel est assez fréquent avec trois inflorescences pour cinque espèces.
2 Les feuilles caulinaires sont subsessiles dans les *Spicatae*, nettement pétiolées dans *aquatica* et *arvensis*; l'interversion de ces caractères sera une autre marque d'hybridité.
3 La face interne du tube de corolle est constamment glabre dans les formes légitimes des trois *Spicatae* et plus ou moins velue dans *aquatica* et *arvensis*; toute exception à cette règle sera un criterium d'hybridité.
4 La base du calice est toujours plus ou moins velu ou pubescente dans les *Verticillatae* légitimes; une parfaite nudité en ce point, coincidant presque toujours avec la glabréité de la face interne de la corolle, provient d'un croisement entre les *Mentha arvensis* et *viridis*.
5 Quand on voit sur une *Verticillatae*, en dehors de toute état pathologique our tératologique, une abréviation des feuilles caulinaires moyennes, relativement aux supérieures aussi bien qu'aux inférieures, d'où résulte dans l'ensemble un aspect claviforme, on peut conclure à l'intervention d'une *Spicata*, principalement du *rotundifolia*.

Many of these hybrids have also been discussed by Harley (1975).

1.6.20 *Mentha* ×*carinthiaca* Host, *Fl. Austriac.* 2: 149. 1831. Type: in Carinthia in Humidis inter Villach et Bleyberg, (Holotype, W5186!)

- *Mentha wohlwerthiana* F.W. Schultz, Jahresber. *Pollichia* **12**: 29. 1854.
- *Mentha* ×*malinvaldi* E.G. Camus, *Bull. Herb. Boissier* **1**(1): 191. 1893.

Distribution: This is accepted as a hybrid of *M. arvensis* × *M. suaveolens*, supported by resynthesis (Harley, 1975). Morphologically, this resembles *M. arvensis*. Two types exist at W; the one chosen here most closely matches the description, while W5189 is most likely *M.* ×*gracilis*.

1.6.21 *M.* ×*dalmatica* Tausch, *Syll. Pl. Nov.* 2: 249. 1828. Type: "Dalmatia," *Tausch* (Type Probably PRC in Old, Unsorted Material), $2n = 48$

- *Mentha haynaldiana* Borbás, *Oesterr. Bot. Z.* **30**: 19. 1880; *Oesterr. Bot. Z.* **43**: 60. 1893.
- *Mentha iraziana* Borbás ex Heinr. Braun, *Verh. KK. Zool. -Bot. Ges. Wien* **40**: 485. 1890.

Distribution: this is accepted as a hybrid of *M. arvensis* × *M. longifolia*. Morphologically, this is similar to *M. arvensis* and also has been confused with pubescent collections of *M.* ×*gracilis* (Tucker and Fairbrothers, 1990; Tucker et al., 1991). Until Tausch's type is located, the name of this nothospecies must remain uncertain.

1.6.22 *M.* ×*dumetorum* Schultes, *Observ. Bot.* 108. 1809. Type: "Galicia," *Schultes* (Holotype, M? BM?), $2n = 60, 72, 84, 96$

- *Mentha pubescens* Auct., ? an Willd., *Enum. pl.* **608**. 1809.
- *Mentha hirta* Willd., *Enum. pl.* **608**. 1809.
- *Mentha nepetoides* Lej., *Rev. Fl. Spa.* **116**. 1824.
- *Mentha ayassei* Malinv., *Bull. Soc. Bot. France* **24**: 234. 1877.

Distribution: this is a hybrid of *M. aquatica* × *M. longifolia*, supported by resynthesis (Murray and Lincoln, 1972) and RAPD analysis (Khanuja et al., 2000). This has been confused with pubescent collections of *M.* ×*piperita*.

1.6.23 *M.* ×*gracilis* Sole, *Menth. Brit.* 37. t. 16. 1798. Type: Side of a Brook Near Bradford, Wilts, England, *Sole* s.n. (Typotype, BM! Syntypes, BRIST! LINN!), Scotch Spearmint, $2n = 54, 60, 61, 72, 84, 96, 108, 120$

- *Mentha gentilis* Auct., non L.
- *Mentha pratensis* Sole, *Menth. Brit.* **39**. t. 17. 1798.
- *Mentha rivalis* Sole, *Menth. Brit.* **45**. t. 20. 1798.
- *M. mülleriana F.W. Schultz*, *Flora* **37**: 543. 1854.
- *Mentha cardiaca* Gerarde ex Baker, *J. Bot.* **3**: 245. t. 34. 1875.

Distribution: this is a hybrid of M. *arvensis* × *M. spicata*, supported by resynthesis (Tucker and Fairbrothers, 1990; Tucker et al., 1991; Khanuja et al., 2000), often cultivated. The inflorescence varies from subverticillate to subspicate, the former confused with collections of *M. arvensis* subsp. *parietariaefolia* (e.g., *M. gentilis* L.).

1.6.24 *M.* ×*maximilianea* F.W. Schultz, *Flora* 37: 472. 1854; Jahresber. Pollichia 12: 34. 1854. Type: In locis incultis, ad rivulos, fossas, inque dumetis regionis dollinae diluvii vallis Rheni ad pedes montium, in vicinitate viae serratae Maximilianeae dictae prope Weissenburg Copiosissime, *F.W. Schultz* #115 (Holotype, B! Isotypes, B! K!), $2n = 72, 84, 108$

- *Mentha suavis* Guss., *Pl. Rar.* **387**. t. 66. 1826, non *Mentha suavis* Hoffm. ex Sm. in *Rees*, *Cycl.* **23**: sub. n. 6. 1812.

- *Mentha schultzii* Boutigny ex F.W. Schultz, *Arch. Fl. France Allem*. **2**: 283. 1854.
- *Mentha rodriguezii* Malinv. in Marès, *Cat. pl. Vasc. Baléares* **335**. 1880.

Distribution: This is accepted as a hybrid of *M. aquatica* × *M. suaveolens*. Morphologically, this is intermediate between both parental species and bears a subspicate inflorescence.

1.6.25 *M.* ×*piperita* L. *Sp. pl.* 576. 1753. Type: Sherard Herbarium, *Sherard* (Typotype, OXF!), Peppermint, $2n = 72, 84, 108$

1.6.25.1 var. *piperita*, Black and Hairy Peppermints

- *Mentha nigricans* Mill., *Gard. Dict*. ed. 8. n. 12. 1768.
- *Mentha rubra* Mill., *Gard. Dict*. ed. 8. n. 9. 1768 (*nomen confusum*).
- *Mentha odora* Salisb., *Prod. Stirp. Chap. Allerton* **80**. 1796.
- *Mentha odorata* Sole, *Menth. Brit*. **21**. t. 9. 1798.
- *Mentha hircina* Hull, *Brit. Fl*. **1**: 127. 1799.
- *Mentha pyramidalis* Ten., *Fl. Napol*. **1**: 34. 1815.
- *Mentha eriantha* K. Koch, *Linnaea* **21**: 650. 1849.
- *Mentha* × *reverchonii* Briq., *Bull. Soc. Bot. Genève* **5**: 676. 1889.

Distribution: this species is a hybrid of *M. aquatica* × *M. spicata*, supported by resynthesis (Murray et al., 1972), AFLP markers (Gobert et al., 2002), and flavonoid data (Voirin et al., 1999), widely cultivated. The leading economic cultivar of var. *piperita*, "Mitcham," can be recreated by crossing a high menthone clone of *M. spicata* with a high menthofuran clone of *M. aquatica* that also has the genes for ketone reductase, resulting in high menthol in the F_1 progeny, a compound not present beyond trace levels in either parent (Murray et al., 1972). This variety typically bears a capitate inflorescence. Pubescent forms may reflect the genetics of either parent but regularly appear spontaneously in vegetatively propagated commercial "Mitcham" peppermint fields as a mutation of one to two genes, so the designation of f. *hirsuta* (Fraser) Graham (*Watsonia* **2**: 32. 1951) is probably the best designation, rather than nothosubsp. *pyramidalis* (Ten.) Harley (*Kew Bull*. **37**: 604. 1983). The hybrids of *M. aquatica* var. *citrata* × *M. spicata* would fall within this variety on the basis of morphology (e.g., *Mentha odorata* Sole), but might deserve recognition at the level of *forma* or cultivar.

1.6.25.2 var. *crispa* (Benth.) W. Koch, *Syn. Fl. Germ. Helv.* 551. 1837. Type: Linnaean Herbarium 730.7 (Lectotype, LINN!), Curly Mint

- *Mentha aquatica* L. var. *crispa* (L.) Benth., *Labiat. Gen. Spec*. **177**. 1833.
- *Mentha aquatica* L. var. *crispa* G. Mey., *Chloris Han*. **291**. 1836.
- *Mentha piperita* L. f. *crispa* (L.) Wirtg., *Herb. Menth. Rhen*. **12**. 1855.
- *Mentha aquatica* L. var. *crispa* (L.) Heinr. Braun ex Top., *Beih. Bot. Centralb*. **30**(2): 171. 1913.

Distribution: associated with cultivation

1.6.25.3 var. *officinalis* Sole, *Menth. Brit.* 15. t. 17. 1798. Type: Swampy Place on Lansdown Called the Wells, it Being the First Spring-Head of Locks-Brook, England, *Sole* s.n. (Lectotype, BRIST! Syntypes, LINN! BM!), White Peppermint

Mentha kahirina Forssk., *Fl. Aegypt-Arab*. **213**. 1775.
Mentha piperita L. var. *officinalis* W. Koch, *Syn. Fl. Germ. Helv*. **551**. 1837.

Distribution: as for var. *piperita*. This bears a subspicate inflorescence. Hybrids that are high in linalool/linalyl acetate (e.g., "Lavanduliodora") belong here.

1.6.26 *M.* ×*rotundifolia* (L.) Huds., *Fl. Angl.* 221. 1762. Type: Burser Herbarium (Lectotype, UPS!)

- *Mentha spicata* L. var. *rotundifolia* L., *Sp. pl.* **576**. 1753, $2n = 24$.
- *Mentha niliaca* Juss. ex Jacq., *Hort. Bot. Vindob.* **3**: 46. t. 87. 1777.
- *Mentha amaurophylla* Timb.-Lagr., *Bull. Soc. Bot. France* **7**: 257. 1860.
- *Mentha nouletiana* Timb.-Lagr., *Bull. Soc. Bot. France* **7**: 259. 1860.
- *Mentha* ×*timbali* Rouy, *Fl. France* **11**: 372. 1909.
- *Mentha villosa* Auct., non Huds.

Distribution: This is accepted as a hybrid of *M. longifolia* × *M. suaveolens* (Harley, 1972, 1975).

1.6.27 *M.* ×*smithiana* R. Graham, *Watsonia* 1: 89. 1949. Type: under a Wet Hedge in the Road from Watton to Saham Church, Norfolk, England, Smith (Holotype, LINN! Isotypes K!), Red Mint, Sometimes Cultivated, $2n = 54$, 98, 108, 120

Mentha rubra Sm., *Trans. Linn. Soc. Lond.* **5**: 205. 1800, non *Mentha rubra* Mill., *Gard. Dict.* ed. 8. n. 9. 1768.

Distribution: this is accepted as a hybrid of *M. aquatica* × *M. arvensis* × *M. spicata*.

1.6.28 *M.* ×*verticillata* L., *Syst. Nat.* ed. 10. 1099. 1759. Type: Linnaean Specimen in Linnaean Herbarium 730.13 (Lectotype, LINN!), $2n = 42$, 54, 78, 84, 90, 96, 120, 132

- *Mentha sativa* L., *Sp. pl.* ed. 2. 805. 1763.
- *Mentha dentata* Moench, *Methodus* **380**. 1794.
- *Mentha agrestis* Sole, *Menth. Brit.* **33**. t. 14. 1798.
- *Mentha paludosa* Sole, *Menth. Brit.* **49**. t. 22. 1798.
- *Mentha acutifolia* Sm., *Trans. Linn. Soc. Lond.* **5**: 203. 1800.
- *Mentha abruptifolia* Borbás ex Heinr. Braun, *Verh. KK. Zool. -Bot. Ges. Wien* **40**: 440. 1890.
- *Mentha pluriglobula* Borbás, *Term. Füz.* **16**: 52. 1893.
- *Mentha verticillata* L. var. *pluriglobula* (Borbás) Hayek, Repert. *Spec. Nov. Regni Veg.* **30**: 387. 1927.
- *Mentha verticillata* L. f. *pluriglobula* (Borbás) Janković in Josifović Fl. SR Srbjie **6**: 519. 1974.
- *Mentha bulgarica* Borbás, *Magyar Bot. Lapok* **4**: 53. 1905.

Distribution: This is accepted as a hybrid of *M. aquatica* × *M. arvensis*. The inflorescence varies from subverticillate to subcapitate.

1.6.29 *M.* ×*villosa* Huds., *Fl. Angl.* ed. 2. 250. 1778. Type: Hudson Herbarium (Lectotype K!), $2n = 36$

1.6.29.1 var. *villosa*

- *Mentha niliaca* Auct., non Willd., *Sp. pl.* **3**: 76. 1801.
- *Mentha gratissima* G.H. Weber in Wigg., *Prim. Fl. Holsat.* **43**. 1780.
- *Mentha nemorosa* Willd., *Sp. pl.* **3**: 75. 1801.
- *Mentha lamyi* Malinv., *Bull. Soc. Bot. France* **27**: 335. 1880; *Bull. Soc. Bot. France* **28**: 76. 1881.
- *Mentha mosoniensis* Heinr. Braun, *Verh. KK. Zool. -Bot. Ges. Wien* **40**: 373. 1890.
- *Mentha scotica* R. Graham, *Watsonia* **4**: 119. 1958.
- *Mentha digenea* Briq. ex Petrak in Rech. fil., *Fl. Aegaea* **543**. 1943.

Distribution: This species is accepted as a hybrid of *M. spicata* × *M. suaveolens*, supported by resynthesis (Harley, 1972, 1975).

1.6.29.2 var. *alopecuroides* (Hull) Briq., *Bull. Herb. Boissier* 4: 679. 1896. Type: Reperitur in aquosis in Cantio et Essexia, England, *Sole* s.n. (Neotype, Here Designated, LINN! Syntypes BRIST! BM!), Apple Mint Bowles Mint, Egyptian Mint

- *Mentha alopecuroides* Hull, *Brit. Fl.* **1**: 126. 1799.
- *Mentha rotundifolia* Sole, *Menth Brit.* **9**. t. 4. 1798.
- *Mentha velutina* Lej., *Rev. Fl. Spa.* **115**. 1824.
- *Mentha sylvestris* L. var. *alopecuroides* Baker, *J. Bot.* **3**: 238. 1865.
- *Mentha* ×*niliaca* Jacq. var. *alopecuroides* (Hull) Fraser, *Bot. Soc. Exch. Club Brit. Isles* **8**: 220. 1927.
- *Mentha* ×*villosa* Huds. nm. *alopecuroides* (Hull) Harley, *J. Linn. Soc., Bot.* **65**: 253. 1972.
- *Mentha rotundifolia* (L.) Huds. subsp. *alopecuroides* (Hull) Malagarriga Heras, *Acta Phytotax. Barcinon.* **18**: 9. 1977.

Distribution: as for var. *villosa*, possibly a backcross (Harley, 1975), often cultivated

1.6.29.3 var. *nicholsoniana* (Strail) Harley, *Watsonia* 18: 212. 1990. Type: unknown (Type unknown)

- *Mentha nicholsoniana* Strail, *Bot. Soc. Exch. Club Brit. Isles* **1**: 186. 1908.
- *Mentha* ×*niliaca* Jacq. var. *nicholsoniana* (Strail) Fraser, *Bot. Soc. Exch. Club Brit. Isles* **8**: 217. 1927.

Distribution: as for var. *villosa* (Harley, 1975)

1.6.30 *M.* ×*villoso-nervata* Opiz, Nomencl. Bot. 1: 60. 1831. Type: Unknown (Holotype, PR? PRC? PRM?), "American" Spearmint, $2n = 36$

Distribution: This is accepted as a hybrid of *M. longifolia* × *M. spicata*, often cultivated. Simple selfing of many selections of *M. spicata* with $2n = 48$ will also produce plants with $2n = 36$; these two origins cannot be satisfactorially distinguished, so this nothospecific name

is the best alternative. This hybrid usually appears almost morphologically indistinguishable from *M. spicata*, but some forms are recognizable (Harley, 1975).

EXCLUDED SPECIES

1.6.31 *M. cunninghamii* (Benth.) Benth. In DC., *Prod.* 12: 174. 1848. Type: Richard Cunningham, 1833, "On Wet Ground at the Village of Mangamuka on the Hokianga River" (Holotype, K!) $2n = 72$

Micromeria cunninghamii Benth., *Labiat. Gen. Spec.* **730**. 1835.

Distribution: New Zealand (North Island, South Island, Stewart Island, Chatham Island)
Habitat: lowland to higher montane grassland and rather open places

Acknowledgments

Analytical research was supported by the State of Delaware and the Cooperative State Research, Education, and Extension Service (#801–15–01C). Thanks are extended to Drs. David E. Fairbrothers and Raymond M. Harley for critical reading of the preliminary manuscript.

APPENDIX 1.1

Data Matrix Used in Maximum Parsimony and Neighbor-Joining Analyses

MIBR	00002	10100	00011	01001	0000?	?0
MEAQ	22002	10102	0221A	21031	10002	31
MEAR	21011	10100	00210	31001	10002	20
MEAU	1B120	10100	0121A	11010	11002	20
MECA	21111	10100	01210	31001	10002	30
MECE	11220	10000	11211	11140	10003	00
MECU	01000	00A00	00011	31001	10002	2?
MEDA	2B11A	10102	01211	21A01	11012	2?
MEDI	11110	00100	01A11	11010	10002	40
MEGA	12221	10000	01201	10041	10002	10
MEGR	00011	10100	00111	11020	1000?	?0
MEJA	21110	00102	01110	21001	10004	00
MELA	1?101	10100	01211	21020	1100?	??
MELO	2B101	10A01	02210	31011	10002	00
MEPU	10010	00100	01201	10030	11101	A0
MERP	11010	00100	01A11	C1011	1000?	??
MERQ	00010	00100	00101	11031	10000	00
MESA	12120	00100	00111	21021	1100?	?0
MESP	2B101	1A001	02210	31011	10002	10
MESU	2B001	11AA1	02210	31011	10002	00

A = 0&1, B = 1&2, C = 2&3, missing data = ?
Taxon key. MIBR = *Micromeria brownei* var. *pilosiuscula*, MEAQ = *Mentha aquatica*, MEAR = *M. arvensis*, MEAU = *M. australis*, MECE = *M. cervina*, MEGA = *M. gattefossei*, MEDI = *M. diemenica*, MEGR = *M. grandiflora*, MERQ = *M. requienii*, MEPU = *M. pulegium*, MERP = *M. repens*, MELA = *M. laxiflora*, MESA = *M. sautureioides*, MEDA = *M. dahurica*, MEJA = *M. japonica*, MECA = *M. canadensis*, MELO = *M. longifolia*, MESP = *M. spicata*, MESU = *M. suaveolens*, MECU = *M. cunninghamii*.

REFERENCES

Allan, H.H. (1982). *Flora of New Zealand. Volume I. Indigenous Tracheophyta*. P.D. Hasselberg, Wellington.

Arnold, M.L. (1997). *Natural Hybridization and Evolution*, Oxford University Press, New York.

Ashlock, P.D. (1984). Monophyly: its meaning and importance. In: *Cladistics: Perspectives on the Reconstruction of Evolutionary History*, Duncan, T. and Stuessy, T.F., Eds., Columbia University Press, New York, pp. 39–46.

Beck, C.B., Ed. (1976). *Origin and Early Evolution of Angiosperms*. Columbia University Press, New York.

Bentham, G. (1835). *Micromeria*. In: *Labiatarum Genera et Species*, Bentham, G., James Ridgway and Sons, London, pp. 380–381.

Bentham, G. (1848). Mentha. In: *Prodromus Systematis Naturalis Regni Vegetabilis*, DeCandolle, A., Ed., Victoris Masson, Paris, Vol. 12, pp. 164–177, 698.

Borissova, A. (1954). Labiatae novae. *Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk SSSR*, 16, 274–285.

Bouman, F. and Meeuse, A.D.J. (1992). Dispersal in Labiatae. In: *Advances in Labiate Science*, Harley, R.M. and Reynolds, T., Eds., Royal Botanic Gardens, Kew, pp. 193–202.

Braun, H. (1886). Ueber einige Arten und Formen der Gattung *Mentha*, mit besonderer Berüksichtigung der in Oesterreich-Ungarn wachsenden Formen, *Verh. KK. Zool. -Bot. Ges. Wien*. 40, 351–508, pl. 7, 8.

Briquet, J. (1889). Fragmenta monographiae Labiatarum. Fascicule 1er. Révision systématique des groupes spécifiques et subspécifiques dans le sous-genre *Menthastrum* du genre *Mentha*. *Bull. Soc. Bot. Genève*, 5, 20–107.

Briquet, J. (1896). *Preslia*, *Mentha*. In: *Die Natürlichen Pflanzenfamilien*, Engler, A. and Prantl, K., Eds., Wilhelm Engelmann, Leipzig, Vols. 3a and 4, pp. 317–324.

Britton, N.L. and Brown, A. (1913). *Mentha*. In: *An Illustrated Flora of the Northern United States and Canada*, Britton, N.L. and Brown, A., Charles Scribner's Sons, New York, Vol. 3, pp. 148–153.

Bunsawat, J., Elliott, N.E., Hertweck, K.L., Sproles, E. and Alice, L.A. (2004). Phylogenetics of *Mentha* (Lamiaceae): evidence from chloroplast DNA sequences, *Syst. Bot*., 29, 959–964.

Cantino, P.D. and Wagstaff, S.J. (1998). A reexamination of North American *Satureja s.l.* (Lamiaceae) in light of molecular evidence, *Brittonia*, 50, 63–70.

Cantino, P.D., Harley, R.M. and Wagstaff, S.J. (1992). Genera of Labiatae: status and classification. In: *Advances in Labiate Science*. Harley, R.M. and Reynolds, T., Eds., Royal Botanic Gardens, Kew, pp. 511–522.

Cockerell, T.D.A. (1926). The antiquity of the Labiatae or mint family, *Nature*, 118, 696.

Crisp, M.D., West, J.G. and Linder, H.P. (1999). Biogeography of the terrestrial flora. In: *Flora of Australia*, 2nd ed., ABRS/CSIRO, Australia. Vol. 1, pp. 321–367.

Cronquist, A. (1987). A botanical critique of cladism, *Bot. Rev*., 53, 1–52.

Doroszenko, A.M. (1987). Taxonomic studies on the *Satureja* complex. Ph.D. Thesis, Edinburgh University.

Fraser, J. (1927). *Menthae* Britannicae. *Bot. Soc. Exch. Club Brit. Isles*, 8, 213–247.

Fraser, J. (1929). Notes on *Mentha*. *Bot. Soc. Exch. Club Brit. Isles*, 8, 809–810.

Friis, E.M., Chaloner, W.G. and Crane, P.R. (1987). *The Origins of Angiosperms and Their Biological Consequences*, Cambridge University Press, Cambridge.

Funk, V. (1981). Special concerns in estimating plant phylogenies. In: *Advances in Cladistics: Proceedings of the First Meeting of the Willi Hennig Society*, Funk, V.A. and Brooks, D.R., Eds., New York Botanical Garden, Bronx, pp. 73–86.

Galen, C. (1999). Why do flowers vary? *Bioscience*, 49, 631–640.

Gandoger, M. (1889). *Mentha*. In: *Flora Europae terrarumque adjacentium*, Gandoger, M., F. Sary Biblioth, Paris, pp. 151–168.

Gobert, V., Moja, S., Colson, M. and Taberlet, P. (2002). Hybridization in the section *Mentha* (Lamiaceae) inferred from AFLP markers, *Am. J. Bot*., 89, 2017–2023.

Godley, E.J. (1979). Flower biology in New Zealand, *New Zealand J. Bot*., 17, 441–466.

Graham, A. (2003). Geohistory models and Cenozoic paleoenvironments of the Caribbean region, *Syst. Bot*., 28, 378–386.

Greuter, W. (2000). *International Code of Botanical Nomenclature (Saint Louis Code)*. Koeltz Scientific Books, Königstein.

Harley, R.M. (1963). Taxonomic studies in the genus *Mentha*. Ph.D. Thesis, University of Oxford, England.

Harley, R.M. (1972). Notes on the genus *Mentha* (Labiatae). In: *Flora Europaea*. *Notulae systmaticae ad floram Europaeam spectantes*, Heywood, V.H., Ed., No. 12; *J. Linn. Soc*., *Bot*., 65, 223–269.

Harley, R.M. (1975). 445. *Mentha* L. In: *Hybridization the Flora of the British Isles*, Stace, C.A., Ed., Academic Press, London, pp. 383–390.

Harley, R.M. and Brighton, C.A. (1977). Chromosome numbers in the genus *Mentha* L, *Bot. J. Linn. Soc*., 74, 71–96.

Harley, R.M. and Paucar, A.G. (2000). List of species of tropical American *Clinopodium* (Labiatae) with new combinations, *Kew Bull*., 55, 917–927.

Hedge, I.C. (1992). A global survey of the biogeography of the Labiatae. In: *Advances in Labiate Science*, Harley, R.M. and Reynolds, T., Eds., Royal Botanic Gardens, Kew, pp. 7–17.

Hill, R.S. (1994). *History of the Australian Vegetation: Cretaceous to Recent*, Cambridge University Press, Cambridge, MA.

Hill, R.S., Truswell, E.M., McLoughlin, S. and Dettmann, M.E. (1999). Evolution of the Australian flora: fossil evidence. In: *Flora of Australia*., 2nd ed., ABRS/CSIRO, Australia. 1, pp. 251–320.

Ikeda, N. (1961). Thremmatological investigation of the genus *Mentha* by means of cytogenetics, *Sci. Rep. Fac. Agric., Okayama University*, 36, 1–264.

Ikeda, N. and Ono, S. (1969). Studies on *Mentha aquatica* L. and its genome analysis I. (Genome analysis in the genus *Mentha*, Part 6), *Jpn. J. Breed*., 19, 302–309.

Ikeda, N., Shimizu, S., Karasawa, D., Origasa, T. and Ono, S. (1970). *Mentha arvensis* L. var. *piperascens* Mal. which grows wild in the north-eastern part of Japan II. General characters and cytogenetical analysis. *Sci. Rep. Fac. Agric*., *Okayama University*, 36, 1–11.

Jackson, B.D. (1928). *A Glossary of Botanic Terms*. Unwin Brothers, Woking.

Jamzad, Z. (1987). *Eremostachys llanata* and *Mentha mozaffarianii*, two new Labiatae from Iran, *Iran, J. Bot*., 3, 111–116.

Kaufmann, M. and Wink, M. (1994). Molecular systematics of the Nepetoideae (Family Labiatae): phylogenetic implications from rbcL gene sequences, *Z. Naturforsch*., 49c, 635–645.

Keng, H. (1977). Labiatae. In: *Flora Malesiana*, van Steenis, C.G.G.J., Ed., Sijthoff & Noorhoff, Alphen aan der Rijn, The Netherlands, Vol. 8(2), pp. 301–394.

Khanuja, S.P.S., Shasany, A.K., Srivastava, A. and Kumar, S. (2000). Assessement of genetic relationships in *Mentha* species, *Euphytica*, 111, 121–125.

Kokkini, S. and Papageorgiou, V.P. (1982). Morphological, cytological and chemical investigations of *Mentha spicata* L. in Greece. In: *Aromatic Plants: Basic and Applied Aspects*, Margaris, N., Koedam, A. and Vokou, D., Eds., Martinus Nijhoff Publication, The Hague, pp. 131–140.

Laing, R.M. and Blackwell, E.W. (1964). *Plants of New Zealand*. 7th ed., Whitcombe & Tombs, Christchurch.

Li, X.-W. and Hedge, I.C. (1994). Lamiaceae. In: *Flora of China. Verbenaceae through Solanaceae*, Wu, Z.-I. and Raven, P.H., Eds., Missouri Botanical Garden, St. Louis, Vol. 17, pp. 50–299.

Lloyd, D.G. and Barrett, S.C.H., Eds., (1996). *Floral Biology: Studies on Floral Evolution in Animal-Pollinated Plants*, Chapman & Hall, New York.

Malinvaud, E. (1901). Classification des espèces et hybrides du genre *Mentha, Compt. -Rend. Congr. Soc. Savantes Paris Dep. Sec. Sci*., 1900, 174–176.

Malinvaud, E. (1903). Classification des espèces et hybrides due genre *Mentha*. De l'application du principe de la "subordination des caractères" à l'étude des groupes critiques, particulariè-rement dans le genre *Mentha, Compt. -Rend. Congr. Soc. Savantes Paris Dep. Sec. Sci*., 1903, 132–135.

McDade, L. (1990). Hybrids and phylogenetic systematics I. Patterns of character expression in hybrids and their expression for cladistic analysis, *Evolution*, 44, 1685–1700.

McDade, L. (1992). Hybrids and phylogenetic systematics II. The impact of hybrids on cladistic anlaysis, *Evolution*, 46, 1329–1346.

McDade, L. (1997). Hybrids and phylogenetic systematics III. Comparision with distance methods, *Syst. Bot*., 22, 669–683.

McDade, L. (2000). Hybridization and phylogenetics: special insights from morphology. In: *Phylogenetic Analysis of Morphological Data*, Wiens, J.J., Ed., Smithsonian Inst, Press, Washington, D.C., pp. 146–164.

Médail, F. and Verlaque, R. (1997). Ecological characteristics and rarity of endemic plants from southeast France and Corsica: Implications for biodiversity conservation, *Biol. Conserv*., 80, 269–281.

Metcalf, E.H. (1936). Studies in the genus *Mentha*. Ph.D. Thesis, University of Reading, Reading, England.

Mildenhall, D.C. (1980). New Zealand and Late Cretaceous and Cenozoic plant biogeography: a contribution, *Palaeogr*., *Palaeoclimatol*., *Palaeoecol*., 31, 197–233.

Miller, P. (1768). *The Gardeners Dictionary*, 8th ed., Miller, P., London.

Múlgura, M.E. (1999). Verbenaceae. In: *Catálogo de las plantas vasculares de la República Argentina II*, Missouri Botanical Garden, Press, St. Louis, pp. 1136–1170.

Murray, M.J. and Lincoln, D.E. (1970). The genetic basis of acyclic oil constituents in *Mentha citrata* Ehrh, *Genetics*, 65, 457–471.

Murray, M.J. and Lincoln, D.E. (1972). Oil composition of *Mentha aquatica–M. longifolia* F_1 hybrids and *M. dumetorum, Euphytica*, 21, 337–343.

Murray, M.J., Lincoln, D. and Marble, P.M. (1972). Oil composition of *Mentha aquatica* × *M. spicata* F_1 hybrids in relation to the origin of *M.* ×*piperita*, *Can. J. Genet. Cytol*., 14, 13–29.

Paton, A.J., Springate, D., Suddee, S., Otieno, D., Grayer, R.J., Harley, M.M., Willis, F., Simmonds, M.S.J., Powell, M.P. and Savolainen, V. (2005). Phylogeny and evolution of basil and allies (Ocimeae, Labiatae) based on three plastid DNA regions, *Mol. Phylogen. Evol*., 31, 277–299.

Pérard, A. (1876). Classification du genre *Mentha*, *Bull. Soc. Émul. Allier*, 1876, 1–59.

Qian, H. (2002). A comparison of the taxonomic richness of temperate plants in East Asia and North America, *Am. J. Bot*., 89, 1818–1825.

Prather, L.A., Wiese, A.K., Posto, A.L. and Williams, R.A. (2002). Monophyly and phylogeny of *Monarda* (Lamiaceae): evidence from the Internal Transcribed Spacer (ITS) region of nuclear ribosomal DNA, *Syst. Bot*., 27, 127–137.

Prodan, I. (1925). *Menthae* novae Romaniae et Iugoslaviae, *Bull. Inform. Grăd. Bot. Univ. Cluj*, 5, 103–123.

Raven, P.H. and Axelrod, D.I. (1974). Angiosperm biogeography and past continental movements, *Ann. Missouri Bot. Gard*., 61, 539–673.

Rogers, G. and Walker, S. (2002). Taxonomic and ecological profiles of rarity in the New Zealand vascular flora, *New Zeal. J. Bot*., 40, 73–93.

Schultz, F. (1854). Untersuchungen über die Arten, Abarten und Bastarden der Gattung *Mentha*, mit besonderer Rücksicht auf die in der Pflaz vorkommenden Formen, *Jahresber. Pollichia*, 12, 27–44.

Sennen. (1927). Nos découvertes en Cardagne, *Bull. Soc. Bot. Fr*., 73, 641–680.

Shinners, L.H. (1962). Micromeria brownei and its allies (Labiatae), Sida, 1, 94–97.

Swofford, D.L. (2001). *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4b8*. Sinauer Associates, Sunderland, Massachusetts.

Thorne, R.F. (1972). Major disjunctions in the geographic ranges of seed plants, *Quart. Rev. Biol*., 47, 365–411.

Tobler, W.R., Mielke, H.W. and Detwyler, T.R. (1970). Geobotanical distance between New Zealand and neighboring islands, *BioScience*, 20, 537–541.

Topitz, A. (1911). Ueber einige neue Formen der Gattung *Mentha*, *Deutsche Bot. Monatsschr*., 22, 42–44.

Topitz, A. (1913). Beiträge zur Kenntnis der Menthenflora von Mitteleuropa, *Beih. Bot. Centralbl*., 30, 138–264.

Topitz, A. (1914a). Diagnoses formarum novarum generic *Menthae* praecipue ex auctoris scripto: Beiträge zur Kenntnis der Menthenflora von Mitteleuropa, *Repert. Spec. Nov. Regni Veg.*, 14, 17–42 (Latin version of German in *Beih. Bot. Centralbl.*, 30, 143–179).

Topitz, A. (1914b). Diagnoses formarum novarum generic *Menthae* praecipue ex auctoris scripto: Beiträge zur Kenntnis der Menthenflora von Mitteleuropa, *Repert. Spec. Nov. Regni Veg.*, 14, 49–75 (Latin version of German in *Beih. Bot. Centralbl.*, 30, 179–243).

Topitz, A. (1915). Diagnoses formarum novarum generic *Menthae* praecipue ex auctoris scripto: Beiträge zur Kenntnis der Menthenflora von Mitteleuropa. *Repert. Spec. Nov. Regni Veg.*, 14, 81–96 (much modified Latin version of German in *Beih. Bot. Centralbl.*, 30, 243–255).

Topitz, A. (1916). Ungarische Minzen. Magyar menták, *Magyar Bot. Lapok*, 15, 125–168.

Trautman, R. (1925). *Mentha*. In: *Különlenyomat dr. Jávorka Sándor: Magyar Flóra (Flora Hungarica)*, Trautman, R., Vol. 3, pp. 905–970.

Truswell, E.M., Kershaw, A.P. and Sluiter, I.R. (1987). The Australian-south-east Asian connection: evidence from the palaeobotanical record. In: *Biogeographical evolution of the Malay Archipelago*, Whitmore, T.C., Ed., Oxford University Press, Oxford, pp. 32–49.

Tucker, A.O. and Chambers, H.L. (2002). *Mentha canadensis* L. (Lamiaceae): a relict amphidiploid from the Lower Tertiary, *Taxon*, 51, 703–718.

Tucker A.O. and Fairbrothers, D.E. (1981). A euploid series in an *F*1 interspecific hybrid progeny of *Mentha* (Lamiaceae), *Bull. Torrey Bot. Club.*, 108, 51–53.

Tucker, A.O. and Fairbrothers, D.E. (1990). The origin of *Mentha* × *gracilis* (Lamiaceae). I. Chromosome numbers, fertility, and three morphological characters, *Econ. Bot.*, 44, 183–213.

Tucker, A.O., Harley, R.M. and Fairbrothers, D.E. (1980). The Linnaean types of *Mentha* (Lamiaceae), *Taxon.*, 29, 233–255.

Tucker, A.O., Hendriks, H., Bos, R. and Fairbrothers, D.E. (1991). The origin of *Mentha* × *gracilis* (Lamiaceae). II. Essential oils, *Econ. Bot.*, 45, 200–215.

Tyagi, B.R. (2003). Cytomixis in pollen mother cells of spearmint (*Mentha spicata* L.), *Cytologia*, 68, 67–73.

Valverde, R.M. (1993). Sinopsis y distribución del género *Micromeria* Bentham, *Bot. Complut.*, 18, 157–168.

Verlaque, R., Aboucaya, A., Cardona, M.A. and Contrandriopoulos, J. (1991). Quelques exemples de spéciation insulaire en Méditerranée occidentale, *Bot. Chron.*, 10, 137–154.

Verlaque, R., Contandriopoulos, J. and Aboucaya, A. (1995). Cytotaxonomie et conservation de la flore insulaire: les especes endémiques ou rares de Corse, *Ecol. Med.*, 21, 257–268.

Verlaque, R., Médail, F., Quézel, P. and Babino, J.-F. (1997). Endémisme végétale et paléogeographie dans le Bassin Méditerranéen, *Giobios*, 21, 159–166.

Voirin, B., Bayet, C., Faure, O. and Jullien, F. (1999). Free flavonoid aglycones as markers of parentage in *Mentha aquatica*, *M. citrata*, *M. spicata* and *M.* × *piperita*, *Phytochemistry*, 50, 1189–1193.

Wagner, W.H. (1983). Reticulistics: the recognition of hybrids and their role in cladistics and classification. In: *Advances in cladistics*, Vol. 2: proceedings of the second meeting of the Willi Hennig Society, Platnick, N.I., and Funk, V.A., Eds., Columbia University Press, New York, pp. 63–79.

Wagstaff, S.J., Olmstead, R.G. and Cantino, P.G. (1995). Parsimony analysis of the cpDNA restriction site variation in subfamily Nepetoideae (Labiatae), *Am. J. Bot.*, 82, 886–892.

Wagstaff, S.J., Martinsson, K. and Swenson, U. (2000). Divergence estimates of *Tetrachondra hamiltonii* and *T. patagonica* (Tetrachondraceae) and their implications for austral biogeography. *New Zealand J. Bot.*, 38, 587–596.

Wanntorp, H.-E. (1983). Reticulated cladograms and the identification of hybrid taxa. In: *Advances in cladistics*, Vol. 2: Proceedings of the second meeting of the Willi Hennig Society, Platnick, N.I. and Funk, V.A., Eds., Columbia University Press, New York, pp. 81–88.

White, M.E. (1990). *The Flowering of Gondwana.*, Princeton University Press, Princeton.

Wiens, J.J., Ed., (2000). *Phylogenetic Analysis of Morphological Data*, Smithsonian Inst. Press, Washington, D.C.

Wilson, P. and Thomson, J.D. (1996). How do flowers diverge? In: *Floral Biology: Studies on Floral Evolution in Animal-Pollinated Plants*, Lloyd, D.G. and Barrett, S.C.H., Eds., Chapman & Hall, New York, pp. 88–111.

Windley, B.F. (1977). *The Evolving Continents*. John Wiley & Sons, New York.

Xifreda, C.X. (1999). Lamiaceae. In: *Catálogo de las plantas vasculares de la República Argentina II*. Missouri Botanical Garden Press, St. Louis, pp. 768–781.

2 Anatomy, Physiology, Biosynthesis, Molecular Biology, Tissue Culture, and Biotechnology of Mint Essential Oil Production

Massimo Maffei, Cinzia M. Bertea, and Marco Mucciarelli

CONTENTS

2.1 ANATOMY

Essential oils are lipophilic substances produced by specialized secreting tissues called glandular trichomes. According to Fahn (1979, 1988a, 1988b), glandular trichomes secreting essential oils are the base for the economic importance of several plant families, including the Lamiaceae. In peppermint, the site of terpene biosynthesis has been localized to the secretory cells of the glandular trichomes (Gershenzon et al., 1989; McCaskill et al., 1992), mainly located on the leaf and stem surfaces (Amelunxen, 1965; Fahn, 1979). Two types of glandular trichomes occur on peppermint: small, capitate glandular trichomes, with a single secretory head cell; and peltate glandular trichomes, with an eight-celled apical cluster of secretory cells, subtended by a stalk and a basal cell, the latter embedded between the ordinary epidermal cells (Fahn, 1979; Maffei et al., 1989, Werker, 1993). Peltate trichomes are enveloped by a large cuticular sheath, which accumulates the essential oil in the underlying storage space (Figure 2.1). Capitate glandular trichomes have only limited storage capacity, and their secretion, which is extruded to the outside through a porous cuticle, appears to consist mainly of a complex mixture of carbohydrates, lipids, and proteins (Werker et al., 1985; Ascensão and Pais, 1998). Whatever the exact nature of the capitate gland secretory products, it is clear that the bulk of the monoterpenes of peppermint essential oil is produced by and stored in the peltate glandular trichomes (Turner et al., 2000a).

Maffei et al. (1986, 1989), using scanning electron microscopy to estimate gland numbers and densities on developing leaves of peppermint and its cv. lavanduliodora, found that young leaves contained fewer glandular trichomes than older leaves, indicating an evident gland production during leaf growth. In the case of peppermint, considerable variation in monoterpene content between individual trichomes was observed, although the trend toward production of menthol and related isomers did correlate with leaf size and age (Maffei et al., 1989). The variation in oil content was taken as evidence that glands of different ages occur in close proximity. More recently, Turner et al. (2000a) have shown that new glands are continually produced during leaf growth and that newly initiated glands do occur together with mature glands in growing regions, such that neighboring glands within the same leaf zone are often of different ages. Turner and colleagues (2000a) have also examined the pattern

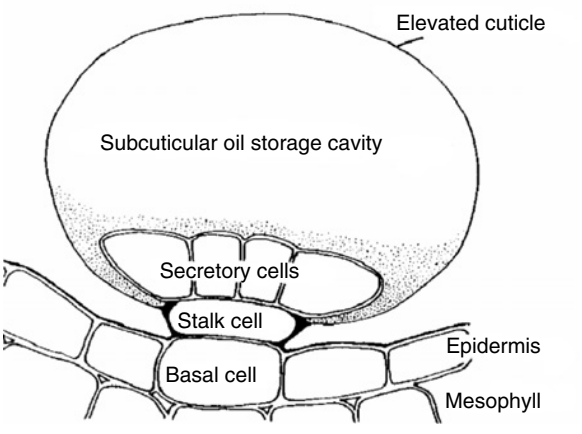


FIGURE 2.1 Schematic diagram of a peppermint leaf peltate glandular trichome illustrating the placement of these epidermal structures and the relationship of the disk of secretory cells to the stalk and basal cells and to the subcuticular storage space. (From Turner et al., *Plant Physiol.*, 120, 879–886, 1999. With permission.)

of peltate glandular trichome initiation and ontogeny on developing peppermint leaves by studying the populations of peltate glands in each of seven developmental stages within sampling areas of leaf apical, mid-, and basal zones for both lower and upper epidermises (Figure 2.2). The results confirm previous results (Maffei et al., 1989; Shanker et al., 1999) that new peltate glands continue to form until leaf expansion ceases and that regions of active gland initiation are unevenly distributed. The distribution of gland initiation reflects the basipetal pattern of leaf maturation, with relatively immature regions at the leaf base continuing to produce oil glands long after gland production has stopped at the leaf apex. The proportion of glands in the secretory stage as a function of leaf development and the direct observations of living glands over a period of 33 h indicate that a period of only 20 to 30 h of secretory activity is required for filling of the gland storage compartment with essential oil (Turner et al., 2000a).

From an ultrastructural point of view, peltate glands arise as epidermal protuberances (initials) that divide asymmetrically to produce a vacuolate basal cell, a stalk cell, and a cytoplasmically dense apical cell. Further divisions of the apical cell produce a peltate trichome with one basal cell, one stalk cell, and eight glandular (secretory) disk cells (Figure 2.3 through Figure 2.6) (Turner et al., 2000b). The secretory phase coincides with the separation and filling of the subcuticular oil storage space (Figure 2.5), the maturation of glandular cell leucoplasts in which monoterpene biosynthesis is known to be initiated, and the formation of extensive smooth endoplasmic reticulum at which hydroxylation steps of the monoterpene biosynthetic pathway occur (Figure 2.5 and Figure 2.6). In the three-celled

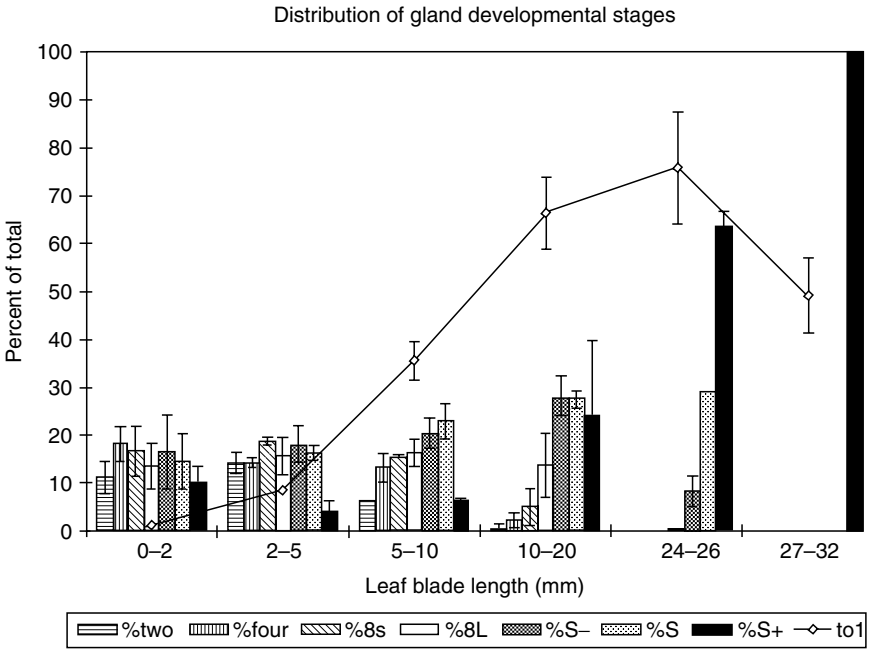


FIGURE 2.2 Distribution of peltate trichome developmental stages among leaves of various ages. Bars indicate the percentage of the total gland number represented by each development stage (left axis). The line graph indicates the total number of peltate glands (right axis) for each leaf size class. Peltate gland stages: 2 two-celled apical discs; 4 four-celled 8s, eight-celled, small; 8L, eight-celled, large; S2, early state; S, middle secretary stage; S1, postsecretory stage. Error bars represent Ses. (From Turner et al., *Plant Physiol.*, 124, 655–663, 2000a. With permission.)

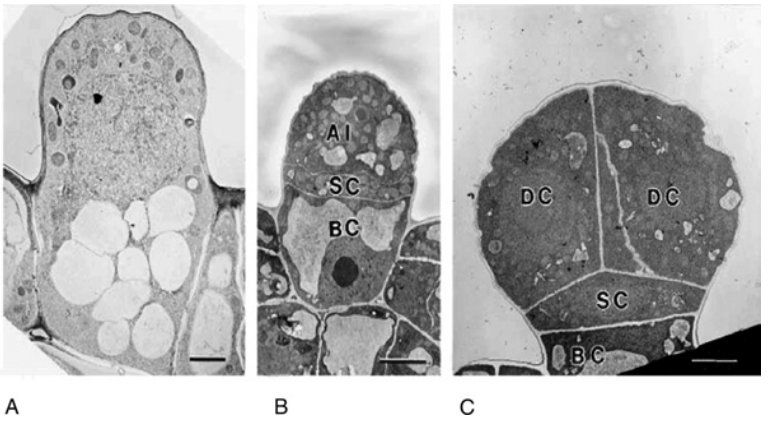


FIGURE 2.3 Early presecretrory state in peppermint glandular trichomes. A, Cryofixed glandular trichome initial consisting of a single cell with a vacuolate basal region and an apical region containing the nucleus, numerous mitochondria, and plastics, but few vacuoles, bar 5.2 μm. B, Chemically fixed glandular trichome initial after periclinal cell divisions, with a vacuolate basal cell (BC), narrow stalk cell (SC), and the apical disk initial cell (AI), bar 5.5 μm. (From Turner et al., *Plant Physiol.*, 124, 665–679, 2000b. With permission.)

glandular hairs where the subcuticular space is very narrow, an extraplasmatic space develops between the cytoplasm and the cell wall (Amelunxen, 1964).

Circumstantial evidence based on ultrastructural correlation, specific labeling, and subcellular fractionation studies indicates that at least the early steps of monoterpene biosynthesis occur in plastids. (4 S)-Limonene synthase, which is responsible for the first dedicated step of monoterpene biosynthesis in mint species, appears to be translated as a preprotein bearing a long plastidial transit peptide. Turner and colleagues (1999), by means of immunogold labeling using polyclonal antibodies raised to the native enzyme, have demonstrated the specific localization of limonene synthase to the leucoplasts of peppermint secretory cells of peltate glandular trichomes during the period of essential oil production (Figure 2.7). Labeling was shown to be absent from all other plastid types examined, including the basal

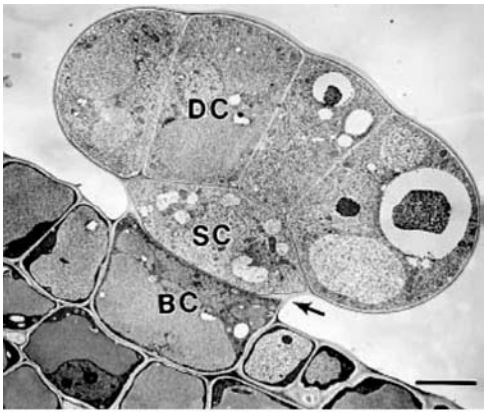


FIGURE 2.4 Transverse section through a chemically fixed presecretory peltate gland with an eight-celled apical disc. BC, Basel cell; SC, stalk cell; DC, disk cell. Arrow indicates a thickening stalk cell lateral wall, bar 5.5 μm. (From Turner et al., *Plant Physiol.*, 124, 665–679, 2000b. With permission.)

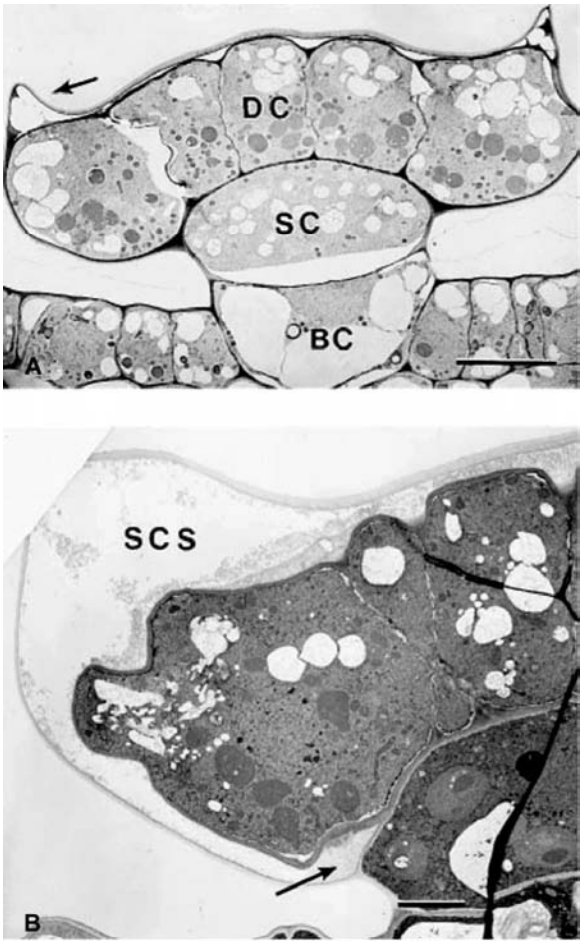


FIGURE 2.5 Secretory stage. A, Cryofixed early-secretory stage peltate gland. DC, Apical disk cell; SC, stalk cell; BC, basal cell. Arrow indicates the lateral rim of raised cuticle, bar 5.10 μm. B, Chemically fixed secretory stage peltate gland. Arrow indicates the lowermost extension of the subcuticular oil storage (SCS) at the juncture of the stalk and disk cells, bar 5.5 μm. (From Turner et al., *Plant Physiol.*, 124, 665–679, 2000b. With permission.)

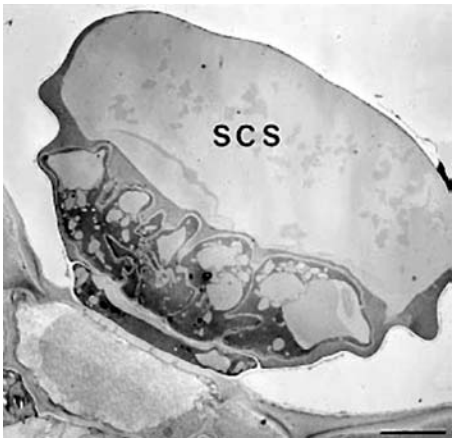


FIGURE 2.6 Postsecretory stage. A, Chemically fixed postsecretory stage peltate gland showing a large SCS containing lipid above the fibrillar material coating the glandular disk cells, bar 5.10 μm. (From Turner et al., *Plant Physiol.*, 124, 665–679, 2000b. With permission.)

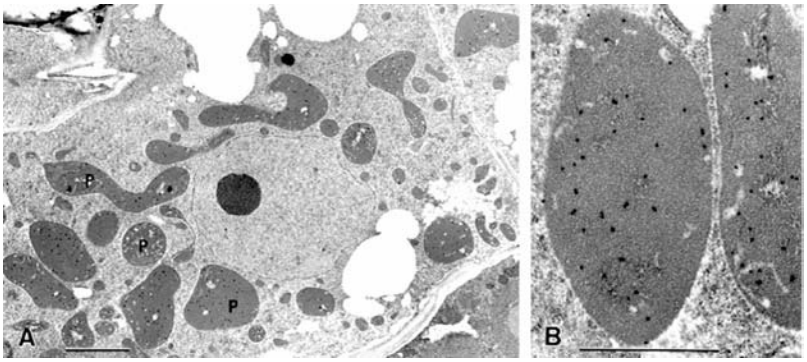


FIGURE 2.7 A, Immunogold labeling against limonene synthase. Colloidal gold particles strongly label leucoplasts (P) of a secretory stage glandular cap cell, whereas cytoplasm and other organelles are essentially unlabeled, bar 5.2 mm. B, High magnification of a leucoplast from a secretory stage glandular cap cell showing immunolabeling against limonene synthase, bar 1.5 μm. (From Turner et al., *Plant Physiol*., 120, 879–886, 1999. With permission.)

and stalk cell plastids, confirming that the leucoplastidome of the oil gland secretory cells is the exclusive location of limonene synthase. However, succeeding steps of monoterpene metabolism in mint appear to occur outside the leucoplasts of oil gland cells.

Morphometry, microfluorimetry, and electron microscopy were also used by Maffei and coworkers (Berta et al., 1993) to investigate the size, ploidy level, and morphology of the nucleus of the individual cells forming the peltate and capitate glandular trichomes of peppermint. Nuclear hypertrophy was observed in the secretory cells of both kinds of trichomes. This was explained by polyploidization and by variations in chromatin structure, which is related to increased translational activity. Endopolyploidy often occurs in metabolically active cells, probably concomitant with DNA amplification, whereas chromatin organization represents a general control of gene expression (Baluska, 1990).

It is clear from this short review that the role of glandular trichomes in secreting essential oils is the base for the economic exploitation of plants, such as those belonging to the genus *Mentha*, producing essential oils. According to McCaskill and Croteau (1999b), the goal of bioengineering for the development and metabolism of glandular structures can be focused on increasing product yield (qualitatively and quantitatively). Alternatively, particular commercial crops can be used to introduce the production of novel compounds to improve the resistance to herbivory or to provide a renewable source of specific chemicals (McCaskill and Croteau, 1999b). Furthermore, glandular structures may be engineered to produce biopharmaceuticals relevant to clinical medicine, in what is now called medical molecular farming (Daniell et al., 2001).

2.2 PHYSIOLOGY

The economic importance of mint oils has prompted a series of investigations into the physiology of the plants. These are focused on many aspects of the mint physiology: from nutrition to photosynthetic responses, from water stress to the effect of light irradiation, and from genotypic variation to phenotypic plasticity. Whereas some mint species have a narrow array of economically important compounds (i.e., spearmint, pennyroyal), others, such as peppermint, produce tens of compounds that can affect the quality of the produced oil. The latter case makes studies on mint physiology of paramount importance.

2.2.1 Effects on Essential Oil Production

For growers, who find striking differences in harvest yields and oil composition with changes in weather conditions, the interaction with the environment is one of the first problems that needs to be solved. Many authors have described the physiological and morphological responses of some *Mentha* species to changes in environmental conditions (Burbott and Loomis, 1967; Clark and Menary, 1980; White et al., 1987; Maffei, 1988, 1990; Maffei and Codignola, 1990; Sacco et al., 1992; Maffei et al., 1999; Maffei and Scannerini, 1999, 2000; Farooqi et al., 1999).

Leaf and flower oil terpene composition and several plant morphological characteristics of 17 *Mentha* ×*verticillata* hybrids were analyzed by Maffei (1990) during two growing seasons. The data obtained were used to study the phenotypic plasticity, the genotypic variation, and the genetic variation for phenotypic plasticity (discussed later). All plants showed high levels of phenotypic plasticity for both oil chemical and morphometrical parameters. High degrees of genotypic variation were found among the plants for oil components while a higher phenotypic plasticity was observed for morphological parameters. Temperatures and rainfall data were collected during the growing seasons and correlated to the data obtained from plant oil and morphology. Low levels of phenotypic plasticity and high degrees of genotypic variation were found to form outliers in the population of *Mentha* ×*verticillata* hybrids. The results obtained confirmed a significant effect of environmental conditions on the physiology and morphology of the genus *Mentha*.

To look for the possible influence of photosynthesis and photorespiration on terpene metabolism in peppermint, Maffei and Codignola (1990) examined the volatile oil composition in the peltate glandular trichomes in relation to: 1,5-ribulose bisphosphate carboxylase (rubisco) and glycolate oxidase (GO) activities; total protein and chlorophyll content and chlorophyll (Chl) a/b ratio; the chloroplast ultrastructure, some chloroplast morphometrical parameters, and the photorespiratory organelle complex (mitochondrion–microbody–chloroplast) ultrastructure; and the effect of 3-(3,4(dichlorophenyl)-1,-1-dimethylurea (DCMU) and 1,1′- dimethyl-4,4′ bipyridinium dichloride (paraquat) on the oil composition tested on peppermint leaf disks. The percentage of monoterpene alcohols and esters increased, along with rubisco and GO activities, with leaf age, whereas the percentage of monoterpene ketones and the Chl a/b ratio decreased. The decline in the Chl a/b ratio and the increased number of thylakoids per granum in the chloroplasts suggested a relative increase in photosystem II (PSII) activity (compared with PSI) and thus an increase in $NADPH_2$ levels with leaf development. Photosynthetic electron transport inhibition made by DCMU and paraquat on peppermint leaf disks caused a decline in the content of monoterpene alcohols, suggesting that the photosynthetic $NADPH_2$ production may be at least partially responsible for the progressive transformation of monoterpene ketones to alcohols (Figure 2.8). Increased rates of carbon availability were also correlated with oil synthesis before the full leaf expansion.

Paraquat was also found to affect the carvone biosynthesis and induced leaking of monoterpenes from the oil-secreting structures in a strain of *Mentha* ×*verticillata* (Maffei et al., 1993a). The addition of $NADPH_2$ to the paraquat treatment increased both limonene and total monoterpene content by probably supplying the loss of endogenous $NADPH_2$ oxidized by paraquat dications and potentially used for monoterpene biosynthesis. Exogenous superoxide dismutase (SOD) increased the total monoterpene content by partly scavenging the toxic effect of paraquat-induced superoxide activity on carvone biosynthesis (Table 2.1).

The apparent loss of essential oil during the maturation of several oil crops has been observed for a long time. During the 1980s, the accumulated evidence was overwhelming that oil components stored in glandular structures could undergo metabolic turnover (Croteau, 1986). Since terpenoids are produced as chemical defenses and the metabolic cost

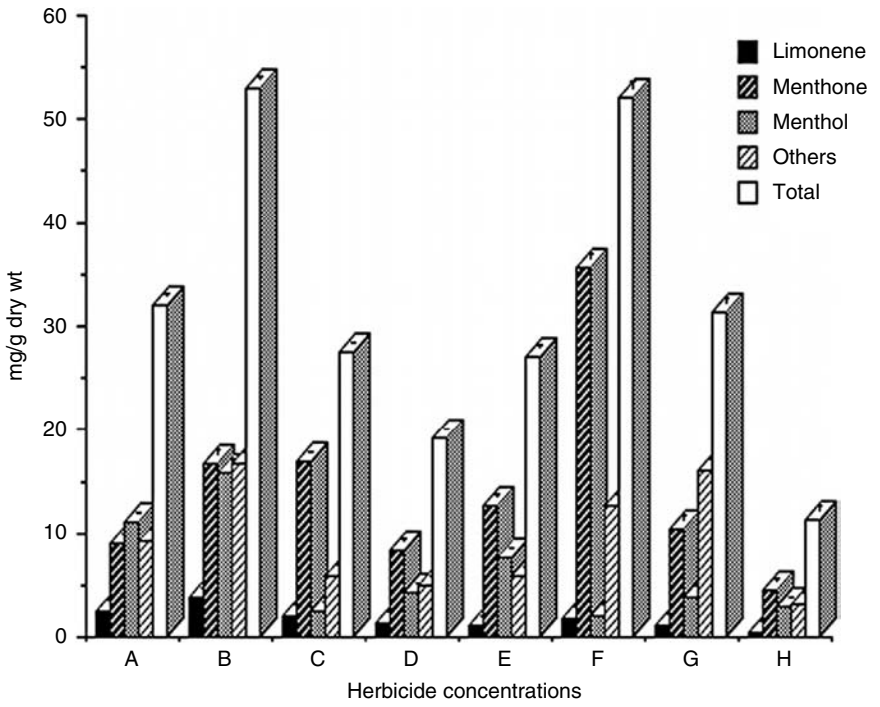


FIGURE 2.8 Effect of paraquat, DCMU, and paraquat + DCMU on oil composition in peppermint (*Mentha piperita*) leaf discs from 760-mm-long leaves. A = control; B = paraquat $1\times10^{-7}M$; C = paraquat $1\times10^{-6}M$; D = paraquat $1\times10^{-5}M$; E + DCMU $1\times10^{-7}M$; F = DCMU $1\times10^{-6}M$; G = DCMU $1\times10^{-5}M$; H = paraquat $1\times10^{-5}M$ + DCMU $1\times10^{-6}M$. Standard deviations are indicated for each mean value. (From Maffei and Codignola, *Biochem. Syst. Ecol.* 18, 493–502, 1990. With permission.)

of defense is envisioned as the amounts of carbon, energy, and other resources used in the biosynthesis and storage of defense compounds (Chew and Rodman, 1979), it is reasonable to deduce that cost increases as a function of the rate of metabolic turnover (Fagerstrom, 1989).

TABLE 2.1
Total Oil Production (μg/g f wt) from Leaf Disk Distillations, Incubation Medium, and Flask Head Space Extracts. (± = SE)

Specifications	(−)-Carvone	(−)-*trans*–Carveol	(−)-Limonene	Total
Paraquat $1\cdot10^{-3}M$	954 ± 67	54 ± 4.1	24 ± 2	1032 ± 72
Paraquat $1\cdot10^{-6}M$	1083 ± 61	9 ± 0.5	360 ± 18	1452 ± 87
Control	1263 ± 56	60 ± 2.1	351 ± 11	1674 ± 64
Paraquat $1\cdot10^{-3}M$ + $NADPH_2$ + DTT	804 ± 59	32 ± 0.1	506 ± 36	1312 ± 94
Paraquat $1\cdot10^{-6}M$ + $NADPH_2$ + DTT	930 ± 56	14 ± 0.2	432 ± 18	1366 ± 79
Control + $NADPH_2$ + DTT	936 ± 32	4 ± 0.1	135 ± 7	1075 ± 35
Paraquat $1\cdot10^{-3}M$ + SOD	1052 ± 76	4 ± 0.1	287 ± 23	1353 ± 98
Paraquat $1\cdot10^{-6}M$ + SOD	649 ± 26	12 ± 0.5	188 ± 12	849 ± 39
Control + SOD	765 ± 28	10 ± 0.4	222 ± 8	997 ± 35

Source: Maffei et al., 1993a.

Mihaliak and coworkers (1991) demonstrated that the rapid monoterpene turnover previously observed using detached stems does not occur in intact plants. Moreover, when $^{14}CO_2$ pulse labeling experiments were carried out with intact plants of four taxonomically distant oil-producing species no significant mono-, sesqui-, or diterpene turnover was observed (Gershenzon et al., 1993). According to Gershenzon and coworkers (1993), at present there is no reliable evidence for the turnover of any class of secondary metabolites.

2.2.1.1 Nutrition and Essential Oil

Recent concern with nitrate contamination to groundwater has encouraged the study of crop yield response to N fertilization. The optimum N rate for peppermint oil yield ranges from 150 to 85 kg/A, depending on weather and soil conditions (Mitchell and Farris, 1996). Nitrogen fertilization results in highly significant effects on dry matter and oil yields. The optimal fertilizer rate can be calculated accordingly, by fitting models to the data provided by Colwell (1994). The broken lines model describes yield, Y, as a function of N applied according to

$$Y = b_0 + b_1(\mathrm{N} - b_2 - |\mathrm{N} - b_2|) \tag{2.1}$$

where b_0 is the maximum yield, b_1 is the slope of the line, and b_2 is the optimum fertilizer rate (Mitchell and Farris, 1996). Usually, differences in environmental conditions between following years has a minor effect on the optimum fertilizer rates. Mitchell and Farris (1996) reported that similarities in yield responses occurred during two contrasting weather scenarios that were wetter and hotter than the 30-year mean. The same authors concluded that the rate of 125 kg/A was adequate for yearly spring N fertilization of peppermint.

2.2.1.2 Phenotypic Plasticity and Multivariate Analyses of Oil Components

Plasticity is shown by a genotype when the expression of its individual characteristics is changed by environmental influences. Since all changes in the character of an organism that are not genetic are environmental, plasticity is applicable to all intragenotypic variability (Bradshaw, 1965). The changing terpene composition from season to season is clearly a plastic phenomenon, which has been thoroughly studied in many genera including the genus *Mentha* (Maffei, 1988, 1990; Sacco et al., 1992). As noted earlier, phenotypic plasticity, genotypic variability, and genotypic variation for phenotypic plasticity have been studied in the genus *Mentha* in relation to essential oil composition. The simplest way to calculate plastic responses is by means of statistics (Marengo et al., 1991; Maffei et al., 1993b). Figure 2.9 shows the mean squares obtained from the two-way ANOVA calculated on the main oil components from some *M. piperita* "lavanduliodora" (as *Mentha lavanduliodora*) during two growing seasons (Sacco et al., 1992).

High levels of genotypic variation were found for linalool, whereas high degrees of phenotypic plasticity were shown by linalyl acetate, 1,8-cineole/limonene ratio (R_2), and geraniol. The greatest variation among genotypes in response to the environment ($P \times V$ interaction) was shown by R_2, linalool and linalyl acetate. Very low values were always observed for linalool/linalyl acetate ratio (R_1) and limonene, 1,8-cineole, β-caryophyllene, and α-terpineol. The analysis of oil composition data by means of multivariate methods requires more complex calculations, which can be made easily by using several statistic software packages available for most of the personal computers (Maffei et al., 1993b). Principal component analysis (PCA) provides significant insights into the structure of any numerical data matrix. By generating new orthogonal variables (the PCs), it concentrates the maximum possible amount of variance in as few PCs as possible. These new variables can

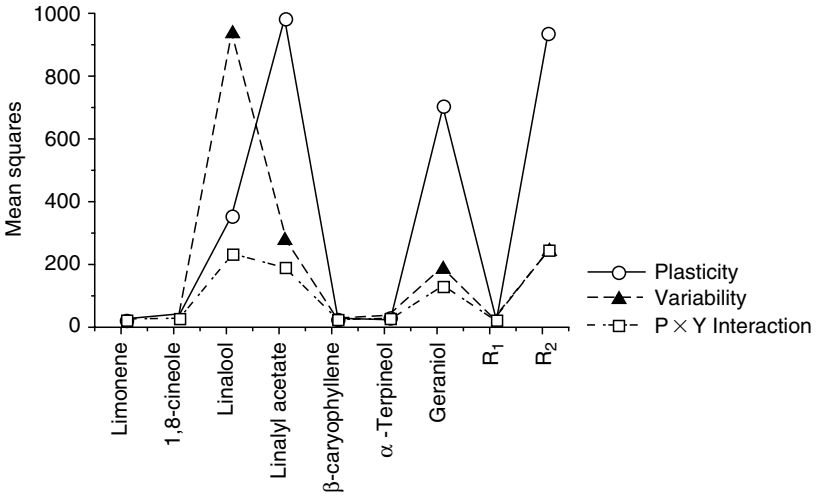


FIGURE 2.9 Plot of the mean squares (MS) values versus oil component of the *Mentha viridis lavanduliodora* F_1 hybrids. MS from the 2 years represent the plasticity, MS from the F_1 hybrids represent the genotypic variation. (From Sacco et al., *J. Essent. Oil Res.*, 4, 491–496, 1992. With permission.)

then be used instead of the original ones. The component loadings are the coefficients of the original variables, which define each PC, while the projections of the experimental points on the new variables are called "scores" (Marengo et al., 1991). These methods of analysis have also been applied to partition plants of the same species (i.e., *Mentha piperita*), but from different geographic areas (Figure 2.10), or plants of different species growing in the same place (Figure 2.11). In all cases, there is always a clear indication about the location of a given plant in the discriminant space of the analysis, which offers further help for the recognition and evaluation of the oil composition data (Maffei et al., 1993b).

2.2.1.3 Effect of Light

Light has been demonstrated to be able to alter oil quality and yield. In a study performed in Lucknow (India), three major cultivated *Mentha* species (*M. canadensis* [as *M. arvensis*], *M. aquatica* var. *citrata* [as *M. citrata*], and *M. gracilis* [as *M. cardiaca*]) were grown under short days, normal days, or long days for 60 cycles (Farooqi et al., 1999). The species grew better under long-day conditions. The long-day treatment resulted in flowering in *M. aquatica* var. *citrata*, which normally does not flower under Indian light conditions. The oil concentration and biogenesis were maximal in short-day plants. The photoperiodic treatment also affected the oil composition as demonstrated in a series of papers (Ruminska and Neweglowska, 1965; Burbott and Loomis, 1967; Clark and Menary, 1980; White et al., 1987; Maffei et al., 1989; Farooqi et al., 1999). Besides photoperiod, light quality has been demonstrated to affect mint oil composition. The response of peppermint essential oil to blue light + white light (W) was a 40% reduction in the total content, caused by a general decrease in *p*-menthanes, with particular reference to menthol. The combined effect of blue light + W clearly affects the expression of the genes that control the enzymes—geranyl pyrophosphate (−)-limonene cyclase; pulegone reductase, and menthone reductase—but no effects were found on menthofuran synthase. A direct comparison of night blue light and blue light + W treatments indicates that menthol and menthofuran formation are particularly affected by

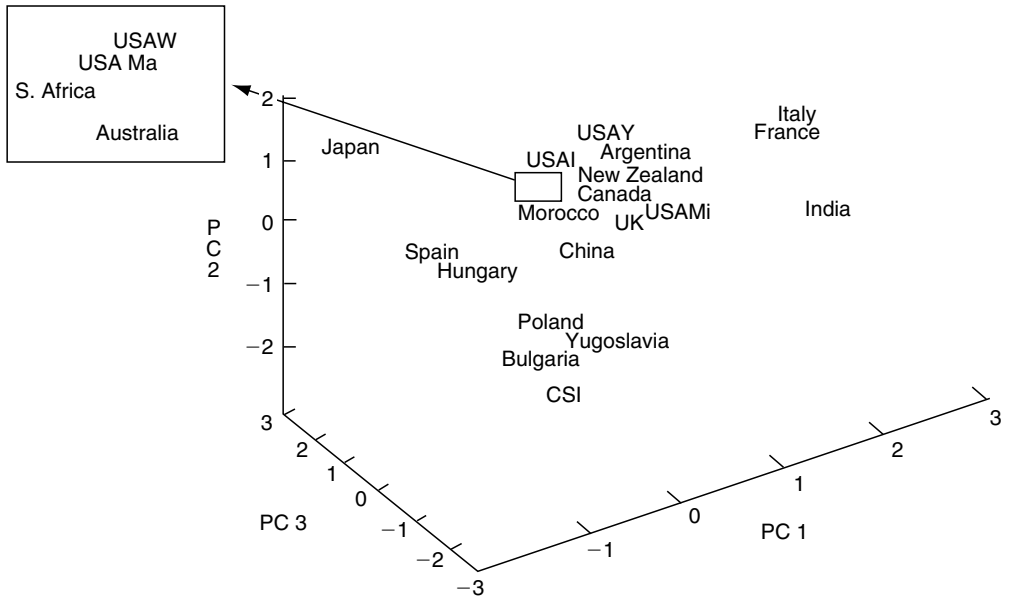


FIGURE 2.10 Scatter plot of some *Mentha piperita* on the three principal components. Data were obtained from oil data of *Mentha piperita* plants coming from different origins. A clear partition was evident among plants indicating a clear differentiation according to the geographic provenance. (From Maffei et al., *Acta Horticulturae*, 330, 159–169, 1993b. With permission.)

blue light (Maffei and Scannerini, 1999). In UV-A + W irradiated peppermint plants, the increased synthesis of menthofuran was responsible for the total terpenoid increase and appeared to depend on activation of specific genes such as those related to the cytochrome P-450 pulegone hydroxylase, which appears to be involved in menthofuran synthesis (see section on biochemistry later). Menthol increase as a consequence of the combined use of UV-A + W was found to depend on an enhanced reduction of its precursor, menthone (Maffei et al., 1999). Total oil

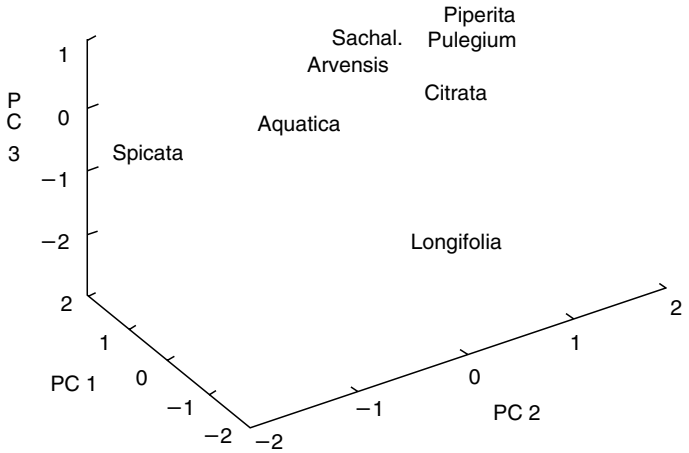


FIGURE 2.11 Scatter plot of different *Mentha* species cultivated in the same geographic area (From Maffei et al., *Acta Horticulturae*, 330, 159–169, 1993b. With permission.)

content was significantly increased in peppermint when irradiated with UV-B and no qualitative differences were observed in the direct comparison of irradiated plants and controls. However, significant quantitative differences were found for many oil components. In particular, 1,8-cineole, γ-terpinene, and isomenthol were the only compounds for which no significant differences were found. All other monoterpenes were significantly increased by UV-B radiation with the sole exception of menthol, which showed a drastic reduction. The content of the oxygenated monoterpenes, menthone, menthofuran, and menthyl acetate, was particularly enhanced by UV-B irradiation. With regard to sesquiterpenes, both germacrene D and β-caryophyllene were enhanced by UV-B treatment (Maffei and Scannerini, 2000).

These results show that in peppermint the essential oil composition is affected in a differential way by different wavelengths. The finding that UV-A (360 nm) stimulates the biosynthesis of some monoterpenes (i.e., menthol), which are suppressed by UV-B (280 to 320 nm) and blue light (450 nm), indicates that some of the reported light effect on terpene biosynthesis may be wavelength-dependent (Maffei and Scannerini, 2000). Table 2.2 resumes the effects of blue light, UV-A, and UV-B irradiations on peppermint.

2.2.1.4 Allelopathy

Higher plants synthesize a great variety of terpenoids, which can be involved in allelophatic interactions. Monoterpenoids are the major components of some essential oils and have been recognized as the main allelochemicals in higher plants: they have toxic effects on seed

TABLE 2.2
Oil Chemical Composition of Essential Oils Distilled from *Mentha Piperita* Plants Irradiated with White Light (W), Blue Light (B), UV-A, and UV-B. Data are Expressed as mg/Kg Fresh Weight ± s.e.m ()

Compound	Control (W)	B[a]	UV-A[b]	UV-B[c]
α-Pinene	10.27 (1.25)	18.61 (1.66)	3.80 (0.77)	17.61 (2.04)
β-Pinene	11.33 (1.04)	20.91 (1.22)	5.60 (0.49)	16.20 (1.42)
Sabinene	7.44 (0.25)	13.90 (0.84)	4.05 (0.82)	12.20 (1.14)
Myrcene	11.79 (0.35)	30.53 (1.03)	6.21 (1.02)	20.75 (0.89)
Limonene	22.28 (3.49)	54.28 (2.63)	11.55 (1.09)	28.74 (1.34)
1,8-Cineole	76.67 (4.63)	120.11 (4.58)	29.89 (1.08)	68.89 (5.24)
γ-Terpinene	8.31 (2.73)	9.47 (2.20)	3.32 (0.28)	9.47 (1.07)
(E)-β-Ocimene	1.66 (0.14)	3.30 (0.81)	0.92 (0.14)	3.51 (1.17)
Menthone	365.46 (46.87)	424.74 (34.73)	127.42 (6.63)	445.97 (41.19)
Menthofuran	150.65 (67.98)	341.54 (19.56)	212.14 (12.10)	369.25 (22.21)
Isomenthone	39.40 (9.10)	51.61 (4.18)	18.12 (1.57)	43.38 (1.12)
Menthyl acetate	24.75 (1.67)	49.36 (2.51)	14.11 (079)	392.67 (33.27)
α-Terpineol	11.91 (3.97)	20.17 (1.24)	6.94 (0.58)	15.84 (2.51)
β-Caryophyllene	60.75 (16.98)	51.80 (6.52)	38.27 (2.06)	90.94 (4.45)
Menthol	780.88 (89.77)	350.94 (15.51)	518.98 (43.52)	173.30 (12.58)
Germacrene D	32.78 (5.73)	74.08 (12.28)	14.90 (1.24)	62.28 (5.65)
Piperitone	22.48 (5.52)	29.67 (2.72)	22.29 (3.52)	49.75 (8.91)
TOTAL	1668.81 (78.88)	1665.02 (92.51)	1038.51 (52.49)	1820.75 (91.87)

Source: [a]From Maffei and Scannerini, *J. Essent. Oil Res.*, 11, 730–738, 1999. With permission; [b]From Maffei et al., *Photobiol. B: Biology*, 52, 105–110, 1999. With permission; [c]From Maffei and Scannerini, *J. Essent. Oil Res.*, 12, 523–529, 2000. With permission.

germination (Robinson, 1983; Rice, 1984), growth of some bacterial strains (Knobloch et al., 1989; Economou and Nahrstedt, 1991), development and growth of some insects (Lee et al., 1999), and on the growth of pathogenic fungi (Adam et al., 1998).

The mechanism by which peppermint essential oil and some of its oil components affect cucumber germination, respiration, and membrane potential has been studied in a series of works by Maffei and coworkers (Maffei, 1999; Maffei and Camusso, 2000; Maffei et al., 2001; Mucciarelli et al., 2001). The complete essential oil inhibited 50% of root and mitochondrial respiration (IC_{50}) when used at 324 and 593 ppm, respectively. (+)-Pulegone was the most toxic compound, with a 0.08 mM and 0.12 mM IC_{50} for root and mitochondrial respiration, respectively. (−)-Menthone followed (+)-pulegone in its inhibitory action (IC_{50} values of 1.11 mM and 2.30 mM for root and mitochondrial respiration, respectively), whereas (−)-menthol was the less inhibitory compound (IC_{50} values of 1.85 mM and 3.80 mM, respectively). A positive correlation was found for (+)-pulegone, (−)-menthone, and (−)-menthol between water solubility and respiratory inhibition (Figure 2.12). The uncoupling agent, carbonyl-cyanide- *m*-chlorophenyl-hydrazone (CCCP), lowered (−)-menthol and (−)-menthone inhibition and annulled (+)-pulegone inhibition of mitochondrial respiration, whereas salicyl-hydroxamic acid (SHAM) (2-hydroxybenzohydroxamic acid), the alternative oxidase (AO) inhibitor, increased (−)-menthone inhibition and annulled both (+)-pulegone and (−)-menthol inhibitory activity (Mucciarelli et al., 2001). Preliminary data on (−)-menthol-induced increase of cucumber root cytosolic free calcium ions, $(Ca^{2+})_i$ (Maffei and Camusso, 2000), suggest that (−)-menthol could play an important role in early events in the signal transduction pathway. Ca^{2+} channels are activated by membrane depolarization; by implication, signals that elicit membrane depolarization, such as the action on some monoterpenes, could trigger entry of Ca^{2+} from the external medium (Sanders and Bethke, 2000). Increasing the concentration of peppermint essential oil from 5 ppm to 50 ppm was also found to cause

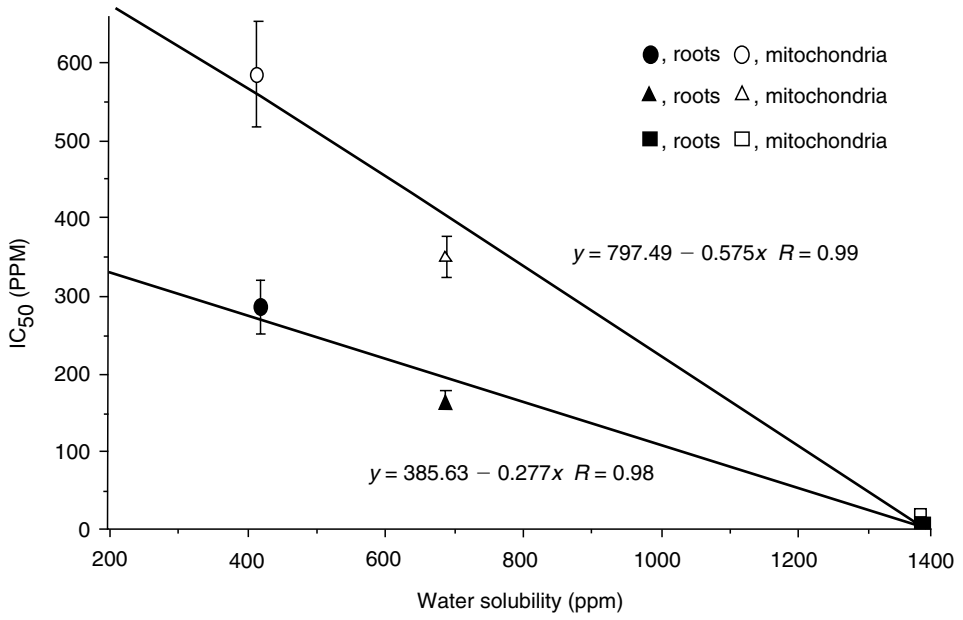


FIGURE 2.12 Plot of the concentration of (−)-menthol, (−)-menthone, and (+)-pulegone able to cause 50% of inhibition of respiration (IC_{50}) against their water solubility. A positive correlation was found between respiratory inhibition and water solubility of compounds. Bars indicate standard deviation. (From Mucciarelli et al., *Phytochemistry*, 57, 91–98, 2001. With permission.)

a decrease in cucumber root membrane potential (V_m) hyperpolarization of 10 to 3 mV, whereas concentrations from 100 to 900 ppm caused an increasing depolarization of V_m (from 5 to 110 mV) (Figure 2.13). When tested at 300 ppm, (+)-menthyl acetate, (−)-limonene, and 1,8-cineole did not exert any significant effect on V_m, whereas (+)-menthofuran (73 mV), (+)-pulegone (85 mV), (+)-neomenthol (96 mV), (−)-menthol (105 mV), and (−)-menthone (111 mV) showed an increased ability to depolarize V_m. A plot of the log of octanol–water partition coefficient (K_{ow}) against their depolarizing effect showed a significant negative correlation, suggesting that among all monoterpenoids increased membrane depolarization depends on lower K_{ow}. However, among monoterpene ketones, alcohols, and furans, increased membrane depolarization is associated with a decline in water solubility (Maffei et al., 2001).

A direct comparison of the data indicates that (+)-pulegone, which acts as a possible uncoupling agent of cucumber root and mitochondria respiration, has little effect on membrane potential, whereas (−)-menthol shows an almost 100-fold V_m depolarizing action with respect to (+)-pulegone, although (−)-menthol has less inhibitory action on root respiration. The fact that (−)-menthol is disruptive to membrane potential, whereas (+)-pulegone inhibits root respiration more readily could be an explanation as to why plants make mixtures of monoterpenes; perhaps they can have different modes of action. For allelopathic purposes, it may be useful for a plant to have more than one kind of weapon at its disposal (Maffei et al., 2001).

We started the chapter with the examination of glandular trichomes and the way of secretion. By going through the physiology and allelopathic effects of mint terpenoids, we arrived at the conclusion that since monoterpenes can cause extensive membrane and respiratory damages, they are clearly toxic to plant cells. Perhaps this is why they are often found to be sequestered in secretory structures (Gershenzon et al., 2000).

We now examine the biochemical machinery responsible for essential oil production in mints, whereas at the end of the chapter we discuss the biotechnological production of mint essential oil in plant cell and tissue cultures.

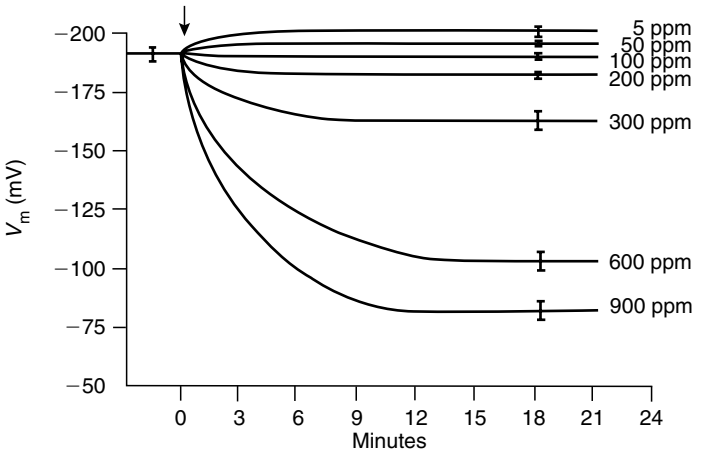


FIGURE 2.13 Effect of increasing peppermint oil concentrations on transmembrane potential (V_m). Essential oil concentrations up to 50 ppm induce a slight hyperpolarization, whereas concentrations greater than 100 ppm cause a drastic membrane depolarization. Arrow indicates the starting time of essential oil perfusion. (From Maffei et al., *Phytochemistry*, 58, 703–707, 2001. With permission.)

2.3 BIOSYNTHESIS AND MOLECULAR BIOLOGY

The biosynthesis of monoterpenes in mints can be divided into four stages (Figure 2.14): (1) the synthesis of the fundamental precursor isopentenyl diphosphate (IPP) from acetyl-CoA; (2) the formation of the allylic prenyl diphosphates from IPP, which serve as the immediate precursors of the different families of isoprenoids; (3) the elaboration of these allylic prenyl diphosphates into the main isoprenoid skeletons by specific isoprenoid synthases; and (4) secondary transformations of the products of these synthases (McCaskill and Croteau, 1997).

2.3.1 Formation of Isopentenyl Diphosphate

The first stage leads to the synthesis of the central C_5 precursor of isoprenoid metabolism, IPP. In plants, IPP is produced following two distinct pathways in two different subcellular locations: the cytosolic compartment, containing the enzymes of the acetate/mevalonate pathway

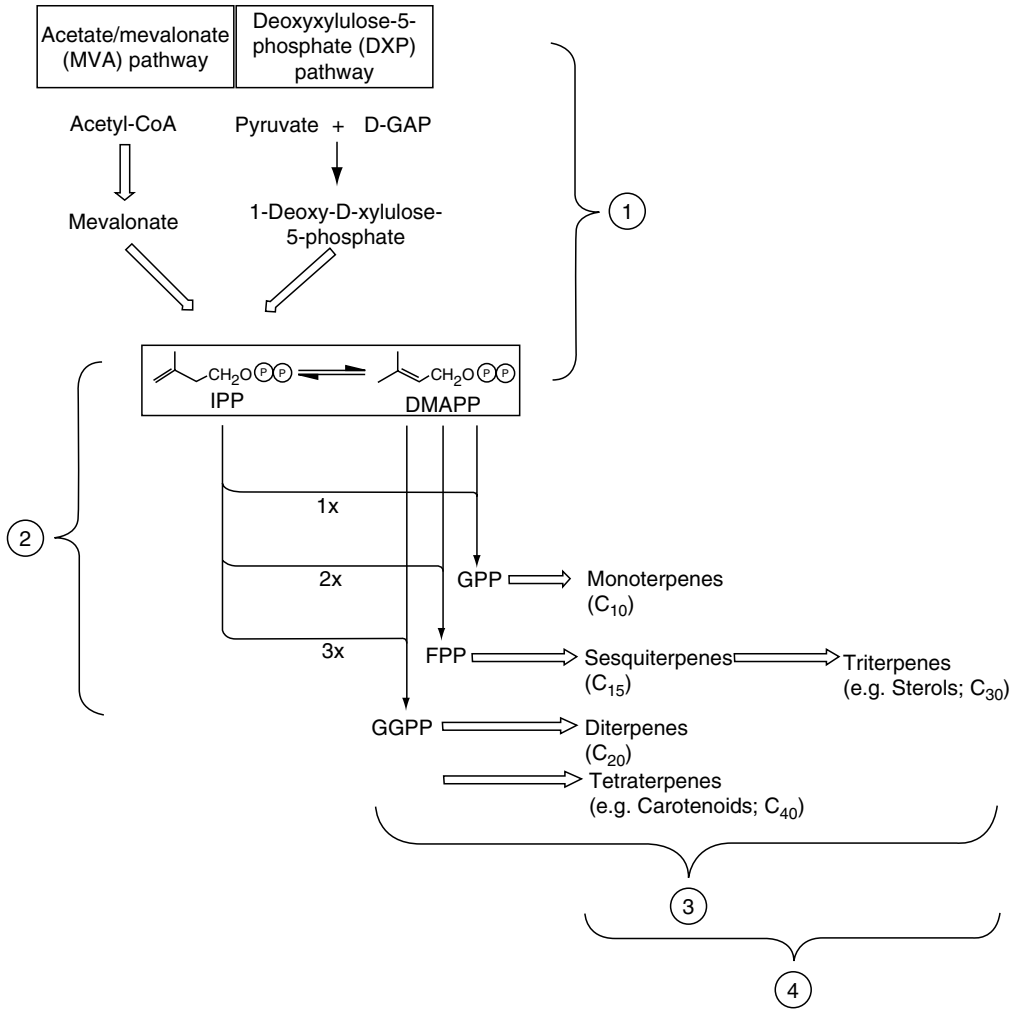


FIGURE 2.14 Steps involved in isoprenoid biosynthesis: (1) formation of isopentenyl diphosphate and dimethylallyl diphosphate, (2) formation of geranyl diphosphate, (3) cyclization of geranyl diphosphate, and (4) secondary transformations.

(Bach, 1995) and the plastids, the site of the recently discovered pyruvate/glyceraldehyde-3-phosphate (GAP) pathway (Schwarz, 1994; Lichtenthaler, 1998). Several studies on the biosynthesis of monoterpenes in Lamiaceae plants have conclusively shown that they are derived from IPP via deoxyxylulose, and not from the classic mevalonate pathway (Eisenreich et al., 1997; Sagner et al., 1998).

Experiments were done on *Mentha ×piperita* and *Mentha pulegium* plantlets, which were fed with [1-^{13}C] glucose or [U-^{13}C] glucose, and several characteristic monoterpenes were isolated by steam distillation and preparative TLC. ^{13}C-enrichment and coupling patterns measured by ^{13}C-NMR showed that the labeling profile of menthone and pulegone from *M. piperita* and *M. pulegium*, respectively, were consistent with an origin from pyruvate/GAP rather than from the acetate/mevalonate pathway (Eisenreich et al., 1997). Recently, it has been demonstrated that in peppermint oil glands, the IPP used for both monoterpene and sesquiterpene biosynthesis is produced in the plastids (McCaskill and Croteau, 1995, 1999a; Lange et al., 1998; Lange and Croteau, 1999a).

The initial step of the mevalonate-independent pathway involves a transketolase-catalyzed condensation of pyruvate and GAP to yield 1-deoxy-D-xylulose-5-phosphate. Genes encoding deoxyxylulose phosphate (DXP) synthase have been recently cloned from peppermint and confirmed by functional expression in *Escherichia coli* (Lange et al., 1998). The subsequent step of this pathway is the rearrangement and reduction of DXP to yield 2-C-methyl-D-erythritol-4-phosphate, catalyzed by a DXP reductoisomerase. The evidence for the existence of peppermint orthologs of this enzyme already cloned in *E. coli* (Takahashi et al., 1998) has been reported by Lange and Croteau (1999a).

The subsequent steps toward IPP are not yet completely elucidated. Although the intermediates following 2-C-methyl-D-erythritol-4-phosphate in the DXP pathway of IPP formation have not yet been identified, their structures can be presumed to be two reduction and dehydration steps, with one phosphorylation step being involved in these final steps of IPP (Lichtenthaler, 1999).

Lange and Croteau (1999c) reported the cloning and expression from peppermint and *E. coli*, of a kinase that catalyzes the phosphorylation of isopentenyl monophosphate as the last step of this biosynthetic pathway leading to isopentenyl diphosphate (Figure 2.15).

These proteins represent a conserved class of the GHMP family of kinases, which include galactokinase, homoserine kinase, mevalonate kinase, and phosphomevalonate kinase, with homologs in plants and several eubacteria. Intact secretory cells isolated from peppermint and incubated with isopentenyl monophosphate are able to produce large amounts of monoterpenes and sesquiterpenes, confirming that isopentenyl monophosphate is the immediate precursor of the deoxyxylulose 5-phosphate (Lange and Croteau, 1999c).

2.3.2 Formation of Geranyl Diphosphate

The second stage begins with the isomerization of IPP to dimethylallyl diphosphate (DPP) catalyzed by isopentenyl diphosphate isomerase. This enzyme has been characterized in a number of plants (Gershenzon and Croteau, 1993) and extensively studied in a number of microorganisms (Street et al., 1994). DPP is a highly reactive compound that undergoes elongation by the sequential addition of one, two, or three IPP molecules to form either geranyl diphosphate (GPP), farnesyl diphosphate (FPP), or geranylgeranyl diphosphate (GGPP) (Lange and Croteau, 1999b). GPP is the ten-carbon precursor of monoterpenes. Its formation depends on a head-to-tail condensation of DPP and IPP operating by the GPP synthase, a specific prenyltransferase (Croteau, 1987; Lange and Croteau, 1999b). This enzyme appears to be abundant only in plant species that produce large amounts of monoterpenes (e.g., essential oil plants), although it is no doubt of much broader distribution, at

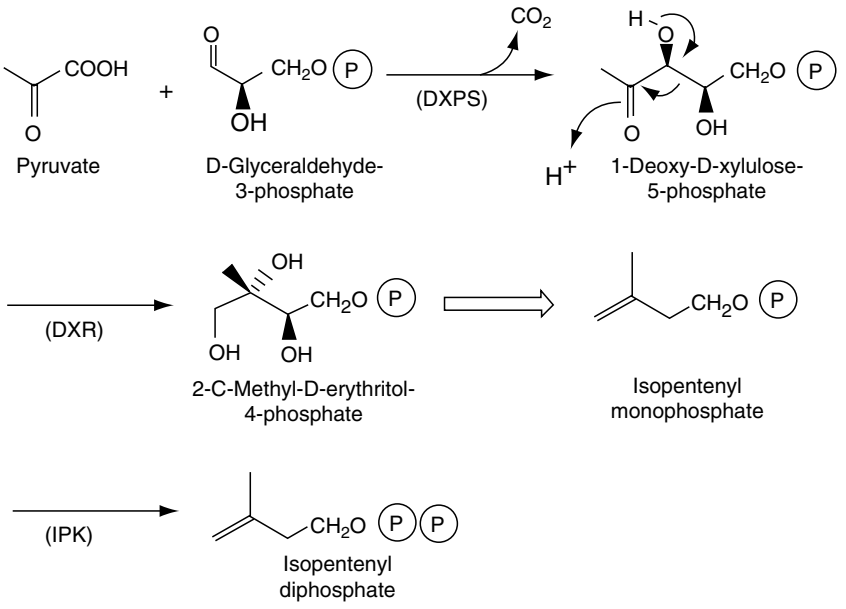


FIGURE 2.15 DXP pathway for the biosynthesis of IPP, and the proposed role of isopentenyl monophosphate kinase (IPK). The circled P denotes the phosphate moiety. The large open arrow indicates several as-yet-unidentified steps. (From Lange and Croteau, *Proc. Natl. Acad. Sci. USA,* 96, 13714–13719, 1999c. With permission.)

least in trace quantities. The reaction catalyzed by GPP synthase involves the initial ionization of the allylic diphosphate to generate a delocalized allylic carbocation. This enzyme-bound carbocation then attacks the double bond of IPP, followed by deprotonation to produce the next allylic diphosphate homolog. In the case of this prenyltransferase, the first condensation product is released from the enzyme (McCaskill and Croteau, 1997). In most instances, GPP synthase is localized to plastids, consistent with this subcellular site of monoterpene biosynthesis (Wise and Croteau, 1999). GPP synthase was purified by Burke and colleagues (1999) from isolated glandular trichomes of spearmint (*Mentha spicata*) (McConkey et al., 2000), and the amino acid sequence information was used to identify two distinct cDNA clones obtained by random sequencing of a peppermint-oil gland library (Lange et al., 2000). Functional expression, combined with the use of antibodies directed to each gene product, demonstrated that GPP synthase is a heterodimer, unlike other homodimeric short-chain prenyltransferases (Burke et al., 1999).

2.3.3 Cyclization of Geranyl Diphosphate to (−)-Limonene

In stage 3, GPP undergoes a range of cyclizations to produce the parent skeletons of different classes of monoterpenes (Figure 2.16) (Wise and Croteau, 1999).

This kind of reactions is carried out by cyclases (the term synthases also includes those enzymes that produce acyclic monoterpenes) (Croteau, 1987). These enzymes catalyze the sequential ionization and isomerization of GPP to linalyl pyrophosphate (LPP) followed by cyclization. These cyclization reactions differ in detail and produce a wide variety of isomers and derivatives of the different structural types, but all the monoterpene synthases identified thus far (about 50) are believed to share the same biochemical properties and basic electrophilic reaction mechanisms (Wise and Croteau, 1999). Isomerization to LPP before cyclization

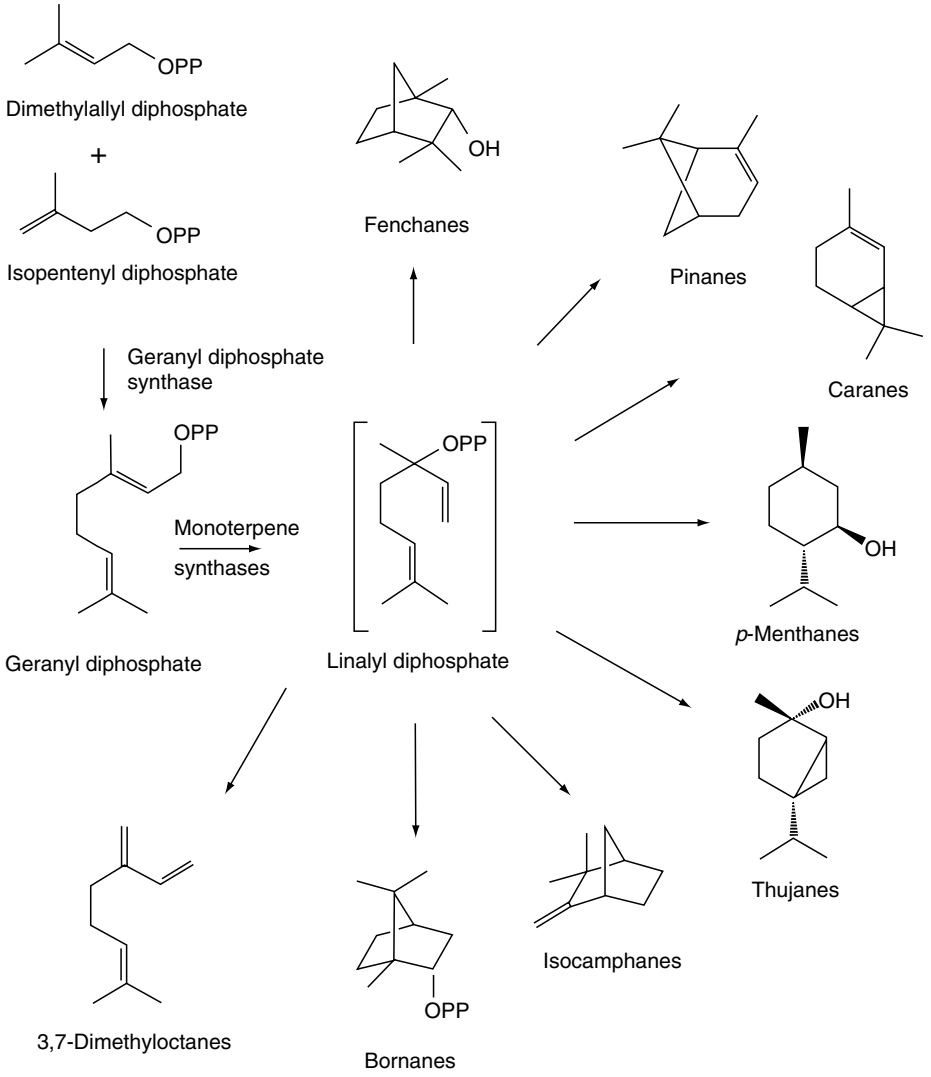


FIGURE 2.16 Formation of geranyl diphosphate and its transformation by monoterpene synthases to representatives of the major structural families. (From Haudenschild and Croteau, *Genetic Engineering*, Plenum Press, New York, NY, 1998. With permission.)

appears to be an essential point of the activity of these enzymes, as the presence of *trans*-2, 3-double bond of GPP prevents direct cyclization of this compound. The absence of such an impediment in the *cis*-isomer of GPP, neryl pyrophosphate (NPP), might at first sight make this compound a more likely precursor of monoterpenes, but studies of multiple cyclases have demonstrated GPP to be their substrate. Cyclization of LPP at the terminal double bond yields the α-terpinyl cation, the universal precursor of cyclic monoterpenes. This cation is highly reactive and its conversion to cyclic structures occurs while it is bound to the cyclase. Thus, the cyclases have in common the ability to generate the α-terpinyl cation from GPP, but each differs in cyclized products that are formed. Most of the monoterpene cyclases produce multiple products, but in each case, it is always present as a single predominant compound. For example, peppermint limonene synthase produces a small amount of α-pinene, β-pinene,

and myrcene in addition to limonene (Alonso et al., 1992). In fact, the analysis of the oil present in glandular trichomes of very young leaves of peppermint showed that 21 components were present, the majority of which are derived from the activity of limonene synthase (Maffei et al., 1989). These include not just *p*-menthane monoterpenes characteristic of this species, but also α-pinene, β-pinene, and myrcene. Cyclases are soluble proteins, either monomeric in the 50 to 70-kDa range or homodimeric with native sizes of about 100 kDa. They all use GPP as the natural substrate as described earlier, and they require a Mg^{2+} or Mn^{2+} as the only cofactor. In the past, a number of monoterpene synthases have been isolated and characterized from members of the mint family, including (−)-4S-limonene synthase, which was purified and characterized (Alonso et al., 1992; Rajaonarivony et al., 1992). It was shown to be a fairly hydrophobic monomer of about 56 kDa, with a pH optimum of 6.7, isoelectric point of 4.35, and k_{cat} of 0.3 sec^{-1}. This enzyme requires only Mn^{2+} as a cofactor and the K_m value measured for GPP–metal complex is 1.8 μM. The basic properties of limonene synthases from both peppermint and spearmint are identical, and their mechanism of action is similar to other terpenoid cyclases of higher plants (Croteau, 1987). Rabbit polyclonal antibodies were generated against SDS-denatured limonene synthase of spearmint, and immunoblotting analysis revealed that these antibodies were specific for the limonene synthase from all *Mentha* species tested, suggesting that this cyclase is similar among these species (Alonso et al., 1993). However, the immunological cross-reactivity was not observed with limonene synthase from other plant species such as *Citrus sinensis* and *Chenopodium ambrosioides* (Croteau and Gershenzon, 1994). Furthermore, no cross-reactivity was observed with other cyclases extracted from Lamiaceae, Asteraceae, and other families. In order to obtain the limonene synthase cDNA, internal amino acid sequences of the purified protein from spearmint oil glands were determined and used to design three oligonucleotide probes, subsequently employed to screen a spearmint cDNA library (Colby et al., 1993). Four clones were isolated; three cDNAs resulted full-length and functionally expressed in *E. coli*. The encoded protein contains a putative N-terminal plastidial transit peptide, consistent with immunocytochemical localization of the mature enzyme in leucoplasts of oil gland secretory cells (Turner et al., 1999). Despite the presence of the transit peptide, the recombinant limonene synthase preprotein is catalytically active, producing the same spectrum of products as the processed native enzyme (Colby et al., 1993).

Common to most cyclases, including the limonene synthase from mint, and prenyltransferases examined so far, is the presence of at least one aspartic acid-rich region with a consensus sequence of (I,L,V)XDDXX(D,E). This sequence is believed to be involved in the binding of the Mg^{2+} or Mn^{2+} salt of the diphosphate substrate (Ashby and Edwards, 1990) and may assist in the initial ionization step of the reaction.

Evidence for this mechanism is related to the observation that cyclases, which bind a single diphosphate ester substrate, contain only a single aspartic acid-rich region, whereas prenyltransferases able to bind two cosubstrates contain two aspartate-rich regions (McCaskill and Croteau, 1997). Based on the sensitivity to amino acid modification reagents, the presence of one or more histidine and cysteine residues at cyclase active sites has also been proposed. Three conserved histidine residues and a conserved cysteine residue in the deduced amino acid sequences for limonene synthase (Colby et al., 1993; Yuba et al., 1996) are *epi*-aristolochene synthase (Facchini and Chappell, 1992), casbene synthase (Mau and West, 1994), and abetadiene synthase (Vogel et al., 1996). Based on the sensitivity of monoterpene synthases to the arginine-directed reagent phenylglyoxal (Savage et al., 1995), the involvement of an active site arginine residue has also been suggested. This hypothesis is also supported by the presence of two strictly conserved and five highly conserved arginine residues in the deduced amino acid sequences of limonene synthase (Colby et al., 1993; Yuba et al., 1996), *epi*-aristolochene synthase (Facchini and Chappell, 1992), and casbene synthase (Mau and West, 1994).

2.3.4 Alternate Transformations of Geranyl Diphosphate

Several *Mentha* species produce acyclic monoterpenes, which constitute relatively small family forms. These molecules include the alcohols geraniol, nerol, and linalool, and the trienes myrcene and the ocimenes. These compounds are biosynthesized starting form GPP.

A few strains and chemotypes of *Mentha* accumulate geraniol, geranyl acetate, and related acyclic derivatives up to 30% in the essential oils, but without the exclusion of the typical cyclic monoterpenes (Lawrence, 1978; Malingré, 1971). The presence of these compounds is probably related to the limited capacity for cyclization, with the consequence that unutilized GPP is hydrolyzed and the geraniol metabolized by conjugation or redox transformations (Croteau and Gershenzon, 1994).

Nerol might derive via citral as a consequence of a redox transformation, and isomerases isolated from different plants are able to catalyze the isomerization of geraniol and geranyl phosphate to nerol and neryl phosphate (Wise and Croteau, 1999).

Linalool and linalyl acetate are the main constituents in the oils of several *Mentha* hybrids, including *M. aquatica* var. *citrata* (as *M. citrata*) (Lawrence, 1978; Todd and Murray, 1968).

S-linalool synthase has been purified to homogeneity from flowers of *Clarkia breweri* (Pichersky et al., 1995) and the corresponding gene has been cloned (Blanc and Pichersky, 1995). This enzyme uses GPP instead of (*S*)-linalyl diphosphate as a precursor and it does not catalyze the conversion of the (*R*)-antipode, suggesting a direct formation of linalool from the geranyl substrate (Pichersky et al., 1995).

2.3.5 Secondary Transformations

The cyclic parent compounds are then transformed in the fourth stage by hydroxylations and subsequent redox reactions, double bond migrations, and conjugations including glycosylations and acylations to yield the isoprenoid endproducts responsible for the great variety of monoterpenoids found in nature as those typical of essential oils (Wise and Croteau, 1999).

The genus *Mentha* provides illustrative examples of the general types of secondary transformations encountered in the monoterpene series. The biosynthesis of the *p*-menthane in mint species is well characterized. The essential oils of mints are distinguished by the position of oxygenation on the *p*-menthane ring (Lawrence, 1981), and extensive classical breeding experiments and both *in vivo* and *in vitro* studies have established the pathways for the formation of (−)-carvone, (−)-menthone, and (−)-menthol (Croteau and Gershenzon, 1994). In peppermint and related species (*Mentha aquatica, Mentha arvensis, Mentha pulegium*), (−)-4*S*-limonene is hydroxylated at the C_3 position to yield (−)-*trans*-isopiperitenol and the peppermint-type species produce almost exclusively monoterpenes bearing an oxygen function at C_3 such as (−)-menthol (responsible for the cooling sensation of peppermint). In spearmint and related species (*Mentha spicata, Mentha ×gracilis, Mentha crispa*), the same substrate is hydroxylated at C_6, to yield (−)-*trans*-carveol and in this case spearmint-type species produce almost exclusively monoterpenes bearing an oxygen function at C_6 such as (−)-carvone (responsible for the typical spearmint flavor) (Lupien et al., 1995) (Figure 2.17).

These reactions are carried out by distinct, regio-, and stereospecific cytochrome P450 oxygenases. The two cytochrome P450s that catalyze these mutually exclusive positional hydroxylations can also be distinguished by differential sensitivity to azole-type inhibitors (Karp et al., 1990). Other examples include hydroxylation of (−)-limonene to perillyl alcohol in perilla (*Perilla frutescens*) (Karp et al., 1990) and hydroxylation of sabinene to produce the oxygenated thujane monoterpenes (Karp et al., 1987).

The (−)-limonene-6-hydroxylase from spearmint has been purified to homogeneity and the pure protein was used to obtain the amino acid sequence employed for the construction of

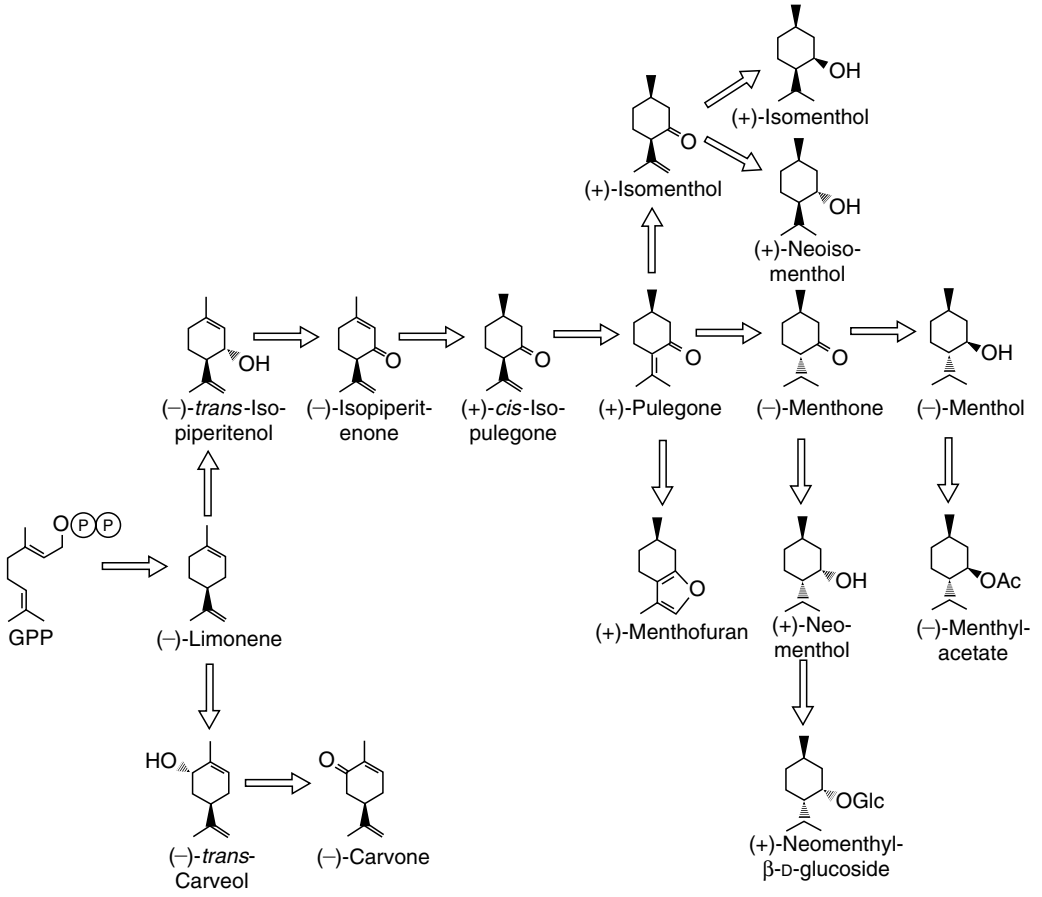


FIGURE 2.17 Monoterpene metabolism in peppermint and spearmint glandular trichomes. (From Lange and Croteau, *Curr. Opin. Plant Biol*., 2, 139–144, 1999b. With permission.)

polyclonal antibodies. Immunoblot analysis demonstrated that (−)-limonene-6-hydroxylase antiserum recognized different hydroxylases from other *Mentha* species, including peppermint 3-hydroxylases (Croteau and Gershenzon, 1994). Cross-reactions were also observed with monoterpene hydroxylases from other species of the Lamiaceae family, indicating some structural similarities among this enzymatic class. However, (−)-limonene-6-hydroxylase results are less efficient than (−)-limonene-3-hydroxylase, since a larger amount of residual limonene is always present in the taxa with C_6-oxygenated monoterpenes than in those with C_3-oxygenated monoterpenes (Lawrence, 1981; Kjonaas and Croteau, 1983; Croteau et al., 1991).

This behavior seems not related to different kinetic characteristics of the two enzymes, but it is probably due to relative limonene synthesis–hydroxylation balances, or to differential compartmentation effects (Croteau and Gershenzon, 1994).

The amino acid sequence of spearmint (−)-limonene-6-hydroxylase was also used to generate an oligonucleotide probe that was employed to screen both a spearmint and a peppermint oil gland cDNA libraries (Lupien et al., 1999). A full-length cDNA clone was isolated from the spearmint oil gland cDNA library and subsequently confirmed as (−)-limonene-6-hydroxylase by functional expression using baculovirus-*Spodoptera* system. In peppermint, two closely related full-length cDNA sequences (PM2 and PM17) were isolated

from the library and were confirmed as (−)-limonene-3-hydroxylase by functional expression as before. Amino acid deduced sequences indicate that both enzymes possess a typical N-terminal membrane anchor, consistent with the membrane location of the native forms, a very similar primary sequence (70% identity), and calculated molecular weights of 56,149 (spearmint) and about 56,560 (peppermint) (Lupien et al., 1999). Subsequent heterologous expression in *E. coli* and *Saccharomyces cerevisiae* (Haudenschild et al., 2000) has provided higher yields of the functional enzymes to allow a more detailed characterization of the mechanism and stereochemistry of the hydroxylation reactions. Recently, a combination of domain swapping and reciprocal site-directed mutagenesis between these two enzymes demonstrated that the exchange of a single amino acid residue in the spearmint (−)-limonene-6-hydroxylase leads to complete conversion to the regiospecificity and catalytic efficiency of the peppermint (−)-limonene-3-hydroxylase (Schalk and Croteau, 2000).

Other insights into the close relationship between C_3 and C_6 oxygenated *p*-menthane monoterpenes were obtained by examining the essential oil produced by a mutant of Scotch spearmint (the mutant 643) (Croteau et al., 1991). This mutant produces primarily C_3 oxygenated *p*-menthane monoterpenes, including significant amounts of menthone, instead of carvone. Cell-free enzyme assays of both the wild-type Scotch spearmint and the mutant demonstrated that both plants contain the same pool of enzymes necessary to produce menthone from (−)-*trans*-isopiperitenol. The wild-type spearmint possessed only the C_6 hydroxylase whereas in mutant 643 this activity was entirely replaced by a C_3 hydroxylase. This new hydroxylase resembled that found in peppermint in differential sensitivity to azole-type inhibitors. Although spearmint clearly contains most of the same terpene-metabolic machinery as peppermint, these enzymes are normally catalytically inefficient because carvone is a poor substrate for these subsequent reaction steps. Studies on cloning and functional expression of 643 3-hydroxylase are still in progress.

These allylic monoterpenols subsequently undergo oxidation, reduction, and isomerization reactions to yield the complex mixture of essential oil components typical of the different mint species. The presence of either (−)-limonene-6-hydroxylase or (−)-limonene-3-hydroxylase is the critical factor in determining whether a *Mentha* species produces a spearmint or peppermint-like essential oil, since the oxygenation pattern of a *p*-menthane monoterpene determines its subsequent metabolism. For instance, in spearmint (−)-*trans*-carveol is oxidized to (−)-carvone, the major monoterpene accumulated in this species, which then originates small amounts of isomeric dihydrocarvones and dihydrocarveols (Gershenzon et al., 1989; Croteau et al., 1991). A similar, but antipodal pathway has been proposed for the formation of (+)-carvone in caraway seed (*Carum carvi*) (Bouwmeester et al., 1998). The metabolic transformations of (−)-*trans*-isopiperitenol in peppermint are much more extensive. A series of redox reactions involving both double bonds and the oxygenated carbon, and a double bond migration, results at the end in the formation of both (−)-menthone and (+)-isomenthone, and all four stereoisomers of menthol (Croteau and Gershenzon, 1994). These pathways appear to be quite different on the chemical level, but the basic reactions are very similar. In fact, the enzymes that catalyze the reactions following limonene hydroxylation share similar properties. This metabolic similarity can be demonstrated by feeding peppermint or spearmint leaf disks with (−)-*trans*-carveol or (−)-*trans*-isopiperitenol, respectively (Croteau and Gershenzon, 1994).

These two allylic alcohols are then enzymatically oxidized to the corresponding α-β-unsaturated ketones, (−)-isopiperitenone, and (−)-carvone. The dehydrogenase responsible for these conversions is present in the soluble supernatants of oil gland extracts from peppermint, spearmint, and Scotch mint (Gershenzon et al., 1989; Croteau et al., 1991). This enzyme has a molecular weight of 66 kDa and uses NAD as an oxidant (Kjonaas et al., 1985).

In peppermint, (−)-isopiperitenone is converted to (+)-*cis*-isopulegone by a specific double bond reductase. This enzyme has been found in oil gland extracts from peppermint, native spearmint, and Scotch spearmint, but in the last two species is present in lower levels (Croteau et al., 1991). The molecular weight of the reductase is about 60 kDa and NADPH is used as a reductant (Croteau and Venkatachalam, 1986).

The isomerization of (+)-*cis*-isopulegone to (+)-pulegone and of (−)-isopiperitenone to piperitenone is catalyzed by the *p*-menthone isomerase. This enzyme, detected in soluble extracts of peppermint, spearmint, and Scotch spearmint, has a molecular weight of 55 kDa and it does not use any cofactors (Kjonaas et al., 1985; Croteau et al., 1991). It is probably present at high levels in all *Mentha* species, as both (+)-*cis*-isopulegone and (−)-isopiperitenone have been observed only in trace amounts (Croteau and Gershenzon, 1994).

(+)-Pulegone and piperitenone may have different metabolic fates in *Mentha* species. The reduction of the double bond of these two substrates leads to (−)-menthone or (+)-isomenthone, and (+)- and (−)-piperitone, respectively. This reaction is NADPH-dependent and it is carried out by two distinct enzymes of opposite stereospecifity (Croteau et al., 1991). The relative proportions of the two reductases producing menthone and isomenthone from (+)-pulegone vary in commercial mints from 3:1 to 10:1 (Battaile et al., 1968; Croteau et al., 1991).

(+)-Pulegone is also the precursor of (+)-menthofuran, the commercially undesirable oil component. Recently, the biosynthesis of menthofuran has been elucidated. The biochemical pathway from pulegone involves a cytochrome P450 catalyzed hydroxylation of the *syn*-methyl group of the (+)-pulegone followed by cyclization to form a hemiketal, and dehydration to give the furan ring. *In vivo* feeding experiments on peppermint shoot tips and young leaves demonstrated that labeled (+)-menthofuran was derived from (+)-pulegone, showing that this compound is produced by an enzymatic oxidation (Fuchs et al., 1999). Bertea and colleagues (2001) isolated a cDNA clone from a peppermint oil gland cDNA library, which was functionally expressed in *S. cerevisiae* and *E. coli* and showed to encode the (+)-menthofuran synthase. This protein, which possesses all the primary structural elements of a cytochrome P450, presents a 35% identity with (−)-limonene-3-hydroxylase from the same source (Bertea et al., 2001).

In peppermint, (−)-menthone and (+)-isomenthone are converted to their corresponding alcohols by two stereospecific, NADPH-dependent keto-reductases (Kjonaas et al., 1982).

Both enzymes possess similar properties such as the molecular weight (about 35 kDa), the cofactor (NADPH), the optimum of pH (about 7.5), kinetic constants, and sensitivity to thiol-directed reagents (Croteau and Gershenzon, 1994).

Most of the enzymes involved in this latter steps have been isolated and characterized and appear to be highly substrate specific (Croteau et al., 1991; Croteau and Gershenzon, 1994).

These secondary enzymatic reactions, subsequent to the limonene cyclization step, occur, not in the plastids, but at the endoplasmic reticulum or in the cytosol. Thus, movement of the parent olefin limonene, from the plastid to the cytosol/ER is required. Nothing is yet known regarding the intracellular trafficking of monoterpenoids. Carrier or transport protein may be involved in this process, as well as in the export of the monoterpenes to the subcuticular storage cavity of the glandular trichomes (Haudenschild and Croteau, 1998).

The *p*-menthane alcohols of *Mentha* species can form conjugates with glucose moieties or with acetate. In fact, they are not stable endproducts of metabolism. Even if most parts of the study have been focused on peppermint (Croteau and Hooper, 1978; Martinkus and Croteau, 1981), the occurrence of this conjugate also in other species indicates that the enzyme responsible for the conjugation reaction has a wide distribution (Croteau and Martinkus, 1979; Sakata and Mitsui, 1975; Sakata and Koshimizu, 1978; Shimizu et al., 1990a,1990b).

(−)-Menthyl acetate is always present in a relatively high concentration (15%) in the essential oil of mature leaves of flowering peppermint (Croteau and Hooper, 1978). The

enzyme responsible for the conversion of (−)-menthol to (−)-menthyl acetate is a soluble acetyltransferase of about 37 kDa, which uses acetyl-CoA as acyl donor. This enzyme is able to acetylate different substrates such as monoterpenoid and nonmonoterpenoid alcohols (Croteau and Hooper, 1978).

In *Mentha*, monoterpene alcohols can occur also as conjugates with glucose. Important are glucosides of (−)-menthol, (+)-neomenthol, (−)-*trans*-carveol, (−)-*cis*-carveol, (−)-dihydrocarveol, and (+)-neodihydrocarveol found in many commercial species (Sakata and Mitsui, 1975; Sakata and Koshimizu, 1978; Croteau and Martinkus, 1979; Shimizu et al., 1990a, 1990b).

In peppermint, a UDP-glucose:monoterpenol glucosyltransferase has been characterized. This soluble enzyme of 46 kDa converts (+)-neomenthol to (+)-neomenthyl-β-D-glucoside and shows a low substrate specificity because it is able to glucosylate at equal rates *in vitro* both (+)-neomenthol and (−)-menthol (Martinkus and Croteau, 1981).

The (+)-neomenthyl-β-D-glucoside is then transported to the rhizomes where it undergoes oxidative degradation, probably necessary to provide acetyl-CoA and to reduce equivalents for rhizome metabolism (McCaskill and Croteau, 1997).

2.3.6 Approaches to the Bioengineering of Monoterpene Biosynthesis

Given the commercial value of mint essential oils, the processes involved in their biosynthesis and secretion are attractive targets for genetic engineering. However, because of the complexity of the monoterpene biosynthetic pathway and the difficulties in purifying the responsible enzymes (Alonso and Croteau, 1993), obtaining gene probes from the corresponding target proteins by classical biochemical approaches has been quite difficult. As part of an ongoing effort to isolate genes of isoprenoid metabolism, the construction of a cDNA library from peppermint oil gland secretory cells allowed Croteau and coworkers to clone and express most part of the genes involved in these pathways (Lange et al., 2000). The partial sequencing (400 to 900 bases) of about 1500 randomly selected cDNAs (expressed sequence tags [EST]) from this library has become a rapid and cost-effective means of gaining information about gene expression in a specialized tissue such as gland cells. Several genes involved in monoterpene biosynthesis have been characterized (Lange et al., 2000) using functional genomics approaches, which combine computational and heterologous expression-based analyses, as previously described.

Studies about monoterpene metabolism have advanced to the point that several approaches may now be considered for the manipulation of essential oil profile. These include the mutagenesis of specific monoterpene synthases to alter their products, the manipulation of a biosynthetic pathway (by altering the activity of an enzyme or by introducing novel enzymes), and the manipulation of the formation of glandular trichomes, the site of essential oil production and accumulation (McCaskill and Croteau, 1997; Lange and Croteau, 1999b).

The mutagenesis of a specific monoterpene synthase can be achieved by using two approaches: site-directed mutagenesis and domain swapping. Changes in the amino acid groups at the active site of an enzyme can influence substrate and cosubstrate binding conformation and compromise the catalysis cycle. For example, in the monoterpene synthases, the modification of specific residues can affect the stability of carbocation intermediates diverting the reaction course along alternate routes. As previously described, experiments of site-directed mutagenesis combined with domain swapping conducted on spearmint (−)-limonene-6-hydroxylase changed the regiospecificity of this enzyme completely, leading to a product hydroxylated at C_3 instead of at C_6 (Schalk and Croteau, 2000).

Another approach to engineering monoterpene formation is the creation of chimeric enzymes that combines functional domains from different synthases. This can be possible

because of the high sequence homology observed for plant isoprenoid synthases. This would allow creating chimeric enzymes with different functional domains able to give novel products (McCaskill and Croteau, 1997). Recent advances in the molecular biology of terpene synthases (Bohlmann et al., 1998) offer a tool for modifying the aroma profile of essential oils by the expression of foreign terpene synthases in transgenic plants to yield oils with novel characteristics.

Recently, *Agrobacterium tumefaciens*-based transformation procedures have been developed for peppermint-transformed calli (Spencer et al., 1993) and teratomas (Berry et al., 1996). The first transgenic peppermint plants were constructed in 1998 by two groups (Niu et al., 1998; Diemer et al., 1998), working independently. However, before molecular engineering technology can be efficiently applied to the essential oils in mint, it is necessary to ensure that transgene expression does not generate complications such as cytotoxicity (Brown et al., 1987). Furthermore, it is critical to find a promoter that selectively regulates gene expression in trichomes, even if studies in this sense are underway (Lange and Croteau, 1999b).

Knowledge of the enzymology and regulation of a metabolic pathway can permit the manipulation of the flux of intermediates through the pathway, with the goal of increasing the accumulation of desirable products or decreasing the accumulation of undesirable ones. The engineering of a metabolic pathway for monoterpenes can involve either the modification of a pathway, to change the composition and yield of the oil, or the introduction of new or altered enzymes, to produce compounds not normally present in the plant. A complete understanding of the various developmental factors related to essential oil formation, such as metabolic branch points in control of flux, possible feedback regulatory properties of intermediates, and transport mechanisms required for the secretion of essential oils, is still lacking. Before manipulating, several enzyme activities will be required to redistribute metabolic flux toward monoterpene biosynthesis, and redirecting significant flux toward monoterpene production may lead to deprivation of essential oil components formed from intermediates, as observed in transgenic tomato plants (Fray et al., 1995).

An example of the effect that partitioning of metabolites by branch points has on the quality of an essential oil is given by the *p*-menthane monoterpenes produced in peppermint, in which (+)-pulegone is the branch point between the formation of (−)-menthone and (−)-menthol, and the commercially undesirable (+)-menthofuran. Therefore, the branch point enzymes producing these two compounds represent promising targets for bioengineering efforts. The approach would involve the overexpression of the reductase, which converts (+)-pulegone to (−)-menthone, thereby reducing the flux of the key intermediate into (+)-menthofuran. An alternate approach would involve the repression of the cytochrome P450 responsible for the conversion of pulegone to (+)-menthofuran by using antisense technology, or the removal of regulatory elements related to the activation of this gene under particular environmental conditions (Burbott and Loomis, 1967).

Using similar approaches, the overexpression of the gene that encodes the dehydrogenase, which converts (−)-menthone to (−)-menthol, should lead to the accumulation of large amounts of the commercially desirable (−)-menthol. In contrast, the silencing of the dehydrogenase responsible for the epimeric reduction of (−)-menthone to (+)-neomenthol could lead to the decrease in catabolic losses of (−)-menthone to the corresponding glucoside (Croteau and Martinkus, 1979; Croteau et al., 1984).

From an ecological point of view, since many monoterpenes such as limonene exhibit insecticidal properties (Pickett, 1991; Gershenzon and Croteau, 1993), the availability of cDNA clones for limonene synthase (Colby et al., 1993; Yuba et al., 1996) raises the possibility of engineering limonene production in crop plants to improve the resistance to insect pests.

Another goal of engineering technology is to increase the tissue density of mint glandular trichomes in which the essential oil is produced and stored, without affecting organoleptic

quality. Work on the regulation of trichome development in *Arabidopsis thaliana* (Hülskamp et al., 1994) may lead to the development of approaches for increasing the density of the oil glands, which could result in a corresponding increase in total oil production. Although this kind of studies involves nonglandular trichomes, the development of glandular trichomes will undoubtedly share many of the same regulatory factors and control elements.

2.3.7 Conclusions and Future Perspectives

The aim of this chapter was to provide an overview of our understanding of the biosynthetic capacity of mint plants, specifically in relation to monoterpenes. Many of the biochemical pathways for monoterpene production have been elucidated, with many enzymes and their corresponding genes now cloned and expressed using different heterologous expression systems. The following are the future objectives in this field:

- Cloning of all the remaining genes of *p*-menthane biosynthesis; defining promoter regions of genes specifically expressed in oil glands; isolating genes involved in trichome formation
- Improving essential oil quality and quantity by optimizing vectors to adjust expression levels of transgenes; using transgenic mint to investigate the regulatory processes involved in the control of pathway fluxes, metabolite profiles, and monoterpene secretion

2.4 TISSUE CULTURE AND BIOTECHNOLOGY

Plant propagation using *shoot cultures*—micropropagation—supplies growers with a highly uniform plant population useful for commercial exploitation and for the maintenance of secure genetic stocks of plant material. Tissue culture propagation for germplasm conservation is essential. First, it allows the proper long-term storage and conservation of plant material, which is essential for crops that produce short-lived seeds and for those that are vegetatively propagated like many mints. Second, it provides an easy and inexpensive way for international exchange of disease-free material (Rech and Pires, 1986).

Importance of such techniques in medicinal and aromatic plants (MAPs) is particularly also for the production or conversion of secondary metabolites of commercial interest by *in vitro* cultured cells. The apparent ease with which the biosynthesis of secondary metabolites is induced and modulated in intact plant tissues and the possibility of obtaining rapidly proliferating cells from these tissues in sterile conditions have consequently stimulated research to obtain terpenoid production *in vitro*. Oil yield can also be improved by laboratory or field applications of chemical and physical external elicitors, as well as phytohormones or microorganism cell culture extracts, through the enhancement of the appropriate biosynthetic enzymes (El Ketawi and Croteau, 1987; Lange and Croteau, 1999b). The industrial scaling up of PAM cell cultures producing oils is therefore of great attraction, both for commercial exploitation and for the development of controlled cell systems in which the biogenesis of monoterpenes can be studied.

The present development of *in vitro* plant propagation and breeding provides a powerful means for the genetic improvement of MAPs. Sexual hybridization imposes narrow limits to the scope of breeding, confining the breeder to the very low variability present in the normal gene pool. Gene transfer to plants via *in vitro* cultures, with the aim of adding specific new agronomic traits, makes possible the overcoming of existing problems encountered with sexual hybridization. Genetic transformation of plants involves the stable introduction of foreign DNA sequences into the nuclear or organelle genome of cells capable of giving rise to a completely transformed plant. Circumventing the sexual process and avoiding the need for

lengthy backcrossing procedures, genetic transformation may accelerate the genetic improvement (Draper and Scott, 1991) of useful plants. To reach this aim, *sterile plant material* capable of rapid regeneration into mature plants is essential.

2.4.1 Tissue Culture and Micropropagation of Mints

Micropropagated shoot cultures are useful as explant sources of cell culture and protoplast preparations. Due to its intrinsic characters and by the means of selectable phenotypic and biochemical markers, the *in vitro* culture system allows practical applicability of large-scale *in vitro* screening of germplasms, clones, and breeding material for the selection of elite genotypes (Shasany et al., 2000).

Since the first studies of Lin and Staba (1963), who showed that an auxin was essential for tissue dedifferentiation and for the *in vitro* establishment of mint callus, there has been considerable research focused on micropropagation of several *Mentha* species (Cellerova, 1992).

Axillary buds have been used as, and still remain, the best source for rapid *in vitro* multiplication of *Mentha piperita*, *M. arvensis*, *M. pulegium*, *M. spicata*, and *M. viridis* (Rech and Pires, 1986). Nodal segments from 1-year-old plants can be grown on Murashige and Skoog (1962) (MS) medium, where, in the absence of exogenous growth regulators, sparse roots and shoots are normally produced. The influence of plant density and different culture conditions on *Mentha spicata* shoots grown *in vitro* has been recently analyzed (Tisserat and Silman, 2000). Shoots can be successfully rooted and potted into the soil for further growth (Rech and Pires, 1986). Rootlets are normally formed at the same time as shoot proliferation, thus rendering unnecessary the subsequent phase of rooting and reducing sensibly the period of transfer of the plantlets to soil.

In *Mentha piperita*, plant regeneration and propagation from pathogen-free material have proved to be useful in the isolation of rust-free regenerants. The latter showed increased productivity with respect to rust-affected plants of field conditions (Nádaská et al., 1990).

Due to juvenile conditions, sterility, and uniform pattern of growth, *in vitro* micropropagated axillary buds or shoots represent an ideal source of plant material for germplasm conservation. Alginate-coated meristems from *in vitro*-grown axillary buds of *Mentha spicata* and other mint species were successfully cryopreserved by a plunge into liquid nitrogen, following vitrification (Hirai and Sakai, 1999). Encapsulated peppermint meristems may be conveniently thawed and transferred to culture media, where they develop shoots and intact plants, also after prolonged times of cryostorage.

2.4.2 Direct Plant Regeneration from Explants

The appearance of genetic instability in the cultured material—*somaclonal variation*—due to the occurrence of chromosome or genetic changes in the regenerating plants is a phenomenon frequently encountered in long-term cultured cells (Evans and Sharp, 1986). Kukreja and colleagues (1991), starting from regenerated shoots of *M. canadensis* (as *M. arvensis*. var. *piperascens*), have isolated 27 somaclones, all differing sensibly for various agronomic traits, i.e., plant height, leaf/stem weight ratio, herb yield, and oil content and composition. However, these genetic changes are often undesirable, especially when clonal reproduction of transformed plants is the goal of regeneration. In this case, as the occurrence of somaclonal variations increases with the duration and extent of the disorganized or undifferentiated culture phase (Karp, 1994), plants regenerated directly from tissues or organs, eventually transformed with physical DNA delivery methods, are preferred. These techniques allow manipulation of entire vegetative plant organs (i.e., leaves), organ meristems, or reproductive structures (i.e., gametes and zygotes) to avoid disturbance of normal plant development and

reduce the *in vitro* culture phase (Draper and Scott, 1991). Direct plant regeneration from cultured explants is a method particularly suited for herbaceous plants, using leaves, corms, bulbs, stems, rhizomes, and tubers (Tisserat, 1985). Starting from cultured apical meristems, usually a cytokinin is added to the medium to overcome apical dominance on shooting and to enhance the branching of lateral buds. This method has been successfully applied to the commercial and large-scale propagation of some important ornamental plants. Regenerated buds from tissue cultures on MS media supplemented with BAP have been obtained from peppermint leaves (Medou et al., 1997) and from internode explants of *Mentha arvensis* (Shasany et al., 1998). The use of BAP (1 to 2 mg/L) or NAA (1 mg/L) has proved useful inducing multiple shoots formation (15 to 20 per explant) in many other species (Rech and Pires, 1986). Best results were obtained using young leaves from *in vitro* micropropagated plants, cultivated in the dark (Medou et al., 1997). The authors demonstrated that bud development starting from specific meristematic areas led to the development of secretory structures. Regeneration frequencies and reproducibility are strongly affected by genotypic effects as demonstrated in the case of adventitious shoots obtained from zygotic embryos (Van Eck and Kitto, 1990) and from leaves (Van Eck and Kitto, 1992). When shoot regeneration arises from very small areas of the cultured leaf tissue (Caissard et al., 1996), the limited area and number of cells involved in the regenerating process reduce probabilities of obtaining transgene integration in regenerating cells. To overcome these limitations, an efficient regeneration system from leaf disks of spearmint and peppermint has been obtained (Faure et al., 1998). The leaf disk regeneration method, combined with the subsequent induction of multiple shooting (10 to 15 shoots per explant) is a useful tool for mint genetic transformation with *A. tumefaciens* (Faure et al., 1998). Through the manipulation of the explant source, the environment of the donor plant, and medium components, Berry and colleagues (1996) improved the frequency of regeneration from petioles and leaf disks of orange mint (*M. aquatica* var. *citrata*), peppermint (*M. piperita* L. cv. Black Mitcham), Native spearmint (*M. spicata*), and Scotch spearmint (*M. gracilis* Sole cv. Baker). The regenerative process appeared to be cytokinin-specific and highly stimulated when N_6-(2-iso-pentyl) adenine (2iP) was used (Berry et al., 1996).

Cultured plant material was also used to obtain new mutants. Induced mutation through γ-irradiation of stolons led to the improvement in peppermint resistance to *Verticillium* wilt in the *cultivars*, "Todd's Mitcham," and "Murray Mitcham" (Murray, 1969).

2.4.3 Mint Plant Regeneration from Callus Cultures

When leaf tissues are incubated under appropriate hormonal conditions, this leads to *in vitro* proliferating cell aggregates, termed *callus cultures*, from which it is possible:

- To develop useful cell lines
- To produce essential oil in cultured cells
- To regenerate new plant clones

Bhaumik and Datta (1989) showed that Japanese mint *M. canadensis* (as *M. arvensis*) requires phenylalanine (500 mg/L) and high phosphate in addition to the ingredients of the MS medium, and these requirements differed for callus initiation, callus growth, and cell growth in suspension. A high auxin–cytokinin ratio (2:1) increased callus growth and the addition of coconut milk acted synergistically with 2,4-D stimulating cell division in young leaf tissues (Bhaumik and Datta, 1989). Later, Kawake (1991) and Kawabe and coworkers (1993) described a complete and more complex *in vitro* procedure, starting from bud material of *M. canadensis* (as *M. arvensis*). Callus and adventitious roots were induced by culture of the

explant on B5 (Gamborg et al., 1968) solid medium containing NAA (4 mg/L). These cultures produced a large amount of adventitious roots when transferred to a liquid medium containing NAA (2 mg/L) and BAP (0.5 mg/L). When recultured for a further 30 days on liquid medium with NAA (0.2 mg/L) and BAP (4 mg/L), green clumped calluses were obtained. Green adventitious buds were obtained after transfer to B5 medium containing NAA (0.1 mg/L) and BAP (4 mg/L), and these buds could then be allowed to regenerate roots and ultimately plants on hormone-free medium (Kawabe, 1991, Kawabe et al., 1993). The use of leaf, shoot, and root explants for callus induction and plant regeneration has also been described in *Mentha spicata* L. (Abou-Mandour and Binder, 1998).

2.4.4 Mint Cell Genome Transformation

Technological advances in genetic engineering have made possible the use of biotechnology for mint crop improvement. The primary constraint to mint oil production is crop yield loss due to pests and pathogens, a situation that is exacerbated by the long-term intensive monoculture. The overexpression of genes that carry resistance to insects or phytopathogens has proved useful to transfer resistance to pests and pathogens in many crops (Martin et al., 1993). As a result, the interest toward the application of biotechnological strategies to peppermint or other commercially valuable mint species to improve mint oil quality and quantity is growing.

2.4.4.1 Protoplast Culture and Cell Manipulation

Peppermint regeneration from protoplasts provides a promising system both for genetic transformation and for the selection of somaclonal variants. Mints have mostly been improved by conventional breeding techniques such as crossing and selection of mutants, resulting in some excellent varieties with disease tolerance (Murray, 1961) or high menthol content (Nakayama et al., 1970). Since *M. piperita* is a triploid sterile hybrid, its breeding by common sexual crossings is impossible unless its ploidy is doubled for fertility with the creation of the hexaploid by chromosome doubling with colchicine treatment. Despite these efforts, no excellent cultivars superior in flavor to Mitcham peppermint have been produced till now.

Genetic manipulation using protoplast culture could therefore enhance mint improvement, enabling the use of techniques such as cell fusion, gene transfer, and somaclonal variation. A prerequisite for applying plant biotechnology is the development of a suitable and reproducible plant regeneration system; according to this, protocols for plant regeneration from protoplasts have been established. Though with a low efficiency of differentiation, Sato and colleagues (1993) succeeded in developing an operative protocol to regenerate peppermint plants from leaf-derived protoplasts. Enzymatically isolated leaf-protoplasts were cultured in a modified B5 medium, supplemented with 1 mg/L NAA and 0.4 mg/L BA. The application of this method allowed the production of 6.8 to 8.9×10^6 protoplasts per g of fresh leaf tissues with a 75% to 80% average viability (Sato et al., 1993). When transferred to the differentiation medium containing 0.1 mg/L NAA and 5 mg/L, BA protoplast-derived green calli developed shoot buds after 3 to 4 weeks of culture. Following rooting on media without hormones, whole plants of normal appearance were recovered after transplanting to the soil (Sato et al., 1993).

More recently, the regeneration efficiency of this procedure (>50%) has been optimized by Jullien and colleagues (1998), with the addition to the media of low concentrations of 2,4-D, the employment of solid media, and stimulating shoot formation and plant regeneration by the use of thidiazuron as exogenous cytokinin. As observed for many other plant species and families, cell and protoplast plating efficiency and regeneration of mints are also strongly dependent on the genotype employed as donor material (Jullien et al., 1998).

Protoplast technology, with the *in vitro* establishment of cell fusion products between distant related species or cultivars, allows the exploitation of *somatic hybridization* in mint, thus overcoming sexual incompatibilities encountered during conventional breeding.

Interspecific somatic hybridization by leaf-derived protoplast fusion was first obtained between peppermint and gingermint *M. gracilis* (as *Mentha gentilis* cv. *variegata*) by the electrofusion method (Sato et al., 1996). One of the regenerants showed three major volatile constituents, menthone, menthol, and linalool, as a result of its interspecific hybrid origin.

Following protoplast fusion between peppermint (cv. Black Mitcham), producing high-quality oil and spearmint, possessing resistance to *Verticillium* wilt, Krasnyanski and colleagues (1999) have identified 28 somatic hybrid plants. Hybrid shoots, regenerated *in vitro*, were identified using RAPD profiles and Southern hybridization. All hybrid plants showed a GC profile typical of spearmint oil and one of them possessed a level of susceptibility to *Verticillium* intermediate between that of the fusion parents (Krasnyanski et al., 1999).

2.4.4.2 Agrobacterium Technology

The use of the *Agrobacterium-mediated gene transfer* system for improvement of commercial mints is now available. To maintain the strict essential oil profile standards of a specific mint cultivar, commercially grown *M.* ×*piperita* cv. Black Mitcham, *M.* ×*gracilis* cv. Baker, and *M. spicata* are commonly vegetatively propagated. The tool of tissue culture in combination with *Agrobacterium* gene transfer technique makes it possible to improve these commercial mints through the introduction of single trait variation or mutations. Effective adventitious shoots and roots regeneration protocols are a fundamental prerequisite for the establishment of such a technique. Starting from the first demonstration of susceptibility of *M. aquatica* var. *citrata* (as *M. citrata*) to *Agrobacterium* transformation by Spencer and coworkers (1990), Berry and colleagues (1996) have evaluated the response of different commercial mints, namely peppermint, Native spearmint, Scotch spearmint petioles, and orange mint leaf disks to cocultivation with a number of *A. tumefaciens* strains. They individuated A281, a hypovirulent agropine vector containing Ti plasmid pTiBo542 as the most responsive and effective *Agrobacterium* strain. It is able to initiate tumor-like callus tissues in peppermint, Native spearmint, and orange mint (Berry et al., 1996). Genomic Southern analysis of tumoral callus DNA and subsequent PCR amplification with specific primers demonstrated T-DNA integration into the mint genomes, their feasibility to transformation, and the potential of this technique for economically important gene-technique applications (Berry et al., 1996). Direct regeneration *in vitro* via shooting and transient GUS expression through either *Agrobacterium* inoculation or biolistic treatment of peppermint has been obtained also by Caissard and collaborators (1996). After these preliminary results, the production of morphologically normal transgenic plants of *M.* ×*piperita* cv. Black Mitcham through the cocultivation of leaf explants with *Agrobacterium* strain EHA 105 and kanamycin selection has been achieved (Niu et al., 1998). Basal leaf explants with petioles excised from leaves closest to the apex of *in vitro* cultured shoots proved to be the best source for *Agrobacterium* infection efficiency and subsequent plant organogenesis; the latter occurred at sites of excision either directly from cells of the explant or via a primary callus (Niu et al., 1998). Eight plants were successfully regenerated in the presence of the cytokinin, thidiazuron, and were verified as transgenic, based on the detection of the transgene by PCR and Southern blot analyses. By measuring transient GUS activity expressed by transformed plant material and transgenic calluses during shoot regeneration, greater transformation efficiency was found in *Agrobacterium*-treated plant material when compared with microparticle bombardment (Niu et al., 1998).

From results based on 200 explants, peppermint transformation via *Agrobacterium* technology has been reported to infer a 10% transformation efficiency (Diemer et al., 1998).

Recently, a substantially improved protocol for *Agrobacterium*-mediated transformation of peppermint has been published by Niu and colleagues (2000). *A. tumefaciens* infection of peppermint leaf explants can now be easily accomplished with an average frequency of transformation of about 20% (percentage of leaf explants that produced transformed plants), thus rendering this biotechnological technique a useful alternative to plant breeding and clonal selection of this crop. A key step in raising the frequency of *Agrobacterium* infection has been the cocultivation of peppermint leaves and the bacterium on tobacco cell feeder layers (Niu et al., 1998, 2000). Feeder cells are hypothesized to secrete phenolics that act as virulence inducers and to promote cell division and growth of explants. Enhanced transformation efficiencies were reached by increasing selection pressure with higher concentration of kanamycin, which is the antibiotic employed for transformant *in vitro* recovery (Niu et al., 2000).

The stable integration of GUS and NPTII genes by *A. tumefaciens*-mediated transfer has been achieved also in *M. canadensis* (as *M. arvensis*) and *M. spicata* (Diemer et al., 1999). From these experiments, one spearmint- and two cornmint-transformed plants have been obtained. Transformed plants have been checked for transformation with specific PCR primers, further evaluated by RT-PCR for the presence or absence of specific transcripts of both genes, whereas the integration of the GUS gene has been confirmed by Southern blot hybridization (Diemer et al., 1999).

Agrobacterium-mediated and *direct gene transfers* into protoplasts using PEG of *Mentha piperita* cv. Black Mitcham were both successfully used to produce stable, transformed peppermint plants with the limonene synthase gene (Krasnyanski et al., 1999). Southern blot analysis revealed that *Agrobacterium*-mediated transformation was superior to the direct DNA uptake into protoplasts with regard to the stability of the insert during transformation (Krasnyanski et al., 1999).

Presently, attempts to achieve other transformation experiments with constructs of genes coding for enzymes involved in monoterpene metabolism are underway. Moreover, in fertile mint species, as well *M. spicata* and *M. canadensis* (as *M. arvensis)*, it should also be possible to assess transgene inheritance in the progeny as well as isolate highly homozygous plants (Diemer et al., 1999), whose genetic stability is much appreciated at the moment of the commercial release of new varietal lines.

As recently stressed by Lange and Croteau (1999b), before molecular engineering technology can be efficiently applied to essential oil production in mint, an additional prerequisite is tissue-specific expression of transgenes. This would ensure that transgenes are expressed directly in glandular trichomes, to avoid potential complication due to cytotoxicity. Although several plant epidermis-specific promoters have been characterized, a promoter that selectively regulates gene expression in glandular trichomes remains to be discovered (Lange and Croteau, 1999b).

2.4.5 Essential Oils of Regenerated and Transformed Mints

Plant regeneration and exogenous growth regulator applications to *in vitro* plant material induce many changes in the chemical profile of volatiles produced by mint tissue cultures.

Abou-Mandour and Binder (1998) reported that *in vitro* cultivation of spearmint regenerants led to plants with one or two shoots containing an essential oil dominated by carvone (75%), whereas all other mono- and sesquiterpenoid compounds accumulated in less than 5% of total oil. Increasing BAP levels increased concentrations of monoterpene hydrocarbons (i.e., limonene from 0.5% up to 13%), reduction of carvone (75% to 53%), and reduction of the content of all sesquiterpenes. In the presence of NAA, increasing content of monoterpene hydrocarbons was observed whereas *cis*- and *trans*-dihydrocarvone were reduced (Abou-Mandour and Binder, 1998).

Attempts have been made to modify the essential oil content of mint plants using *somaclonal variation* through plant regeneration from protoplast cultures. Chaput and colleagues (1996) observed a decrease in menthone and menthol and an increase in carvone for all their protoplast-derived peppermint plants. They have reported the successful culture and plant regeneration of two protoplast-derived hybrid species of mint, *M.* ×*piperita* L. and *M. aquatica* var. *citrata* (as *M. citrata*) (Chaput et al., 1996). Out of the 80 somaclones analyzed by Chaput, only 8 showed an amount of menthol slightly higher (88% to 94%) than that of the control (86%), whereas all the others were lower (Chaput et al., 1996). No genetic analysis of the *in vitro* regenerated plant material has been reported to sustain the use of this technique for successfully developing commercially new mint varieties or chemotypes.

Sato and colleagues (1996) have described the production of an interspecific somatic hybrid plant between peppermint (*Mentha* ×*piperita* cv. Blackmint) and gingermint *M. gracilis* (as *Mentha gentilis* cv. *Variegata*) by the electrofusion method. Shoots raised from these experiments of cell fusion were grown to whole plants, and some of them showed intermediate morphological characters between the parental plants. One of these hybrid variants showed three major volatile constituents when analyzed by GC–MS: menthone, menthol (as main components of peppermint oil), and linalool (the major component of gingermint oil) (Sato et al., 1996). Additional evidence of the hybridity was provided by chromosome counts that showed the halloexaploid condition of the fusion product and by results of the RAPD analysis, which indicated the presence in the somatic hybrid of DNA fragments common to both parents (Sato et al., 1996).

The competence for *in vitro* shoot regeneration and terpene synthesis by *Agrobacterium*-transformed peppermint has been demonstrated, thus inferring that shooting teratomas are a useful system for studying metabolite production by transformed tissues grown *in vitro*. According to the scope of the research, by genetically manipulating the *onc*-genes of *A. tumefaciens*, it should be possible to assess and modify the effect of transformation on morphology, growth, and terpene production by transformed shoots of mint.

Spencer and colleagues (1990) in their early work on *M. aquatica* var. *citrata* (as *M. citrata*) T-gene transformation experiments, published results concerning qualitative and quantitative composition of terpenes isolated from shooty teratomas (galls) grown on solid media after transformation with *A. tumefaciens* T37 strain and compared these results with that obtained from leaves of parent plants. The main components of the *Mentha citrata* oil, linalool, and linalyl acetate were the major components present in both the parent plants (93%) and the shoot cultures (94%). The total yield of oil from shoot cultures was lower than that obtained from leaves of the parent plant, probably owing to the juvenile condition of secretory tissues and the presence of stem material in *in vitro* raised shoots (Spencer et al., 1990).

Similar results have been obtained earlier by Spencer and colleagues (1993) for *A. tumefaciens*-transformed *M. piperita* shoot cultures. They found menthone, menthofuran, menthyl acetate, and (−)-menthol, and menthol and menthofuran were the major components of the essential oil. The application to *M. piperita* of different transformation strategies, namely using either a nopaline strains of *A. tumefaciens*, or disarmed strains carrying a binary vector with the *ipt* sequence (CaMV 35S promoter) coding for isopentenyl-transferase, gave the same phytochemical properties (relatively high menthofuran and low menthone) in all the regenerants (Spencer et al., 1993). (−)-Menthone is metabolically very important as it can be reduced either to (−)-menthol, which accumulates, or converted to (+)-neomenthol, which is glycolized, translocated, and degraded (see Croteau in the previous section). As shown earlier, menthofuran is an oxidation product of (+)-pulegone, a key component in the pathway from (−)-limonene to (−)-menthone. Menthofuran is reported to accumulate in plants grown under oxidative conditions such as long photoperiods of poor light quality with warm night temperatures, whereas under short light photoperiods and cold nights the production of menthone is favored (Spencer et al., 1993 and references therein).

In vitro peppermint shoot cultures are extremely sensible to artificial light and temperature conditions. We have documented the chemical evolution and changes in the terpenic profile of micropropagated peppermint plants (*Mentha piperita* cv. Maine et Loire) as a consequence of extended growing periods in axenic artificial cultures (Mucciarelli et al., 1995). The fact that the *M. piperita* cultures of Spencer and colleagues (1993) were grown at relatively low light intensities and high day and night temperatures probably may have favored menthofuran production. Investigations on the effects of growth conditions and medium composition on the growth and production of monoterpenes by transformed shoot cultures of mint (Spencer et al., 1990, 1993) have been revised later (Hilton et al., 1995). These authors subjected *M. aquatica* var. *citrata* (*as M. citrata*) and *M. piperita* shoot cultures to different environmental conditions of varying periods of light and temperature and found that increased illumination reduced the yields of pulegone and menthofuran, without stimulating the production of either menthol or menthone. Besides, they cultured transformed shoots in a 14-l bioreactor for up to 60 days, gaining a 25×and 35×increase in *M. citrata* and *M. piperita* green biomass, respectively. The total yields of essential oil from the fermentation process were 1.16 g (*M. piperita*) and 0.18 g (*M. citrata*) (Hilton et al., 1995).

When single transgenic peppermint plants were grown to 10% flowering in a greenhouse by Krasnyanski and colleagues (1999), plants regenerated from both *Agrobacterium* and protoplast-derived method of transformation showed essential oil profiles characterized by a high menthone, low menthol, high menthofuran, and pulegone content in comparison with a typical Midwest peppermint (Krasnyanski et al., 1999).

2.4.6 Terpene Production in Mint Cell Cultures

Some lines of *Mentha piperita* callus or cell suspensions can synthetize essential oils and many efforts have been made to optimize culture conditions such as carbon source, exposure time to light, light quality, initial inoculum density, pH of the culture media, agitation, hormone concentration and operating protocols, and type of bioreactor (Kim et al., 1996; and references therein).

The accumulation of monoterpenoids in the culture of different *Mentha* species and hybrids has been reported, i.e., in *M. aquatica, M. aquatica* var. *citrata (as M. citrata), M. longifolia, M. pulegium, M. spicata, M. suaveolens*, etc. (Charlwood and Charlwood, 1983).

Some studies have emphasized the need for high levels of differentiation to achieve the production of monoterpenoid by cell cultures, as those obtained by Hirata and colleagues (1990) with the production of carvone and limonene in sprouting callus of *M. spicata*.

It has been hypothesized that the pH of the medium can influence the observed pattern of secondary metabolism in ways other than those influencing growth and stability of cultures (Banthorpe and Brown, 1990). Many of the secondary metabolites, such as terpenoids, coumarins, glycosides, and esters are unstable to prolonged incubation outside the range of pH 5 to 7 and consequently secondary reactions may occur when metabolites are excreted into the culture medium. Indeed the pH of the culture medium shows great variation during autoclaving or over the culture period as a result of NH_4^+ or NO_3^- uptake by cultured cells (Banthorpe and Brown, 1990). This could influence both the metabolite leakage from cultured cells and the stability of these molecules in the media. Moreover, monoterpenes secreted into the culture medium may be much more susceptible to enzymatic degradation than those sequestered into the secretory hairs because, in culture, plant cells excrete a large amount of hydrolytic and oxidative enzymes into the medium (Spencer et al., 1993, and references therein). Recent studies have demonstrated that plant cell cultures actively catabolize monoterpenes added to the culture medium.

Gathering all these considerations, a substantial increase in dry weight of *M. spicata* cell cultures when cultured in MES-buffered media (pH 5.8) with respect to unbuffered cultures

has been reported (Banthorpe and Brown, 1990). Consequently, studies have been undertaken to test the applicability of cell-recycled air-lift bioreactors for high-density culture of *Mentha piperita* cells. Owing to their low shear stress, simple mechanical structure, and low energy consumption, air-lift bioreactors have found various applications in the area of plant cell culture. In the case of peppermint, Chung and colleagues have finally reported a volumetric productivity of essential oils substantially higher than that obtained from batch culture (Chung et al., 1994). However, plant cell suspensions and cell cultures in bioreactors have not yet fulfilled the needs of the market, yielding secondary metabolites at very low levels.

The low yield of mint cell cultures has been enhanced by the introduction in the culture media of a second phase. Removal of excreted monoterpenes from the culture medium is beneficial because they are toxic to plant tissue (Wink, 1990); moreover, production of chemicals via biotechnology generally requires time-consuming procedures of isolation and purification from dilute, aqueous solutions (Garcia, 1991). The second phase of Li Chropep RP-8, a silica gel with outer SiOH group covalently bounded to C8 hydrocarbons, has been successfully employed to select monoterpene producing cell lines in *Mentha* cell cultures in shaken flasks and air-lift bioreactors (Kim et al., 1996). *Mentha* cells have been conveniently cultured in LS medium supplemented with 0.2 mg/L 2,4-D and 20 g/L of sucrose, with the addition of RP-8, XAD-4, and XAD-7 free or packed in little bags as second phases. An addition of RP-8 gave best results and did not impair cell growth. This treatment increased the ratio of extracellular oil to intracellular oil formation and resulted in stimulated formation of mint essential oils. The latter consisted in α-terpinene, *p*-cymene, and the citrals together with menthone, neomenthol, and neomenthyl acetate with a yield of 6.9 μg/L per day (10% of the total level in parent tissue) (Kim et al., 1996).

Monoterpenes are lipophilic, and solvents such as the water-insoluble triglyceride Myglol, or hexadecane, when used as a second phase in cell culture medium, have been reported to absorb and stabilize monoterpenes secreted by cultures into the culture medium (Spencer et al., 1993; and references therein).

Maximum accumulation of terpenoids has been found in the late exponential phase of the cell culture cycle and is higher in cell suspensions than in callus cultures (Banthorpe, 1996). Industrialization of mint cell culture for the production of secondary metabolites is still limited by low productivity, low growth rate of cells, and high sensitivity to shear. In order to solve these problems, various techniques have been tried.

Enzymatic methods have been developed to permeabilize the tonoplast to stimulate the release of any products to the media without reducing cell viability (Banthorpe, 1996). The most promising techniques are cell immobilization and elicitation. The first trial provides many advantages including reuse of cells and maintenance of high cell-to-cell contact, which is useful for the stable growth of active cells that are to be used in a continuous culture system. Peppermint cells have been immobilized in a polyurethane (PU) foam of $5 \times 5 \times 5$ mm and cultured both in shaken flasks and in a bioreactor, equipped with a round spiral type impeller. The maximum oil productivity was 148 mg/L after 40 days of culture in flasks, using chitosan as an elicitor (Ha et al., 1996). Despite the high viability of mint cells cultured in the bioreactor, the oil productivity was relatively lower than that of the flask system.

In this context, the development of culture systems more properly adapted to plant cell cultures should improve terpene yields of plant origin. From the viewpoint of industrial food and flavor production, the development of biofermentation systems containing natural or transformed microbial cells able to produce and eventually transform monoterpenes has been suggested (Nishimura and Noma, 1996).

Other than monoterpenes, mint cell culture systems have been recently exploited for the production of natural antioxidants as well as rosmarinic acid and related phenolics useful for food processing and nutraceutical applications. With the aim of selecting high rosmarinic acid

clonal lines of *M. spicata*, tissue-culture-based selection techniques have been developed (Al-Amier et al., 1999).

2.4.7 Biotransformations in Cell Cultures

For a great variety of *Mentha* species, the ability of different cell lines to carry out oxidations and reduction of carbonyl groups, alcohols, and double bonds has been demonstrated. This has made it possible to select a single clone or cell line with a desired transforming ability (Banthorpe, 1994).

In their early works on this topic, Aviv and colleagues found that all tested cell lines of *Mentha* spp. were able to transform (−)-menthone to (+)-neomenthol, reducing the carbonyl to hydroxyl group (Aviv et al., 1981). On the contrary, ability to hydrogenate the unsaturated α–β keto bond of (+)-pulegone leading to (+)-isomenthone was restricted to only some of the cell lines (Aviv et al., 1983). These results indicated stereospecificity with respect to precursors and products of biotransforming mint cell cultures and pointed out that plant cells are restricted to some extent in their biotransformation capabilities to components of the biosynthetic pathway of the plant from which they were derived (Aviv et al., 1983).

Biotransformations have also been reported when the usual type of substrates were added to callus cultures of *M. piperita* lines, but the efficiency of conversion was low compared with that of free cell suspensions (Rodov et al., 1983; Banthorpe, 1996).

Immobilization of dispersed cells in either calcium alginate beads or polyacrylamide hydrazide cross-linked with glyoxal as linking agent (PAAH-G) has been reported to stimulate the ability of cells to transform (+)-pulegone to (+)-isomenthone and (+)-menthone to (+)-neomenthol (Galun et al., 1983). Moreover, the distribution of both precursors and product in the medium versus the cell showed that less monoterpenes were retained in cells entrapped in PAAH-G with respect to cell-free suspensions, thus considerably improving their yield.

Cell division and consequent proliferation can be controlled in these immobilized systems through the use of known amounts of auxins or by the use of γ-irradiation (Galun et al., 1985). Exposure of *Mentha* cells to 500 Gy gamma-irradiation causes cell division arrest and the irradiated cells efficiently reduce (−)-menthone to (+)-neomenthol with similar or higher efficiency than nonirradiated cells (Galun et al., 1985). Moreover, the reducing ability is retained for at least 1 week by cells after embedding and immobilization, thus rendering a continuous biotransformation process possible by repeated exposure to the transformation precursor. The advantage of suppressing the mitotic activity is useful to save the metabolic energy required for biotransformation activity and it also improves the mechanical stability of the gel beads (Galun et al., 1985).

In the course of studies examining the biochemical differences of peppermint suspension cells, novel compounds have also been found. Park and colleagues (1994), feeding peppermint cell suspension cultures with various intermediates of the menthol pathway, found that when (−)-isopiperitenone was the original precursor, a previously unreported compound in peppermint, (−)-7-hydroxyisopiperitenone was isolated in addition to all other typical mint metabolites, namely piperitone, (+)-pulegone, (−)-menthone, and (−)-menthol (Park et al., 1994, 1997). In experiments that followed, they found that the biotransformation of (−)-(4R)-isopiperitenone by suspension cultures yielded three new hydroxylated derivatives and two new epoxidized derivatives (Park and Kim, 1998). Peppermint cells biotransformation leading to (−)-7-hydroxyisopiperitenone is inhibited by several cytochrome P450-specific inhibitors as well as carbon monoxide, and this inhibition can be substantially overcome by irradiation with blue light (Park et al., 1999). The authors have interpreted this finding as the result of activation in the cell culture conditions of a dormant P450 gene in the plant. When 21-day-old

suspension cells were treated with isopiperitenone, mRNA of a cytochrome P450 was induced with a timecourse pattern similar to that of the biotransformation cycle in cultured cells (Park et al., 1997). The induction of P450 mRNA and the biotransformation ability were both increased by the addition of methyl jasmonate to the culture system, thus inferring that the induction of the P450 cytochrome is modulated *in vivo* by the jasmonic acid as a signaling molecule (Son et al., 1998).

Most of the studies dealing with monoterpenes transformation in mint cell suspensions involve the incubation of the substrate with the cell suspension for almost 8 to 72 h, when conversions of 10% to 40% are achieved. During this period of culture, enzymatic glycosilation of the terpenoids may occur, especially when using a sugar in the culture medium as cosubstrate. Glycosides have been synthesized by incubating monoterpenoid alcohols or ketones with *Mentha* cells in media supplemented with 2,4-D and myo-inositol, thus reaching the conversion of (+)-menthol into its (+)-β-glucoside (*l*-menthyl β-glucoside) (Berger and Drawert, 1988). These observations have been interpreted as a result of the peculiar metabolism of *in vitro*-cultured *Mentha* cells. These cells are forced to grow continuously by the plant growth regulators, thus transforming even foreign substrates to carbon and energy, before being transferred to primary pathways. The use of glycosides, which are normally better degraded by cells, may facilitate the transport of these substrates to catabolic enzymatic sites (Berger and Drawert, 1988).

Werrmann and Knorr (1993) demonstrated that *l*-(−)- and *d*-(+)-monoterpenyl acetates added to *M. piperita* and *M. canadensis* suspension cultures were degraded within 6 to 12 h in their corresponding alcohols. After further incubation, all synthesized alcohols and acetates were completely degraded. Within 24 h, most of the *l*-(−)-menthol was converted into *l*-(−)-menthyl glucoside, whereas only small amounts of *d*-(+)-menthol and *d*-(+)-neomenthol were glycolized (Werrmann and Knorr, 1993). Despite both *Mentha* species exhibiting the same conversion of *l*-(−)-menthyl acetate to *l*-(−)-menthol, in *M. canadensis* the glycosilation was more enantioselective and stereospecific to *l*-(−)-menthol than in *M. piperita* cell cultures (Werrmann and Knorr, 1993).

Mint cell cultures therefore might have a potential to produce natural *l*-menthol by deacetylation of *l*-menthyl acetate as a natural compound in the essential oil. This and other activities are of considerable interest for their utilization in food and drink industry as well as for pharmaceutical applications.

The low level at which many secondary compounds are normally produced by cells in culture has prompted to assess the feasibility of enzyme immobilization techniques for the production of monoterpenes and sesquiterpenes. Thanks to the recent advances in the discovery and characterization of the key enzymes responsible for the biosynthesis of terpenoids (see previous section, Croteau, 1987, and references therein), terpenoid cyclases have become the most promising targets for this technological exploitation. These enzymes are involved in the transformation of the ubiquitous isoprenoid intermediates, geranyl- and farnesyl-pyrophosphate to the cyclic parent compounds, from which the various terpenes are derived. Therefore, a great array of different techniques of immobilization have been developed through the reversible absorption to beaded resins or anion-exchange resins or through covalent attachment of the enzymes to activated resins (i.e., activated Sepharose) (Miyazaki and Croteau, 1990).

Recent progress in the molecular biology of terpene synthases and especially the identification of new candidate genes involved in essential oil biosynthesis, through their expression and functional evaluation in vectors (*E. coli*), will offer a tool for modifying the aroma profiles of essential oil plants by the expression of these foreign terpene genes both in transgenic plants and in cell-free systems to yield oils with novel properties (Lange and Croteau, 1999b; McCaskill and Croteau, 1999b; Lange et al., 2000)).

2.4.8 Conclusions and Prospects

Cell tissue culture and micropropagation methods for the regeneration of plantlets of *Mentha* species are now available, although great attention must be devoted to the choice of donor or parental plants. When possible, donor plant material must be chosen on the basis of useful and heritable agronomic traits, rather than on their easy response to *in vitro* culture.

Callus, cell suspension, and protoplast cultures have been easily established from most sources of mint organ and tissue explants, and biotransforming cells, immobilized cultures, as well as organs in bioreactors producing considerable quantities of terpenoids can be readily derived therefrom.

Monoterpenoids can be accumulated in many of these *in vitro* culture systems, providing the correct culture conditions and relying on the appropriate exogenous stimuli. The latter may be represented by either exogenously applied natural or artificial substrates or by biotic elicitors, among which are bacterial and fungal cells. However, most of these *in vitro* processes are not yet of commercial relevance.

The readily available *in vitro* cultures of cell and tissue and especially the competence of this material for genetic transformation and plant regeneration are invaluable for studies on the characterization of all the biochemical steps, which still remain unknown in the terpenoid biosynthetic pathway.

The bulk of all these studies should lead to further advances in the field of gene transfer to produce novel desirable secondary metabolites in more healthy and easy-to-grow plants and cell lines.

REFERENCES

Abou-Mandour, A.A. and Binder, G. (1998). Effect of exogenous growth regulators in plant regenerates from tissue cultures of *Mentha spicata* and production of volatiles. *J. Appl. Bot*., 75, 233–243.

Adam, K., Sivropoulou, A., Kokkini, S., Lanaras, T., and Arsenakis, M. (1998). Antifungal activities of *Origanum vulgare* subsp. Hirtum, *Mentha spicata, Lavandula angustifolia* and *Salvia fruticosa* essential oils against human pathogenic fungi. *J. Agric. Food Chem*., 46, 1739–1745.

Al-Amier, H., Mansour, B.M.M., Toaima, N., Korus, R.A., and Shetty, K. (1999). Screening of high biomass and phenolic producing clonal lines of Spearmint in tissue culture using *Pseudomonas* and azetidine-2-carboxylate. *Food Biotechnol*., 13, 227–253.

Alonso, W.R. and Croteau, R. (1993). Prenyltransferases and cyclases. In, Dey, P.M. and Harborne, J.B., Eds, *Methods in Plant Biochemistry. Enzymes of Secondary Metabolism*, Vol. 9. Academic Press, London, UK, pp. 239–260.

Alonso, W.R., Crock, J.E., and Croteau, R. (1993). Production and characterization of polyclonal antibodies in rabbits to (4*S*)-limonene synthase from spearmint (*Mentha spicata*). *Arch. Biochem. Biophys*., 301, 58–63.

Alonso, W.R., Rajaonarivony, J.I.M., Gershenzon, J., and Croteau, R. (1992). Purification of 4*S*-limonene synthase, a monoterpene cyclase from the glandular trichomes of peppermint (*Mentha* ×*piperita*) and spearmint (*Mentha spicata*). *J. Biol. Chem*., 267, 7582–7587.

Amelunxen, F. (1964). Beobachtungen über die Veränderung der Zusammensetzung des Ätherisches Öls in isolierten Welkenden Blättern von *Mentha piperita. Planta*, 62, 321–331.

Amelunxen, F. (1965). Elektronmikroskopische untersuchungen an den drusenschuppen von *Mentha piperita L. Planta Med*., 13, 457–473.

Ascensão, L. and Pais, M.S. (1998). The leaf capitate trichomes of *Leonotis leonurus*: histochemistry, ultrastructure and secretion. *Ann. Bot*., 81, 263–271.

Ashby, M.N. and Edwards, P.A. (1990). Elucidation of the deficiency in two yeast coenzyme Q mutants. Characterization of the structural gene encoding hexaprenyl pyrophosphate synthase. *J. Biol. Chem*., 265, 13157–13164.

Aviv, D., Dantes, A., Krochmal, E., and Galun, E. (1983). Biotransformation of monoterpenes by *Mentha* cell lines: conversion of pulegone-substituents and related unsaturated α-β ketones. *Planta Med*., 47, 7–10.

Aviv, D., Krochmal, E., Dantes, A., and Galun, E. (1981). Biotransformation of monoterpenes by *Mentha* cell lines: conversion of menthone to neomenthol. *Planta Med*., 47, 7–10.

Bach, T.J. (1995). Some new aspects of isoprenoid biosynthesis in plants—a review. *Lipids*, 30, 191–202.

Baluska, F. (1990). Nuclear size, DNA content, and chromatin condensation are different in individual tissues of the maize root apex. *Protoplasma*, 158, 45–52.

Banthorpe, D.V. (1994). Plant tissue culture: scope and limitations. *Nat. Prod. Rep*., 11, 302–348.

Banthorpe, D.V. (1996). *Mentha* species (Mints): *in vitro* culture and production of lower terpenoids and pigments. In: *Biotechnology in Agriculture and Forestry 37*, Bajaj, Y.P.S., Ed, Medicinal and Aromatic Plants, I., Springer-Verlag, Berlin Heidelberg, pp. 202–225.

Banthorpe, D.V. and Brown, G.D. (1990). Growth and secondary metabolism in cell cultures of *Tanacetum, Mentha* and *Anethum* species in buffered media. *Plant Sci*., 67, 107–113.

Battaile, J., Burbott, A.J., and Loomis, W.D. (1968). Monoterpene interconversions: metabolism of pulegone by a cell-free system from *Mentha piperita. Phytochemistry*, 7, 1159–1163.

Berger, R.G. and Drawert, F. (1988). Glyosilation of terpenols and aromatic alcohols by cell suspensions of *Mentha piperita. Z. Naturforsh, C. Biosci*., 43, 415–490.

Berry, C., Van Eck, J.M., Kitto, S.L., and Smigocki, A. (1996). *Agrobacterium*-mediated transformation of commercial mints. *Plant Cell Tiss. Org. Cult*. 44, 177–181.

Berta, G., DeLa Pierre, M., and Maffei, M. (1993). Nuclear morphology and DNA content in the glandular trichomes of peppermint (*Mentha ×piperita*. L.). *Protoplasma*, 175, 85–92.

Bertea, C.M., Schalk, M., Karp, F., Maffei, M., and Croteau, R. (2001). Demonstration that menthofuran synthase of mint (*Mentha*) is a cytochrome P450 monooxygenase: cloning, functional expression, and characterization of the responsible gene. *Arch. Biochem. Biophys*., 390, 279–286.

Bhaumik, C. and Datta, P.C. (1989). Development of Japanese mint tissue culture method. *Indian Perfum*., 33, 165–168.

Blanc, V.M. and Pichersky, S. (1995). Nucleotide sequence of a *Clarkia breweri* cDNA clone of *Ipi1*, a gene encoding isopentenyl pyrophosphate isomerase. *Plant Physiol*., 108, 855–856.

Bohlmann, J., Meyer-Gauen, G., and Croteau, R. (1998). Plant terpenoid synthases: molecular biology and phylogenetic analysis. *Proc. Natl. Acad. Sci. USA*, 95, 4126–4133.

Bouwmeester, H.J., Gershenzon, J., Konings, M.C., and Croteau, R. (1998). Biosynthesis of the monoterpenes limonene and carvone in the fruit of caraway I. Demonstration of enzyme activities and their changes with development. *Plant Physiol*., 117, 901–912.

Bradshaw, A.D. (1965). Evolutionary significance of phenotypic plasticity in plants. *Adv. Genet*., 13, 115–155.

Brown, J.T., Hegarty, P.K., and Charlwood, B.V. (1987). Monoterpene and sesquiterpene biosynthesis in plant cells in culture. 4. The toxicity of monoterpenes to plant cell cultures. *Plant Sci*., 48, 195–201.

Burbott, A.J. and Loomis, W.D. (1967). Effects of light and temperature on monoterpenes of peppermint. *Plant Physiol*., 42, 20–28.

Burke, C.C., Wildung, M.R., and Croteau, R. (1999). Geranyl diphosphate synthase: cloning, expression, and characterization of this prenyltransferase as heterodimer. *Proc. Natl. Acad. Sci. USA*, 96, 13062–13067.

Caissard, J.C., Faure, O., Jullien, F., Colson, M., and Perin, A. (1996). Direct regeneration *in vitro* and transient GUS expression in *Mentha ×piperita. Plant Cell Rep*., 16, 67–70.

Cellerova, E. (1992). Micropropagation of *Mentha*. In: *Biotechnology in Agriculture and Forestry*, Bajaj, Y.P.S., Ed, Vol. 19, High-tech and micropropagation III, Springer, Berlin Heidelberg, New York, pp. 262–276.

Chaput, M.E., San, H., De Hys, L., Grenier, E., David, H., and David, A. (1996). How plant regeneration from *Mentha ×piperita* and *Mentha ×citrata* Ehrh. leaf protoplasts affects their monoterpene composition in field condition. *J. Plant Physiol*., 149, 481–488.

Charlwood, B.V. and Charlwood, K.A. (1983). The biosynthesis of mono- and sesquiterpenes in tissue culture. *Biochem. Soc. Trans*., 11, 592–593.

Chew, F.S. and Rodman, J.E. (1979). Plant resources for chemical defense. In: *Herbivores: Their Interaction with Secondary Plant Metabolites*. Rosenthal, G.A. and Janzen, D.H. Eds, Academic Press, New York, pp. 271–307.

Chung, I.S., Kang, Y.M., Oh, J.H., Kim, T., Lee, H.J., and Chae, Y.A. (1994). Continuous suspend cell culture of *Mentha piperita* in cell-recycled air-lift bioreactor. *Biotechnol. Techn*., 8, 789–792.

Clark, R.J. and Menary, R.C. (1980). Environmental effects of peppermint (*Mentha piperita* L.). II. Effects of temperature on photosynthesis, photorespiration and dark respiration in peppermint with reference to oil composition, *Aust. J. Plant Physiol*., 7, 693–697.

Colby, S.M, Alonso, W.R., Katahira, E.J., McGarvey, D.J., and Croteau, R. (1993). 4*S*-limonene synthase from the oil glands of spearmint (*Mentha spicata*): cDNA isolation, characterization and bacterial expression of the catalytically active monoterpene cyclase. *J. Biol. Chem*., 268, 23016–23024.

Colwell, J.D. (1994). *Estimating Fertilizer Requirements, a Quantitative Approach*. CAB International, Oxon, UK.

Croteau, R. (1986). Biochemistry of monoterpenes and sesquiterpenes of the essential oils. In: *Herbs, Spices, and Medicinal Plants. Recent Advances in Botany, Horticulture and Pharmacology*, Vol. 1. Cracker, L.E., Simon, J.E. Eds, Food Product Press, New York, pp. 81–133.

Croteau, R. (1987). Biosynthesis and catabolism of monoterpenoids. *Chem. Rev*., 87, 929–954.

Croteau, R. and Gershenzon, J. (1994). Genetic control of monoterpene biosynthesis in mints (*Mentha*: Lamiaceae). In: *Genetic Engineering of Plant Secondary Metabolism*, Ellis, B.E., Kuroki, G., and Stafford, H.A., Eds, Plenum Press, New York, NY, pp. 193–228.

Croteau, R. and Hooper, C.L. (1978). Metabolism of monoterpenes. Acetylation of (−)-menthol by a soluble enzyme preparation from peppermint (*Mentha piperita*) leaves. *Plant Physiol*., 61, 737–742.

Croteau, R. and Martinkus, C. (1979). Metabolism of monoterpenes. Demonstrantion of (+)-neomenthyl-β-D-glucoside as a major metabolite of (−)-menthone in peppermint (*Mentha piperita*). *Plant Physiol*., 64, 169–175.

Croteau, R. and Venkatachalam, K.V. (1986). Metabolism of monoterpenes—demonstration that (+)-*cis*-isopulegone, not piperitenone, is the key intermediate in the conversion of (−)-isopiperitenone to (+)-pulegone in peppermint (*Mentha* ×*piperita*). *Arch. Biochem. Biophys*., 249, 306–315.

Croteau, R., Karp, F., Wagschal, K.C., Satterwhite, D.M., Hyatt, D.C., and Skotland, C.B. (1991). Biochemical characterization of a spearmint mutant that resembles peppermint in monoterpene content. *Plant Physiol*., 96, 744–752.

Croteau, R., Sood, V.K., Renstrom, A., and Bushan, R. (1984). Metabolism of monoterpenes: early steps in the metabolism of *d*-neomenthol-β-D-glucoside in peppermint (*Mentha piperita*) rhizomes. *Plant Physiol*., 76, 647–653.

Daniell, H., Streatfield, S.J., and Wycoff, K. (2001). Medical molecular farming: production of antibodies, biopharmaceuticals and edible vaccines in plants. *Trends Pl. Sci*., 6, 219–226.

Diemer, F., Caissard, J.C., Moja, S., and Jullien, F. (1999). *Agrobacterium tumefaciens*-mediated transformation of *Mentha spicata* and *Mentha arvensis. Plant Cell Tiss. Org. Cult*, 57, 75–78.

Diemer, F., Jullien, F., Faure, O., Moja, S., Colson, M., Matthys-Rochon, E., and Caissard, J.C. (1998). High efficiency transformation of peppermint (*Mentha* ×*piperita* L.) with *Agrobacterium tumefaciens. Plant Sci*., 136, 101–108.

Draper, J. and Scott, R. (1991). Gene transfer to plants. In: *Plant Genetic Engineering*, Grierson, D. Ed, Blackie, Glasgow, pp. 38–41.

Economou, D. and Nahrstedt, A. (1991). Chemical, physiological, and toxicological aspects of the essential oil of some species of the genus *Bystropogon. Planta Med*., 57, 347–351.

Eisenreich, W., Sagner, S., Zenk, M.H., and Bacher, A. (1997). Monoterpenoid essential oils are not of mevalonoid origin. *Tetrahedron Lett*., 38, 3889–3892.

El-Ketawi, N.E. and Croteau, R. (1987). Influence of plant growth regulators on oil production in *Mentha* species. *Phytochemistry*, 26, 891–902.

Evans, D.A. and Sharp, W.R. (1986). Application of somaclonal variation. *Biotechnology*, 4, 528.

Facchini, P.J. and Chappell, J. (1992). Gene family for an elicitor-induced sesquiterpene cyclase in tobacco. *Proceed. Natl. Acad. Sci. USA*, 89, 11088–11092.

Fagerstrom, T. (1989). Anti-herbivore chemical defense in plants: a note on the concept of cost. *Am. Nat*., 133, 281–287.

Fahn, A. (1979). *Secretory Tissues in Plants*. Academic Press, London.
Fahn, A. (1988a). Secretory tissues and factors influencing their development. *Phyton*, 28, 13–26.
Fahn, A. (1988b). Secretory tissues in vascular plants—Tansley review No. 14. *New Phytol*., 108, 229–257.
Farooqi, A.H.A., Samgwan, N.S., and Sangwan, R.S. (1999). Effect of different photoperiodic regimes on growth, flowering and essential oil in *Mentha* species. *Plant Growth Regul*., 29, 181–187.
Faure, O., Diemer, F., Moja, S., and Jullien, F. (1998). Mannitol and thidiazuron improve *in vitro* shoot regeneration from spearmint and peppermint leaf disks. *Plant Cell Tiss. Org. Cult*., 52, 209–212.
Fray, R.G., Wallace, A., Fraser, P.D., Valero, D., Hedden, P., Bramley, P.M., and Grierson, D. (1995). Constitutive expression of a fruit phytoene synthase gene in transgenic tomatoes causes dwarfism by redirecting metabolites from the gibberellin pathway. *Plant J*., 8, 693–701.
Fuchs, S., Zinn, S., Beck, T., and Mosandl, A. (1999). Biosynthesis of menthofuran in *Mentha ×piperita*: stereoselective and mechanistic studies. *J. Agric. Food Chem*., 47, 4100–4105.
Galun, E., Aviv, D., Dantes, A., and Freeman, A. (1983). Biotransformations by plant cells immobilised in cross-linked polyacrylmide-hydrazide. *Planta Med*., 49, 9–13.
Galun, E., Aviv, D., Dantes, A., and Freeman, A. (1985). Bioconversion of monoterpenes by γ-irradiated, supported entrapped cells of *Mentha* and *Nicotiana* species. *Planta Med*., 51, 513–525.
Gamborg, O.L., Miller, R.A., and Ojima, K. (1968). Nutrients requirements of suspension cultures of soybean root cells. *Exp. Cell. Res*., 50, 151–158.
Garcia, A.A. (1991). Strategies for the recovery of chemicals from fermentation—a review of the use of polymeric adsorbents. *Biotechnol. Prog*., 7, 33–42.
Gershenzon, J. and Croteau, R. (1993). Terpenoid biosynthesis: the basic pathway and formation of monoterpenes, sesquiterpenes and diterpenes. In: *Lipid Metabolism in Plants*, Moore, Jr, T.S., Ed, CRC Press, Boca Raton, FL, pp. 339–388.
Gershenzon, J., Maffei, M., and Croteau, R. (1989). Biochemical and histochemical localization of monoterpene biosynthesis in the glandular trichomes of Spearmint (*Mentha spicata*). *Plant Physiol*., 89, 1351–1357.
Gershenzon, J., Murtagh, G.J., and Croteau, R. (1993). Absence of rapid terpene turnover in several diverse species of terpene-accumulating plants. *Oecologia*, 96, 583–592.
Gershenzon, J., McConkey, M.E., and Croteau, R. (2000). Regulation of monoterpene accumulation in leaves of peppermint. *Plant Physiol*., 122, 205–213.
Ha, W.-H., Gun-Jo Woo, J.-H. and Hyong, J.-L., (1996). Characteristics of immobilized culture of *Mentha piperita* cells for oil production. *J. Microb. Biotechnol*., 6, 132–136.
Haudenschild, C.D. and Croteau, R. (1998). Molecular engineering of monoterpene production. In: Setlow, J.F., Ed, *Genetic Engineering*, Plenum Press, New York, NY, pp. 267–280.
Haudenschild, C., Schalk, M., Karp, F., and Croteau, R. (2000). Functional expression of regiospecific cytochrome P450 limonene hydroxylases from mint (*Mentha* spp.) in *Escherichia coli* and *Saccharomyces cerevisiae. Arch. Biochem. Biophys*., 379, 127–136.
Hilton, M.G., Jay, A., Rhodes, M.C.J., and Wilson, P.D.G. (1995). Growth and monoterpenes production by transformed shoot cultures of *Mentha citrata* and *Mentha piperita* in flasks and fermenters. *Appl. Microbiol. Biotechnol*., 43, 452–459.
Hirai, D. and Sakai, A. (1999). Cryopreservation of *in vitro*-grown axillary shoot-tip meristems of mint (*Mentha spicata* L.) by encapsulation vitrification. *Plant Cell Rep*., 19, 150–155.
Hirata, T., Murakami, S., Ogihara, K., and Suga, T. (1990). Volatile monoterpenoid constituents of the plantlets of *Mentha spicata* produced by shoot tip culture. *Phytochemistry*, 29, 493–495.
Hülskamp, M., Miséra, S., and Jürgens, G. (1994). Genetic dissection of trichome cell development in *Arabidopsis. Cell*, 76, 555–566.
Jullien, F., Diemer, F., Colson, M., and Faure, O. (1998). An optimising protocol for protoplasts regeneration of three peppermint cultivars (*Mentha ×piperita*). *Plant Cell Tiss. Org. Cult*., 54, 153–159.
Karp, A. (1994). Origins, causes and uses of variation in plant tissue cultures. In: *Plant Cell and Tissue Cultures*, Vasil, I.K. and Thorpe, T.A., Eds, Kluwer, Academic Publisher, Dordrecht, pp. 139–151.
Karp, F., Harris, J.L., and Croteau, R. (1987). Metabolism of monoterpenes: demonstration of the hydroxylation of (+)-sabinene to (+)-*cis*-sabinol by an enzyme preparation from sage (*Salvia officinalis*) leaves. *Arch. Biochem. Biophys*., 256, 179–193.

Karp, F., Mihaliak, C.A., Harris, J.L., and Croteau, R. (1990). Monoterpene biosynthesis—specificity of the hydroxylations of (−)-limonene by enzyme preparations from peppermint (*Mentha piperita*), spearmint (*Mentha spicata*), and perilla (*Perilla frutescens*) leaves. *Arch. Biochem. Biophys*., 276, 219–226.

Kawabe, S. (1991). Volatile monoterpenes constituents of plantlets of *Mentha* species produced by *in vitro* adventitious root culture. *Frag. J. (Jpn)*, 19, 70–74.

Kawabe, S., Watanabe, A., Watase, Y., Murakami, K., and Hosomi, K. (1993). Mass propagation of *Mentha arvensis* by tissue culture. *Shokubutsu Soshiki Baiyo*, 10, 184–187; Chem. Abstr., 119, 197719 (1993).

Kim, T., Kim, T.Y., Bae, G.W., Lee, H.K., Chae, Y.A., and Chung, I.S. (1996). Improved production of essential oils by two-phase culture of *Mentha piperita* cells. *Plant Tiss. Cult. Lett*., 13, 189–192.

Kjonaas, R. B. and Croteau, R. (1983). Demonstration that limonene is the first cyclic intermediate in the biosynthesis of oxygenated *p*-menthane monoterpenes in *Mentha piperita* and other *Mentha* species. *Arch. Biochem. Biophys*., 220, 79–89.

Kjonaas, R.B., Martinkus-Taylor, C., and Croteau, R. (1982). Metabolism of monoterpenes—conversion of *l*-menthone to *l*-menthol and *d*-neomenthol by stereospecific dehydrogenases from peppermint (*Mentha ×piperita*) leaves. *Plant Physiol*., 69, 1013–1017.

Kjonaas, R.B., Venkatachalam, K.V., and Croteau, R. (1985). Metabolism of monoterpenes—oxidation of isopiperitenol to isopiperitenone, and subsequent isomerization to piperitenone by soluble enzyme preparations from peppermint (*Mentha ×piperita*) leaves. *Arch. Biochem. Biophys*., 238, 46–60.

Knobloch, K., Pauli, A., Iberl, B., Weigand, H., and Weis, N. (1989). Antibacterial and antifungal properties of essential oil components. *J. Essent. Oil Res*., 1, 119–128.

Krasnyanski, S., May, R.A., Loskutov, A., Ball, T.M., and Sink, K.C. (1999). Transformation of the limonene synthase gene into peppermint (*Mentha piperita* L.) and preliminary studies on the essential oil profiles of single transgenic plants. *Theor. Appl. Genetics*, 99, 676–682.

Kukreja, A.K., Dahawan, O.P., Mathur, A.K., Ahuja, P.S., and Mandal, S. (1991). Screening and evaluation of agronomically useful somaclonal variations in Japanese mint (*Mentha arvensis* L.). *Euphytica*, 53, 183–191.

Lange, M.B. and Croteau, R. (1999a). Isoprenoid biosynthesis via a mevalonate-indipendent pathway in plants: cloning and heterologous expression of 1-deoxy-D-xylulose-5-phosphate reductoisomerase from peppermint. *Arch. Biochem. Biophys*., 365, 170–174.

Lange, B.M. and Croteau, R. (1999b). Genetic engineering of essential oil production in mint. *Curr. Opin. Plant Biol*., 2, 139–144.

Lange, M.B. and Croteau, R. (1999c). Isopentenyl diphosphate biosynthesis via a mevalonate-indipendent pathway: isopentenyl monophosphate kinase catalyzes the terminal enzymatic step. *Proceed. Natl. Acad. Sci. USA*, 96, 13714–13719.

Lange, M.B., Wildung, M., McCaskill, D., and Croteau, R. (1998). A family of transketolases that directs isoprenoid biosynthesis via a mevalonate-indipendent pathway. *Proceed. Natl. Acad. Sci. USA*, 95, 2100–2104.

Lange, B.M., Wildung, M.R., Stauber, E.J., Sanchez, C., Pouchnik, D., and Croteau, R. (2000). Probing essential oil biosynthesis and secretion by functional evaluation of expressed sequence tags from mint glandular trichomes. *Proceed. Natl. Acad. Sci. USA*, 97, 2934–2939.

Lawrence, B.M. (1978). A study of the monoterpene interrelationships in the genus *Mentha* with special reference to the origin of pulegone and menthofuran, PhD. Thesis, Groningen State University, Groningen, Netherlands.

Lawrence, B.M. (1981). Monoterpene interrelationships in the *Mentha* genus: a biosynthetic discussion. In: *Essential oils*, Mookherjee, B.D. and Mussinan, C.J., Eds, Allured Publ. Co., Wheaton, IL., pp. 1–81.

Lee, S., Tsao, R., and Coats, J.R. (1999). Insecticidal activity of monoterpenoids to western corn rootworm (Coleoptera: Chrisomelidae), twospotted spider mite (Acari: Tetranychidae) and house fly (Diptera: Muscidae). *J. Econ. Entomol*., 92, 56–67.

Lichtenthaler, H.K. (1998). The plant's 1-deoxy-D-xylulose-5-phosphate pathway for biosynthesis of isoprenoids. *Fett/Lipids*, 100, 128–138.

Lichtenthaler, H.K. (1999). The deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. *Ann. Rev. Plant Physiol. Mol. Biol.*, 50, 47–65.

Lin, M-L and Staba, E.J. (1963). Peppermint and spearmint tissue cultures. I. Callus formation and submerged culture. *Lloydia*, 24, 139–145.

Lupien, S., Karp, F., Ponnamperuma, K., Wildung, M., and Croteau, R. (1995). Cytochrome P450 hydroxylases of *Mentha* species. *Drug Metab. Drug Interact.*, 12, 245–260.

Lupien, S., Karp, F., Wildung, M., and Croteau, R. (1999). Regiospecific cytochrome P450 limonene hydroxylases for mint (*Mentha*) species. cDNA isolation, characterization, and functional expression of (−)-4S-Limonene-3-hydroxylase and (−)-4S-Limonene-6-hydroxylase. *Arch. Biochem. Biophys.*, 368, 181–192.

Maffei, M. (1988). Environmental factors affecting the oil composition of some *Mentha* species grown in North West Italy. *Flav. Fragr. J.*, 3, 79–84.

Maffei, M. (1990). Plasticity and genotypic variation in some *Mentha* ×*verticillata* hybrids. *Biochem. Syst. Ecol.*, 18, 493–502.

Maffei, M. (1999). Allelopathic effects of peppermint essential oil in higher plants. Oral communication 2-05. 30th International Symposium on Essential Oils, Leipzig, September 5–8.

Maffei, M. and Codignola, A. (1990). Photosynthesis, photorespiration and herbicide effect on terpene production in peppermint (*Mentha piperita* L.). *J. Essent. Oil Res.*, 2, 275–286.

Maffei, M. and Camusso, W. (2000). Allelopathic effects of peppermint essential oil in higher plants: intracellular calcium variations. Oral communication L-21; 31st International Symposium on Essential Oils, Hamburg, September 10–13.

Maffei, M. and Scannerini, S. (1999). Photomorphogenic and chemical responses to blue light in *Mentha piperita*. *J. Essent. Oil Res.*, 11, 730–738.

Maffei, M. and Scannerini, S. (2000). UV-B effect on photomorphogenesis and essential oil composition in peppermint (*Mentha piperita* L.). *J. Essent. Oil Res.*, 12, 523–529.

Maffei, M., Camusso, W., and Sacco, S. (2001). Effects of *Mentha* ×*piperita* essential oil and monoterpenes on cucumber root membrane potential. *Phytochemistry*, 58, 703–707.

Maffei, M., Canova, D., Bertea, C.M., and Scannerini, S. (1999). UV-A effects on photomorphogenesis and essential oil composition in *Mentha piperita*. *Photochem. Photobiol. B: Biology*, 52, 105–110.

Maffei, M., Chialva, F., and Sacco, T. (1989). Glandular trichomes and essential oils in developing peppermint leaves. I. Variation of peltate trichome number and terpene distribution within leaves. *New Phytologist*, 111, 707–716.

Maffei, M., Gallino, M., and Sacco, T. (1986). Glandular trichomes and essential oils of developing leaves in *Mentha viridis lavanduliodora*. *Planta Med.*, 52, 187–193.

Maffei, M., Mucciarelli, M., and Scannerini, S. (1993a). Paraquat on carvone metabolism in *Mentha*. *J. Essent. Oil Res.*, 5, 651–657.

Maffei, M., Peracino, V., and Sacco, T. (1993b). Multivariate methods for aromatic plants: an application to mint essential oils. *Acta Horticulturae*, 330, 159–169.

Malingré, T.M. (1971). Chemotaxonomisch onderzock von *Mentha arvensis* L. *Pharm. Weekblad*, 106, 165–171.

Marengo, E., Baiocchi, C., Gennaro, M.C., Bertolo, P.L., Lanteri, S., and Garrone, W. (1991). Classification of essential mint oils of different geographic origin applying pattern recognition methods to gas chromatographic data. *Chemometr. Intell. Lab. Sys.*, 11, 75–88.

Martin, G.B., Brommonschenkel, S.H., Chungwongse, J., Frary, A., Ganal, M.W., Spivey, R., Wu, T., Earle, E.D., and Tanksley, S.D. (1993). Map-based cloning of a protein kinase gene conferring disease resistance in tomato. *Science*, 262, 1432–1436.

Martinkus, C. and Croteau, R. (1981). Metabolism of monoterpenes. Evidence for compartmentation of *l*-menthone metabolism in peppermint (*Mentha piperita*) leaves. *Plant Physiol.*, 68, 99–106.

Mau, C.J. and West, C.A. (1994). Cloning of casbene synthase cDNA: evidence for conserved structural features among terpenoid cyclases in plants. *Proceed. Natl. Acad. Sci. USA*, 91, 8497–8501.

McCaskill, D. and Croteau, R. (1995). Monoterpene and sesquiterpene biosynthesis in glandular trichomes of peppermint (*Mentha* ×*piperita*) rely exclusively on plastid-derived isopentenyl diphosphate. *Planta*, 197, 49–56.

McCaskill, D.G. and Croteau, R. (1997). Prospects for the bioengineering of isoprenoid biosynthesis. *Adv. Biochem. Engineer./biotechnol.*, 55, 107–146.

McCaskill, D.G. and Croteau, R. (1999a). Isopentenyl diphosphate is the terminal product of the deoxyxylulose-5-phosphate pathway for terpenoid biosynthesis in plants. *Tetrahedron Letters*, 40, 653–656.

McCaskill, D. and Croteau, R. (1999b). Strategies for bioengineering the development and metabolism of glandular tissues in plants. *Nature Biotechnology*, 17, 31–36.

McCaskill, D., Gershenzon, J., and Croteau, R. (1992). Morphology and monoterpene biosynthetic capabilities of secretory cell clusters isolated from glandular trichomes of peppermint (*Mentha piperita* L.). *Planta*, 187, 445–454.

McConkey, M.E., Gershenzon, J., and Croteau, R.B. (2000). Developmental regulation of monoterpene biosynthesis in the glandular trichomes of peppermint. *Plant Physiol.*, 122, 215–223.

Medou, G., Faure, O., Jullien, F., Colson, M., and Perrin, A. (1997). Caulogenesis and terpenic synthesis *in vitro* in peppermint. *Acta Bot. Gall.*, 144, 371–379.

Mihaliak, C.A., Gershenzon, J., and Croteau, R. (1991). Lack of rapid monoterpene turnover in rooted plants: implications for theories of plant chemical defense. *Oecologia*, 87, 373–376.

Mitchell, A.R. and Farris, N.A. (1996). Peppermint response to nitrogen fertilizer in an arid climate. *J. Plant Nutrit.*, 19, 955–967.

Miyazaki, J.H. and Croteau, R. (1990). Immobilization of cyclase enzymes for the production of monoterpenes and sesquiterpenes. *Enzyme Microb. Technol.*, 12, 841–845.

Mucciarelli, M., Camusso, W., Bertea, C.M., Bossi, S., and Maffei, M. (2001). Effect of (+)-pulegone and other oil components of *Mentha* × *piperita* on cucumber respiration. *Phytochemistry*, 57, 91–98.

Mucciarelli, M., Sacco, T., Bricarello, M., Scannerini, S., and Maffei, M. (1995). Oli Essenziali di *Mentha* × *piperita* micropropagata *in vitro*. *Rivista Italiana Eppos*, 17, 19–30.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*, 15, 473–497.

Murray, M.J. (1961). Spearmint rust resistance and immunity in the genus *Mentha*. *Crop. Sci.*, 1, 175–179.

Murray, M.J. (1969). Successful use of irradiation breeding to obtain *Verticillium* resistant strains of peppermint, *Mentha piperita* L. In: *Induced Mutations in Plants*. IAEA, Vienna, pp. 345–370.

Nádaská, M., Erdelsky, K., and Cupka, P. (1990). Improvement of *Mentha piperita* L. cv. Perpeta by means of *in vitro* micropropagation and stabilization of contained substances. *Biologia* (Bratislava), 45, 955–959.

Nakayama, T., Higashiyama, T., Sakata, I., and Hashizume, T. (1970). A new Japanese peppermint "Shubi" developed in Okayama. *Koryo*, 97, 47–56.

Nishimura, H. and Noma, Y. (1996). Microbial transformation of monoterpenes: flavor and biological activity. *Biotechnol. Improv. Foods Flav.*, 637, 173–187.

Niu, X., Li, X., Veronese, P., Bressan, R.A., Weller, S.C., and Hasegawa, P.M. (2000). Factors affecting *Agrobacterium tumefaciens*-mediated transformation of peppermint. *Plant Cell Rep.*, 19, 304–310.

Niu, X., Lin, K., Hasegawa, P.M., Bressan, R.A., and Weller, S.C. (1998). Transgenic peppermint (*Mentha* × *piperita*) plants obtained by cocultivation with *Agrobacterium tumefaciens*. *Plant Cell Rep.*, 17, 165–171.

Park, S.H. and Kim, S.U. (1998). Modified monoterpenes from biotransformation of (−)-isopiperitenone by suspension culture of *Mentha piperita*. *J. Nat. Prod.*, 61, 354–357.

Park, S.H., Chae, Y.A., Lee, H.J., Lim, Y.H., and Kim, S.U. (1994). Production of (−)-7-hydroxyisopiperitenone from (−)-isopiperitenone by a suspension cell culture of *Mentha piperita*. *Planta Med.*, 60, 374–375.

Park, S.H., Chang, Y.J., Kim, K.H., and Kim, S.U. (1999). Involvement of cytochrome P450 in (−)-(4R)-isopiperitenone oxidation by cell suspension cultures of *Mentha piperita*. *J. Microbiol. Biotech.*, 9, 147–149.

Park, S.H., Kim, K.S., Suzuki, Y., and Kim, S.U. (1997). Metabolism of isopiperitenones in cell suspension cultures of *Mentha piperita*. *Phytochemistry*, 44, 623–626.

Pichersky, E., Lewinsohn, E., and Croteau, R. (1995). Purification and characterization of *S*-linalool synthase, an enzyme involved in the production of floral scent in *Clarkia breweri*. *Arch. Biochem. Biophys*., 316, 803–807.

Pickett, J.A. (1991). Lower terpenoids as natural insect control agent. In: *Ecological Chemistry and Biochemistry of Plant Terpenoids*, Harborne, J.B. and Tomas-Barberan, F.A., Eds, Clarendon Press, Oxford, pp. 297.

Rajaonarivony, J.I.M., Gershenzon, J., and Croteau, R. (1992). Characterization and mechanism of 4*S*-limonene synthase, a monoterpene cyclase from the glandular trichomes of peppermint (*Mentha* × *piperita*). *Arch. Biochem. Biophys*., 296, 49–57.

Rech, L. and Pires, M.J.P. (1986). Tissue culture propagation of *Mentha* spp. by the use of axillary buds. *Plant Cell Rep*., 5, 17–18.

Rice, E.L. (1984). *Allelopathy*. 2nd edn. Academic Press Inc., Orlando, FL.

Rodov, V.S., Reznikova, S.A., and Melnikov, V.N. (1983). Monoterpenes transformation in suspended cultures of *Mentha piperita* cells. *Rastit Resur*., 19, 92–97.

Robinson, T., (1983). *The Organic Constituents of Higher Plants*. 5th edn. Cordus Press, North Amherst, MA.

Ruminska, A. and Neweglowska, A. (1965). Influence of shading of peppermint (*Mentha piperita* L.) on some of its morphological features, on the yield and content of volatile oil and reducing sugars. *Acta Pol. Pharmac*., 22, 480–487.

Sacco, T., Peracino, V., and Maffei, M. (1992). Phenotypic plasticity in *Mentha viridis lavanduliodora*. *J. Essent. Oil Res*., 4, 491–496.

Sagner, S., Eisenreich, W., Fellermeier, M., Latzel, C., Bacher, A., and Zenk, M.H. (1998). Biosynthesis of 2-C-methyl-D-erythritol in plants by rearrangement of the terpenoid precursor 1-deoxy-D-xylulose-5-phosphate. *Tetrahedron Lett*., 39, 2091–2094.

Sakata, I. and Koshimizu, K. (1978). Occurrence of *l*-menthyl-β-D-glucoside and methyl palmitate in rhizoma of Japanese peppermint. *Agric. Biol. Chem*., 42, 1959–1960.

Sakata, I. and Mitsui, T. (1975). Isolation and identification of *l*-menthyl-β-D-glucoside from shubi. *Agric. Biol. Chem*., 39, 1329–1330.

Sanders, D. and Bethke, P. (2000). Membrane transport. In: *Biochemistry & Molecular Biology of Plants*. Buchanan, B., Gruissem, W., Jones, R., Eds, American Society of Plant Physiologists, Rockville, MD, pp. 110–159.

Sato, H., Enomoto, S., Oka, S., Hosomi, K., and Ito, Y. (1993). Plant regeneration from protoplasts of *peppermint* (*Mentha piperita* L.). *Plant Cell Rep*., 12, 546–550.

Sato, H., Yamada, K., Mii, M., Hosomi, K., Okuyama, S., Uzawa, M., Ishikawa, H., and Ito, Y. (1996). Production of an interspecific somatic hybrid between peppermint and gingermint. *Plant Sci*., 115, 101–107.

Savage, T.J., Ichii, H., Hume, S.D., Little, D.B., and Croteau, R. (1995). Monoterpene synthases from gymnosperms and angiosperms: stereospecificity and inactivation by cysteinyl- and arginyl-directed modifying reagents. *Arch. Biochem. Biophys*., 320, 257–265.

Schalk, M. and Croteau, R. (2000). A single amino acid substitution (F3631) converts the regiochemistry of the spearmint (−)-limonene hydroxylase from a C6- to a C8-hydroxylase. *Proceed. Natl. Acad. Sci. USA*, 97, 11948–11953.

Schwarz, M.K. (1994). Terpenbiosynthese in Ginkgo Biloba. PhD Thesis. Eidgenoss. Tech. Hochsch. Zürich, Switzerland.

Shanker, S., Ajayakumar, P.V., Sangwan, N.S., Kumar, S., and Sangwan, R.S. (1999). Essential oil gland number and ultrastructure during *Mentha arvensis* leaf ontogeny. *Biol. Plant*., 42, 379–387.

Shasany, A.K., Khanuja, S.P.S., Dhawan, S., and Kumar, S. (2000). Positive correlation between menthol content and *in vitro* menthol tolerance in *Mentha arvensis* L. cultivars. *J. Biosci*., 25, 263–266.

Shasany, A.K., Khanuja, S.P.S., Dhawan, S., Yadav, U., Sharma, S., and Kumar, S. (1998). High regenerative nature of *Mentha arvensis* internodes. *J. Biosci*., 23, 641–649.

Shimizu, S., Shibata, H., and Maejima, S. (1990a). A new monoterpene glucoside, *l*-menthyl-6′-*O*-acztyl glucoside. *J. Essent. Oil Res*., 2, 21–24.

Shimizu, S., Shibata, H., Karasawa, D., and Kozaki, T. (1990b). Carvyl- and dihydrocarvyl-β-D-glucosides in spearmint (Studies on terpene glycosides in *Mentha* plants, Part II). *J. Essent. Oil Res*., 2, 81–86.

Son, J.S., Chang, Y.J., Choi, Y.D., and Kim, S.U. (1998). Role of jasmonic acid in biotransformation of (−)-isopiperitenone in suspension cell culture of *Mentha piperita. Molecule and Cells*, 8, 366–369.

Spencer, A., Hamill, J.D., and Rhodes, M.J.C. (1990). Production of terpenes by differentiated shoot cultures of *Mentha citrata* transformed with *Agrobacterium tumefaciens* T37. *Plant Cell Rep*., 8, 601–604.

Spencer, A., Hamill, J.D., and Rhodes, M.J.C. (1993). *In vitro* biosynthesis of monoterpenes by *Agrobacterium* transformed shoot cultures of two *Mentha* species. *Phytochemistry*, 32, 911–919.

Street, I.P., Coffman, H.R., Baker, J.A., and Poulter, C.D. (1994). Identification of Cys139 and Glu207 as catalitically important groups in the active site of isopentenyldiphosphate: dimethyl-allyldiphosphate isomerase. *Biochemistry*, 33, 4212–4217.

Takahashi, S., Kuzuyama, T., Watanabe, H., and Seto, H. (1998). A 1-deoxy-D-xylulose 5-phosphate reductoisomerase catalyzing the formation of 2-*C*-methyl-*D*-erythritol 4-phosphate in an alternative nonmevalonate pathway for terpenoid biosynthesis. *Proc. Natl. Acad. Sci. USA*, 95, 9879–9884.

Tisserat, B. (1985). Embyogenesis, organogenesis and plant regeneration. In: *Plant Cell Culture. A Practical Approach*, Dixon, R.A., Ed, IRL Press, Oxford, pp. 79–105.

Tisserat, B. and Silman, R. (2000). Interactions of culture vessels, media volume, culture density, and carbon dioxide levels on lettuce and spearmint shoot growth *in vitro. Plant Cell Rep*., 19, 464–471.

Todd, W.A. and Murray, M.J. (1968). New essential oils from hybridization of *Mentha citrata* Ehrh. *Perfum. Essent. Oil Rec*., 59, 97–102.

Turner, G., Gershenzon, J., Nielson, E.E., Froehlich, and Croteau. R. (1999). Limonene synthase, the enzyme responsible for monoterpene biosynthesis in peppermint, is localized to leucoplasts of oil gland secretory cells. *Plant Physiol*., 120, 879–886.

Turner, G.W., Gershenzon, J., and Croteau, R.B. (2000a). Distribution of peltate glandular trichomes on developing leaves of peppermint. *Plant Physiol*., 124, 655–663.

Turner, G.W., Gershenzon, J., and Croteau, R.B. (2000b). Development of peltate glandular trichomes of peppermint. *Plant Physiol*., 124, 665–679.

Van Eck, J.M. and Kitto, S.L. (1990). Callus initiation and regeneration in *Mentha. Hortsci*., 25, 804–806.

Van Eck, J.M. and Kitto, S.L. (1992). Regeneration of peppermint and orange mint from leaf disks. *Plant Cell Tiss. Org. Cult*., 30, 41–49.

Vogel, B.S., Wildung, M.R., Vogel, G., and Croteau, R. (1996). Abetadiene synthase from grand fir (*Abies grandis*). cDNA isolation, characterization, and bacterial expression of a bifunctional diterpene cyclase involved in resin acid biosynthesis. *J. Biol. Chem*., 271, 23262–23268.

Werker, E. (1993). Function of essential oil-secreting glandular hairs in aromatic plants of the Lamiaceae—a review. *Flav. Fragr. J*., 8, 249–255.

Werker, E., Ravid, U., and Putievsky, E. (1985). Structure of glandular hairs and identification of the main components of their secreted material in some species of the Labiatae. *Isr. J. Bot*., 34, 31–45.

Werrmann, U. and Knorr, D. (1993). Conversion of menthyl acetate or neomenthyl acetate into menthol or neomenthol by cell suspension cultures of *Mentha canadensis* and *Mentha piperita. J. Agric. Food Chem*., 41, 517–520.

White, I.G.H., Iskandar, S.H., and Barnes, M.F. (1987). Peppermint effect of time of harvest on yield and quality of oil. *New Zeal. J. Exper. Agric*., 15, 73–79.

Wink, M. (1990). Physiology of secondary product formation in plants. In: *Proceedings of the Phytochemical Society of Europe 30*, Charlwood, B.V. and Rhodes, M.J.C., Eds, Oxford Science Publications, Clarendon Press, Oxford, pp. 29–30.

Wise, M.L. and Croteau, R. (1999). Monoterpene biosynthesis. In: *Comprehensive Natural Products Chemistry: Isoprenoids*, Cane, D.E., Ed, Elsevier Science, Oxford, UK, pp. 97–153.

Yuba, A., Yazaki, K., Tabata, M., Honda, G., and Croteau, R. (1996). cDNA cloning, characterization, and functional expression of 4*S*-(−)-limonene synthase from *Perilla frutescens. Arch. Biochem. Biophys*., 332, 280–287.

3 Commercial Mint Species Grown in the United States

Mark A. Morris

CONTENTS

3.1 INTRODUCTION

The mint family (Labiatae or Lamiaceae) is composed of many species grown commercially for their oils or leaves, including basil, lavender, peppermint, sage, spearmint, and rosemary. Not only are such species desired for their flavors and fragrances, but many have been used for centuries as folk remedies and at present they are regaining mainstream acceptance for their medicinal properties (Brown, 1995; Beckstrom-Sternberg and Duke, 1996; Newall et al., 1996). One example is peppermint, considered to provide relief for a wide range of ailments, from tension headaches to irritable bowel syndrome (Blumenthal, 2003).

In the last century, the U.S. Mint Industry was very innovative and research oriented. Findings on topics ranging from basic agronomy to pest and weed control have been reported in a diversity of peer-reviewed journals, mint industry publications, and extension bulletins. The objective of this review, which focuses on agricultural practices employed in the production of U.S.-grown peppermint (*Mentha ×piperita*), Native spearmint (*Mentha spicata*), and Scotch spearmint (*Mentha ×gracilis*) for essential oil, is to update the current literature on U.S. mint agronomy and suggest areas where additional research is needed.

3.2 HISTORY OF U.S. PEPPERMINT AND SPEARMINT PRODUCTION

3.2.1 Origins and Varietal Identification

The impact of different agricultural practices often depends on the particular mint species or variety grown. For this reason, an important aspect of mint agriculture is correct identification of the species and variety to be cultivated.

Identifying species in the genus *Mentha* is complicated by the extensive hybridization thought to have occurred within this group (Tucker and DeBaggio, 2000). Because the ability to determine species based on sexual crosses is limited, pinpointing the hypothesized origins of specific blocks of peppermint and spearmint is currently based on a combination of data, including plant morphology, essential oil composition, chromosome numbers, and the artificial resynthesis of species (Murray et al., 1972; Tucker and Chambers, 2002). There are many variables to consider with each of these techniques. For example, both morphology and essential oil composition of *Mentha* species may vary depending on environmental factors. Varietal identification is even more challenging because differences in morphology or oil quality are often barely discernible. For these reasons, genetic analysis may soon play a key role in distinguishing among mint species and varieties. Interestingly, such methods may also help clarify the botanical origins of mint species themselves.

Three different genetic approaches have been used to determine the genetic relationships and possible origins of peppermint and spearmint. The first method focused on randomly amplified polymorphic DNA (RAPD). Benefits of RAPD include simplicity and low cost because prior knowledge of a plant's genetic sequence is not required. Fenwick and Ward (2001) studied RAPD's ability to accurately identify peppermint and spearmint varieties. Despite testing hundreds of primers, they were unable to distinguish among six of the eight commercial peppermint and native spearmint types. Shasany et al. (2002) evaluated RAPD to assess the genetic relationship among 15 native spearmint accessions from India. The authors

concluded that the results of RAPD analysis were better for this purpose than the traditional approach of comparing plant morphologies and chemistries. Even so, few researchers currently use RAPD analysis because results are not as consistent as with other genetically based methods.

Amplified fragment length polymorphism (AFLP) is one of the more reliable methods used to characterize genetic relationships among plants, and has been used successfully to group different mint plants into major taxonomic clusters (Gobert et al., 2002). Like RAPD, AFLP requires no knowledge of genetic sequences. However, it is difficult to use and may not be sensitive enough to distinguish among closely related individuals.

Analysis of single sequence repeats (SSRs), also called microsatellite DNA markers, is another approach to distinguish different mint plants. Microsatellites are very reliable and easy to use, but their application requires genetic sequencing information that can be difficult and expensive to obtain. Liu and Blouin (in preparation) have developed 22 SSR markers capable of differentiating among several species and varieties in the genus *Mentha*. Additional SSR markers are needed, however, to distinguish among other closely related mint plants.

3.2.1.1 Peppermint

Peppermint (*Mentha ×piperita*) is considered to be a hybrid of *M. aquatica* and *M. spicata* (Murray et al., 1972; Tucker and DeBaggio, 2002). This species was first cultivated in the Mediterranean basin and by the late 1700s was commercially grown in England. The peppermint variety White Mitcham was introduced into Massachusetts from England sometime around the late 1700s or early 1800s, and production soon migrated westward to the states of New York, Michigan, Ohio, and Indiana (Nelson, 1950; Landing, 1969; Vessels, 1984). The peppermint variety Black Mitcham was later imported into Michigan from England around 1883. Because of superior vigor compared with White Mitcham, Black Mitcham or varieties derived from it have become the standards of U.S. peppermint production.

In addition to Black Mitcham, there are six peppermint varieties available in the U.S. recognized by the Mint Industry Research Council (MIRC). The varieties Murray Mitcham, Roberts Mitcham, Todd Mitcham, M-83-7, and B-90-9 were all derived from Black Mitcham through mutation breeding aimed at developing peppermint more resistant to Verticillium wilt disease. (A discussion of Verticillium resistance is presented in the section on disease management.)

3.2.1.1.1 Production Trends

After the arrival of Black Mitcham, peppermint acreage shifted to new areas of the Northeast and then westward as the productivity of established growing regions declined and demand for peppermint oil increased. The decline of U.S. peppermint-growing regions is most often blamed on Verticillium wilt, although other factors, such as the mint flea beetle (MFB) (*Longitarsus ferrugineus*), could also have contributed. The flea beetle was responsible for decimating peppermint plantations in England as well as the midwestern and western U.S. (Vessels, 1984).

Peppermint production moved westward into Idaho in 1903, western Oregon around 1909, western Washington State in 1917, and south-central Washington by 1927. Commercial peppermint cultivation was attempted in California but was not widely successful, possibly because of unacceptable oil quality (Landing, 1969). Today most U.S. peppermint oil is produced in states located north of the 40th parallel, areas considered to produce the best quality oil (Green, 1963).

From the mid-1960s until 1987, U.S. peppermint acreage fluctuated between 60,000 acres (ca. 25,000 ha) and 107,000 acres (ca. 40,500 ha) then peaked at 149,000 acres (ca. 60,300 ha) in 1995 (Figure 3.1). Acreage erosion began in 1996 with an economic downturn in foreign markets, primarily in the Pacific Rim countries, and continues today because of intense global cost pressures and trends toward high-intensity mint flavors (Figure 3.1).

With U.S. peppermint growers facing increased competition from foreign producers, domestic acreage has shifted from the high-cost growing regions of central and western Oregon to south-central Washington and western Idaho, where yields are higher (Figure 3.2). Thanks to this regional shift and to the U.S. Mint Industry's substantial commitment to agricultural research, although U.S. mint acreage has declined, yields have increased: from an average of about 60 lb of oil per acre (67.25 kg/ha) in the 1960s to more than 100 lb of oil per acre (ca. 112 kg/ha) (Figure 3.3). The largest yield increases have been in south-central Washington and Idaho (Figure 3.4).

3.2.1.2 Spearmint

Perhaps because spearmint has never been as popular a flavor as peppermint, there is less information on it. Two species of spearmint are grown commercially in the U.S. today: Scotch spearmint and Native spearmint.

Scotch spearmint (*Mentha* ×*gracilis*) is believed to have originated in Europe and imported into the U.S. from Scotland. Commercial interest in this variety increased around 1908 because it yielded more than Native spearmint (Landing, 1969). Four cultivars of Scotch spearmint are recognized today by the U.S. Mint Industry: standard Scotch, cvs. 213, 227, and 770.

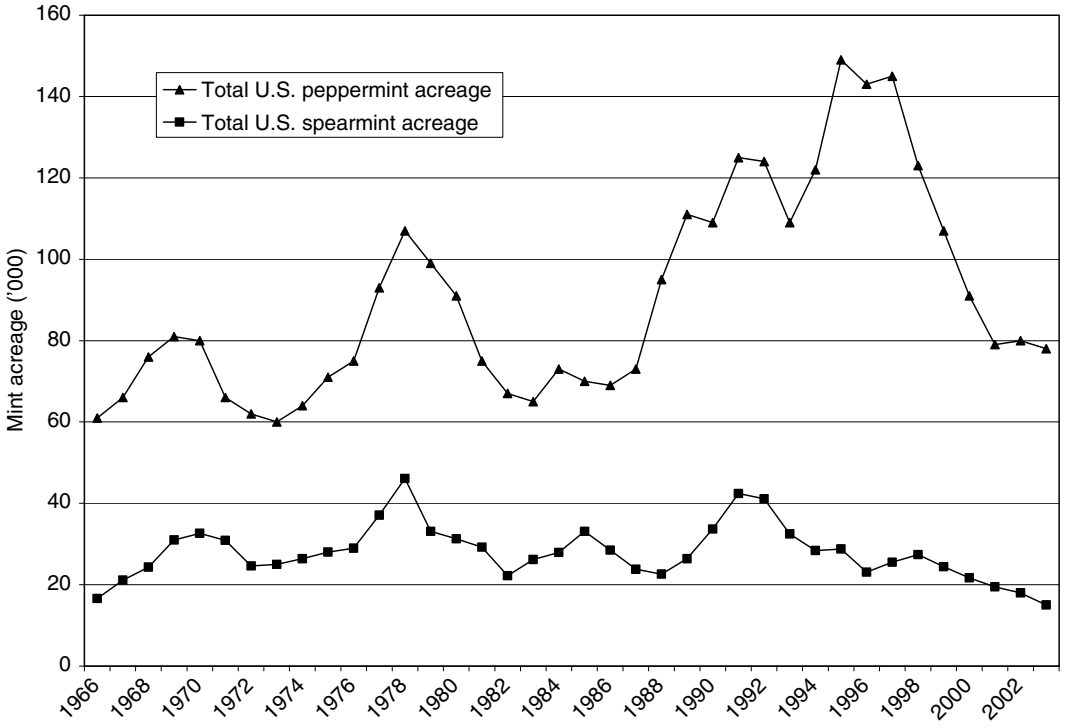


FIGURE 3.1 U.S. peppermint and spearmint acreage trends from 1966 through 2003.

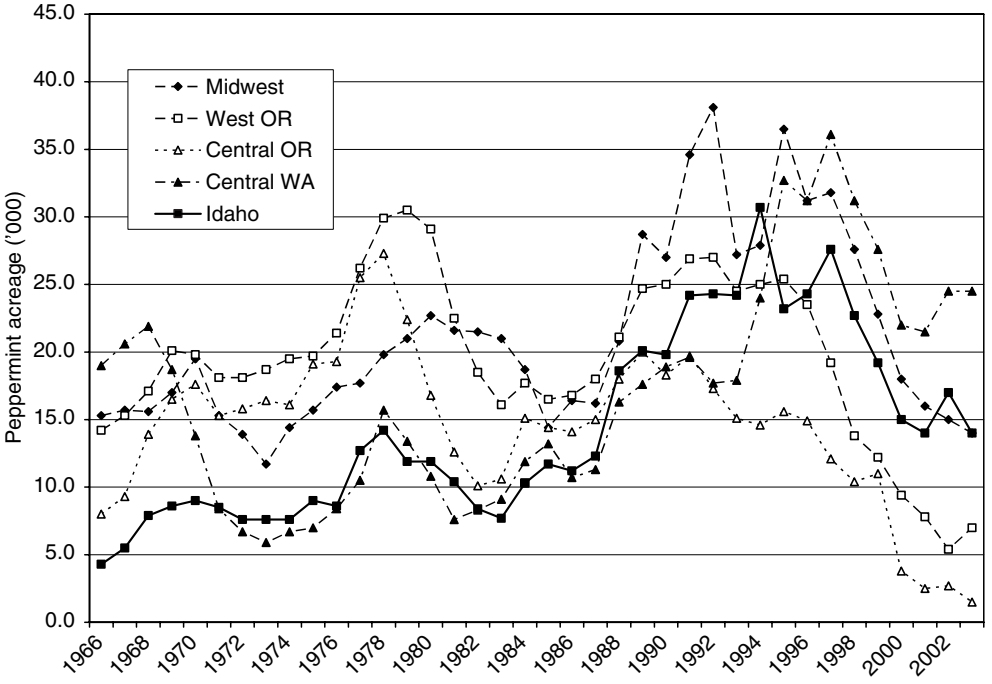


FIGURE 3.2 Peppermint acreage trends for the major U.S. mint-producing regions from 1966 through 2003.

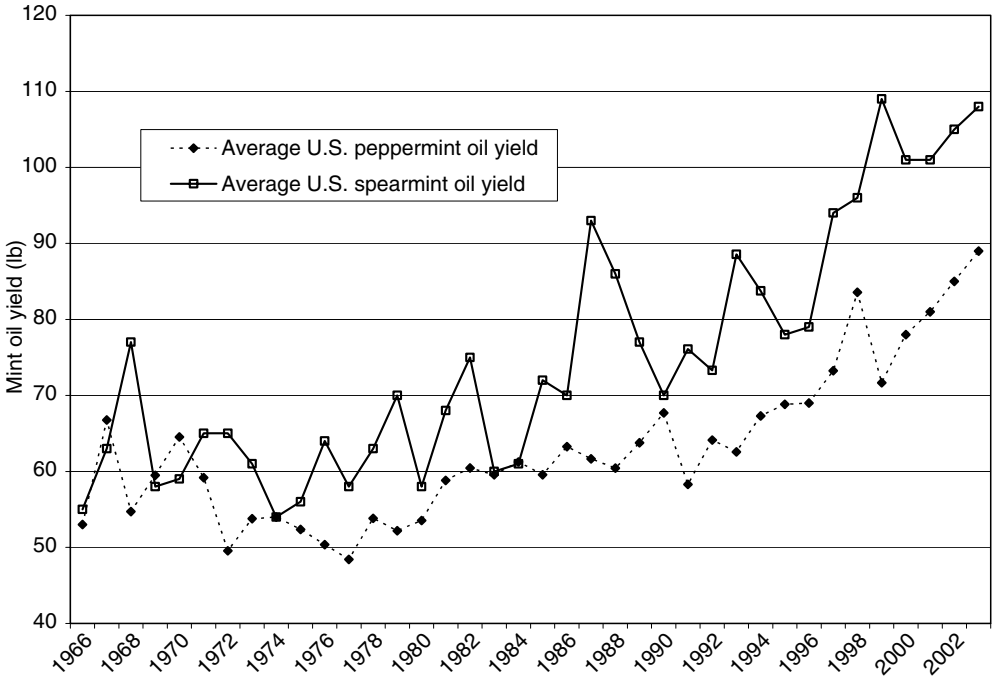


FIGURE 3.3 U.S. peppermint and spearmint oil yield trends from 1966 through 2003.

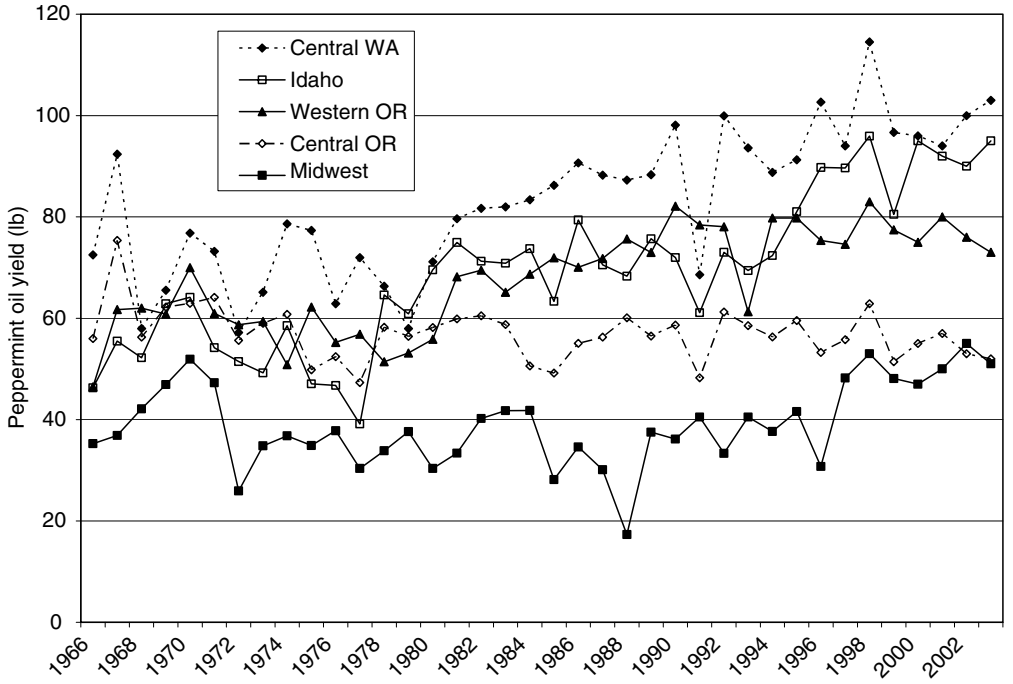


FIGURE 3.4 Peppermint oil yield trends for the major U.S. mint-producing regions from 1966 through 2003.

Native spearmint is usually referred to as *Mentha spicata*, although *M* × *villoso-nervata* has been proposed (Tucker and DeBaggio, 2000). Believed to be of European origin, Native spearmint was brought to the U.S. by colonists in the late 1700s or early 1800s and subsequently spread throughout the country (Landing, 1969). In the 19th and early 20th century, Scotch spearmint generally yielded 50% more oil than Native spearmint; however, this is not always the case today. When grown in the rich fertile soils of south-central Washington, Native spearmint is capable of yielding upward of 200 lb of oil per acre. Perhaps the variety of Native spearmint also is different today from what it was in the early 1800s. The U.S. Mint Industry recognizes two varieties of Native spearmint: standard Native and N-83-5.

3.2.1.2.1 Production Trends

Although spearmint acreage has always trailed that of peppermint, acreage trends are similar (Figure 3.1). Spearmint acreage ranged from 10,000 acres (ca. 4050 ha) in 1966 to 46,000 acres (ca. 18,620 ha) in 1978 (Figure 3.1). Like peppermint, spearmint acreage has shrunk since the mid-1990s; as of 2003, spearmint acreage had decreased to 18,000 acres (ca. 7285 ha). However, like peppermint, spearmint oil yields have increased since the 1960s (Figure 3.3), with the largest increase occurring in the growing regions of the western U.S. (Figure 3.5).

3.3 MINT AGRONOMY IN THE UNITED STATES

Mint cultivation is truly a combination of both art and science, and there is no simple, straightforward recipe for growing peppermint and spearmint. However, because the global market for peppermint oil has become increasingly competitive, agricultural practices that maintain oil quality while managing production costs are more crucial today than ever before.

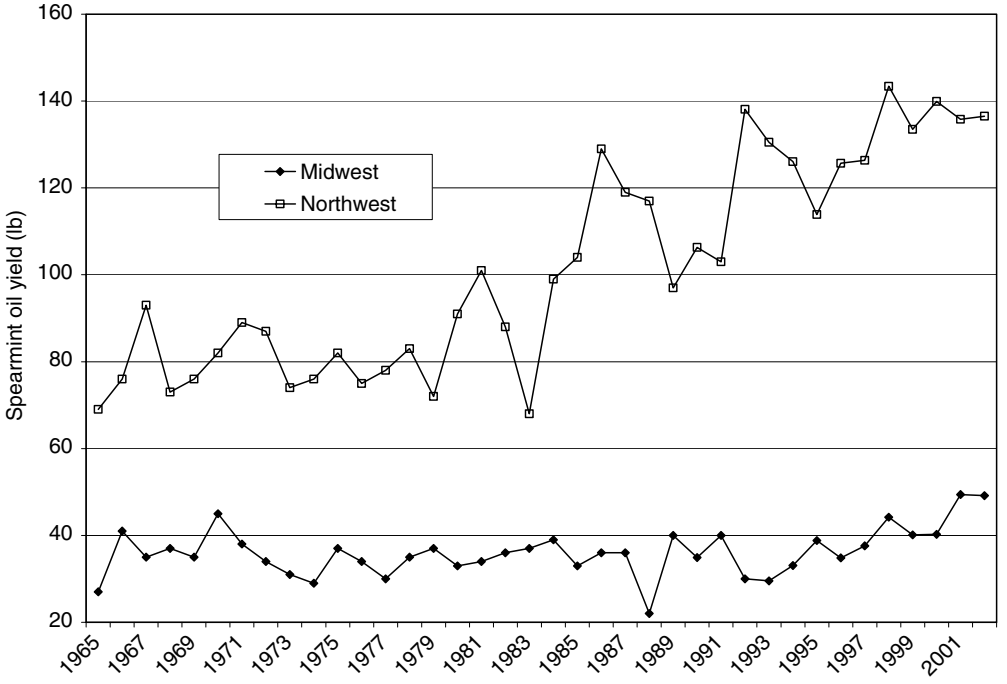


FIGURE 3.5 Spearmint oil yield trends for mint-producing regions of the Midwest and western U.S.

To cultivate mint successfully, the grower must consider many factors, from what mint cultivar to grow to when the crop should be harvested for its oil. Mint growers and researchers from different regions vary widely in their views regarding agricultural practices. One reason for this diversity of opinion is the considerable variation in research data among and within growing regions. Another confounding factor is the variable success of applying research and growing practices from one mint cultivar to another. Whatever agricultural practices a grower chooses to adopt, the bottom-line consideration of cost must be weighed if U.S. mint growers wish to remain competitive in today's more complex global mint market.

3.3.1 Selection of the Agricultural Ecosystem

One of the first considerations in establishing a mint farm is whether the location is suitable. Peppermint and spearmint can survive under a wide range of climatic and soil conditions, but the ultimate question is whether they can be grown profitably. While profitable mint production continues to depend on oil quality as well as cost, the focus has increasingly shifted to reducing oil cost, and many components of the agricultural ecosystem can influence a mint farm's profitability.

3.3.1.1 Climate

Day length, temperature, and precipitation affect mint oil yield and quality, and these climatic factors are important considerations when deciding where to plant a mint farm.

3.3.1.1.1 Day Length

Peppermint grown under long-day conditions often yields more oil than plants grown under short-day conditions (Langston and Leopold, 1954; Burbott and Loomis, 1967; Clark and

Menary, 1980a). In fact, day length in excess of 15 h is recommended for satisfactory oil yields (Green, 1963). Langston and Leopold (1954) concluded that a long photoperiod is necessary for, among other things, floral differentiation and oil synthesis. Most peppermint production occurs at latitudes above the 40th parallel where the day length is considered optimal. However, peppermint is increasingly grown near the 28th parallel in places like India, where lower production costs may justify lower oil yields and differences in oil quality.

Other environmental factors, like temperature and elevation, can interact with photoperiod to counteract the effect of short day length and allow commercial peppermint production at lower latitudes. One model proposed by Dwayne Johnson, director of the Yellowjacket Southwestern Research Center in Colorado, states that the increase in temperature for every 100 miles traveled southward is negated by a 1000-foot increase in elevation. Therefore, Yellowjacket, Colorado, should have a similar climate as Kalispell, Montana. Higher elevations also result in higher levels of ultraviolet light and a thinner atmosphere, but less is known about how these factors affect mint production.

Differences in plant morphology also exist between plants grown under long-day and short-day conditions. Long-day plants often have larger leaves, a more erect habit, and fewer stolons than to short-day plants. These morphological changes may contribute to differences in oil yield, and there is some debate as to whether the photoperiod also affects oil composition. Burbott and Loomis (1967) observed that oil composition remained constant at different photoperiods. In contrast, in studies by Clark and Menary (1979, 1980a), mint grown in long-day conditions produced oil with more menthone and menthol and less menthofuran than short-day conditions.

3.3.1.1.2 Temperature

Variations in seasonal and diurnal temperature patterns influence not only mint growth, plant development, and oil quality (Biggs and Leopold, 1955; Burbott and Loomis, 1967), but also mint survival. For example, extreme cold during the winter months may damage a mint stand despite adequate temperatures during the growing season. Even if winter survival is poor during one out of 4 years, profitable mint production may not be realized.

Different mint species vary in their tolerance to cold. Scotch spearmint and peppermint are more cold tolerant than Native spearmint. In Bio-freezer studies, Welty et al. (1997) showed that peppermint stolons survived better than Scotch spearmint stolons at 10°F (−12.2°C), and Scotch spearmint stolons survived better than native spearmint stolons. Stolons from all three species died at 0°F (−17.8°C), while greater than 95% survival was observed for all three species at 20°F (−6.7°C). In contrast, Landing (1969) reported that Scotch spearmint was less frost tolerant than Native spearmint, suggesting again the possible existence of more than one original Native spearmint cultivar. Other conditions can help a mint field withstand occasional cold-temperature extremes—for example, if the earth is insulated with a layer of snow, the stand is vigorous and soil moisture is adequate.

Assuming that mint plants can survive regional subfreezing temperatures, the duration of the growing season is another factor affecting commercial mint production. Peppermint prefers a growing season of at least 117 frost-free days. This observation is based on the number of frost-free days in central Oregon, which has the highest incidence of frost among all U.S. mint-producing regions (Table 3.1). Freezing temperatures in mid- to late spring can cause economic injury to newly emerged mint, especially just-planted rooted tip-cuttings. A growing season of adequate duration is especially important in regions where mint is harvested twice per season.

Heat units measured in growing degree-days (calculated as the average temperature over a 24-h period minus the lower developmental threshold, i.e., the temperature at which plant growth ceases) is a method of quantifying how warm a region is during the growing season.

TABLE 3.1
Climatic Data and Elevation for Five Diverse U.S. Mint-Producing Regions

	Madras, Oregon	Prosser, Washington	Caldwell, Idaho	Kalispell, Montana	LaGrande, Oregon
Latitude	44°30′	46°20′	43°30′	48°15′	45°10′
Elevation (ft) (m)	3000 (914.4)	900 (274.3)	2300 (701)	3200 (975.4)	2700 (823)
Mean precipitation (in) (mm)	8.80 (22.35)	8.20 (20.83)	8.00 (20.32)	15.02 (38.15)	18.79 (47.63)
Mean number of frost-free days (28°F) (−2.2°C)	117	185	175	150	140
First frost-free day (28°F)	May 20	May 01	April 21	May 04	May 09
Last frost-free day (28°F)	September 14	October 10	October 13	October 01	October 02
Total growing degree-days (40°F Base)	4048	4940	4872	3124	3880

See Table 3.1 for accumulated heat units for selected mint-growing regions of western U.S. (A base threshold of 40°F [4.4°C] is often used in heat unit calculations, but it must be experimentally verified.) Data show that Prosser, Washington was the warmest region with 4940 heat units, while Kalispell, Montana, was the coolest with 3124 heat units. In all regions measured, the majority of heat units were accumulated from May through August.

Diurnal temperature patterns during the growing season also affect peppermint growth, development, and oil quality. In a greenhouse study, Clark and Menary (1980a) showed that oil yield was higher when night temperature was 68°F (20°C) than when it was 50°F (10°C). The higher night temperature also resulted in changes in oil composition by causing the plant to grow more rapidly and produce menthofuran and pulegone in abundance. In contrast, plants grown at cooler night temperature did not accumulate menthofuran and pulegone, but instead produced higher cineole and menthone levels. These results confirm an earlier growth-chamber study conducted by Burbott and Loomis (1967) in which they compared night temperatures of 77°F (25°C) and 46°F (7.8°C). Again, lower night temperatures resulted in lower menthofuran and pulegone levels.

The relationship between daytime and nighttime temperatures also affects oil yield. Mint grown at day temperatures of 60°F (15.6°C) and 70°F (21.1°C) produced higher oil yield as night temperatures rose. At a day temperature of 80°F (26.7°C), however, oil yield decreased with increasing night temperature (Biggs and Leopold, 1955). The presence of an upper developmental threshold for peppermint and spearmint needs to be experimentally determined, not only for mint growth but also for its effects on both oil yield and oil quality.

Temperature also affects plant morphology, which influences both oil composition and yield. In one study, leaf size and side branching were maximized at a constant temperature of 70°F (21.1°C), while the longest internode length occurred at a constant temperature of 80°F (26.7°C) (Biggs and Leopold, 1955). Peppermint plants grown at a constant temperature of 90°F (32.2°C) had weak stems and small leaves, but this could have resulted from moisture stress and temperature effect. Moisture stress and cool night temperatures produce smaller leaves, which lead to an increase in oil yield and improved oil composition (Loomis, 1978). Smaller leaves contain as much oil as larger leaves, and because they minimize shading of the lower leaves, the plant retains the more mature lower leaves and more leaves overall. This is important because high quality oil results from a combination of young and old leaves (Loomis, 1978), and obviously more leaves produce higher oil yields.

All these findings combined explain why the U.S. Mint Industry has long believed that warm days and cool nights result in excellent peppermint oil quality. The optimal diurnal

temperature fluctuation for peppermint yield and oil quality is in the range of 85°F to 90°F (29.4°C to 32.2°C) during the day and 55°F to 60°F (12.8°C to 15.6°C) during the night (DeFrancisco, 2002).

Temperature also plays a role in regulating flowering, the timing of which is particularly important since oil composition and yield are at optimum levels at flowering. Peppermint grown at a constant temperature of 70°F (21.1°C) and 80°F (26.7°C) initiated flowering in a shorter amount of time and had fewer nodes, whereas plants grown at a constant temperature of 60°F (15.6°C) or 90°F (32.2°C) took almost three times longer to flower and had more nodes (Biggs and Leopold, 1955). Peppermint grown at a constant 70°F or under an 80°F day or 60°F night regime flowered at approximately the same time (Biggs and Leopold, 1955), indicating that peppermint may be amenable to a thermal accumulation model.

In unregulated field conditions, however, Hollingsworth (1981) did not find any correlation between flower initiation and either time or temperature. In field studies by Talkington (1983), only peppermint stem length and the number of nodes increased with higher temperatures; primary leaf number, number of lateral branches, and flower bud formation were not highly correlated with time or temperature. When developmental characteristics were compared between the years 1979 and 1980, the results were even less clear. Average temperatures in 1979 were warmer than in 1980, yet average stem length was shorter and the number of primary nodes was fewer. The author suggests that other environmental or agronomic factors may influence the development of these features in peppermint. For instance, water and fertility stress can induce peppermint to flower early (Loomis and Burbott, 1977).

3.3.1.1.3 Precipitation

Precipitation can greatly impact the productivity of a mint-producing area (Lacy et al., 1981). Average annual rainfall for selected mint-growing regions of western U.S. is shown in Table 3.1.

Rainfall early in the growing season can reduce the need for supplemental irrigation in the spring. In western U.S., precipitation falling as snow in the higher elevations is crucial to ensure adequate water for irrigation during the growing season and after the harvest. In the Midwest, mint growers often do not use supplemental irrigation on muck soils, and timely rainfall during the growing season is often necessary to achieve economic yields.

Precipitation can have negative effects on the crop as well. Too much rainfall in winter or spring may result in flooding, which can kill mint and spread diseases such as Verticillium wilt. Precipitation at or near harvest is especially problematic, reducing yields by shattering leaves (Bullis et al., 1948), damaging oil glands, or increasing the membrane permeability of oil glands resulting in oil loss through evaporation (Croteau, 1977). Rainfall that occurs during harvest can also reduce distillation efficiency (Watson and St. John, 1955).

3.3.1.2 Site Selection

Once an acceptable geographic region has been selected, field location must be determined. Factors to consider before planting a mint stand include soil type, availability of irrigation, slope, field history, and adjacent crops.

3.3.1.2.1 Soil Type

The best soil types for growing mint are deep, well-drained soils with loose texture, good organic matter, and a pH range of 6.0 to 7.5 (Green, 1963; Martin et al., 1976; Jackson et al., 1983). Muck soils prevalent throughout the Midwest growing region are excellent for mint production, as are loamy soils commonly found throughout western U.S. (Green, 1963; Lacy et al., 1981). Although mint prefers well-drained soils, it also requires soils capable of retaining adequate moisture under the normal range of mint irrigation regimes. Soils that

contain too much sand often require more supplemental irrigation to keep mint roots hydrated. Soils with too much clay reduce root penetration and tend to retain too much moisture, resulting in root rot.

Soil type also influences soil temperature, which is important in a shallow-rooted crop like mint. In fact, soil temperature is often thought to have a greater impact on plant growth than ambient temperature because roots are more sensitive to temperature extremes (Nielsen, 1974). The first peppermint planting in Michigan is an example of this. Planted into sandy soils, the stand was unable to survive the harsh winter (Ellis et al., 1941), whereas Midwest peppermint stands planted in soils with a higher level of organic matter have a much greater chance of winter survival.

Soil texture also has harvest implications. Single-cut mint (mint harvested once a year) can be grown effectively on some sandy soils, but double-cut mint (harvested twice a year) may not fare as well. The thick canopy of a single-cut crop serves to shade the soil during the hottest part of the season, preventing the soil from getting too hot or too dry. On the other hand, after the first cut of a double-cut harvest regime, the soil surface is exposed to direct sun and may become too hot and dry for healthy regrowth.

3.3.1.2.2 Water Availability

A suitable mint-growing region has affordable, adequate irrigation that is available when needed. Mint plants can be severely injured if water is unavailable during summer periods of peak temperatures. Winter survival can be jeopardized if water is not available to irrigate the crop after harvest until the onset of fall precipitation.

3.3.1.2.3 Slope

A steeply sloped field can increase production cost by increasing the time needed to harvest. Heavy harvest equipment may have difficulty in negotiating a steep slope, and harvesting perpendicular to the slope is not without its own hazards. Consequently, harvesting can only be accomplished while going downhill, which also can be hazardous. Steep slopes also increase soil erosion due to water runoff.

3.3.1.2.4 Regional Crop and Vegetation History

Knowledge of previous cropping history can minimize harm to new mint stands. For example, certain previously applied herbicides can carry over and devastate new mint fields. In one instance, chlorsulfuron was used to control weeds on nonirrigated wheat fields. When irrigation became available and the field was planted with mint, severe injury occurred. This was especially problematic in fields with high-pH soils because chlorsulfuron degrades much slower under alkaline conditions. Fields with a history of noxious weeds, arthropod pests, and diseases can also cause problems for new mint plantings.

There are also benefits associated with using different crops in rotation with mint and surrounding vegetation. An effective crop rotation can break insect, disease, and weed cycles, and planting the correct crop (for example, alfalfa) in between mint harvests can increase soil tilth and organic matter. Neighboring fields should also be considered; native vegetation and crops such as alfalfa hay may serve as habitat for natural enemies of mint pests.

3.3.2 Rootstock Selection, Propagation, and Planting

Planting healthy, vigorous rootstock is an important part of successful mint production. Because peppermint and spearmint are functionally sterile, new mint fields are established by planting rooted tip cuttings or by digging stolons from existing fields and planting them into new fields (Green, 1963; Lacy et al., 1981). Stolons are defined as underground stems, also referred to as rhizomes, runners, or rootstock.

To avoid spreading harmful pests, diseases, and weeds, which can reduce the vigor of a new plantation, growers must be diligent when selecting rootstock. Although more research is needed to verify the effectiveness of the method, one way to measure rootstock vigor is to quantify carbohydrate levels in mint rhizomes (Mitchell et al., 1998).

A good way to ensure the health of a new mint stand is to plant rootstock that has been professionally inspected. In a typical certification program, rootstock age does not exceed five generations because older plants may be less vigorous, even in situations where pests are adequately managed.

3.3.2.1 Nuclear Rootstock

Rootstock production begins by taking rooted tip cuttings from plants grown in the greenhouse or by tissue culture. The best time to plant nuclear rootstock is during April or May to avoid frost injury (Green, 1963; Martin et al., 1976; Lacy et al., 1981); later plantings can result in heat stress.

Although use of tissue culture may be a good way to eliminate diseases such as Verticillium wilt and rapidly expand plantlets in a small space, certain tissue culture techniques may affect mint growth and development.

Peppermint plants produced by meristematic tissue culture exhibited thicker stems, longer internodes, larger yet fewer leaves, and less oil (Crowe and Lommel, 1994; Welty and Prestbye, 1994; Crowe et al., 1995). However, the meristem effect on peppermint tended to subside with age, whereas meristem-derived spearmint sometimes yielded more than spearmint that was not meristemed (Welty and Prestbye, 1994).

Several explanations have been offered for the change in growth habit observed in meristemed peppermint. One idea was that meristeming caused a genetic change in the plant. Another view cited a previously unidentified virus, which was removed by meristeming. Crowe et al. (1995) demonstrated that when this virus is not present, peppermint grew too vigorously, producing longer internodes, thicker stems, and larger but fewer leaves; when they reintroduced the virus into the same plant, noticeable stunting occurred, with smaller and more abundant leaves. They named this new virus Peppermint Stunt Virus (PSV) (Lommel and Trembay, 1997). Lommel (personal communication) also suggested that based on the complexity of the genetic analysis, there was probably more than one such virus infecting peppermint. Because peppermint oil yield increased in its presence, PSV could be a beneficial virus in a commercial crop species.

To eliminate soil diseases such as Verticillium wilt without losing PSV, Wang and Reed (2003) took 3-mm to 4-mm shoot tips from Verticillium-infected peppermint and grew them in tissue culture. Because most of the plants obtained by this method were free of Verticillium and also retained the PSV, such techniques could be a valuable part of a certified mint rootstock program.

3.3.2.2 Foundation Rootstock

Foundation rootstock is the first generation of certified mint grown under field conditions, and is generated by planting nuclear rooted tip cuttings obtained from a registered propagator. Regions such as southwestern Idaho and south-central Washington are ideal for growing mint rootstock because of their long growing seasons, warm temperatures, and sandy or silty loam soils, which allow good root penetration. (In some crops like potato and strawberry, rootstock performance is improved when grown in more northerly latitudes; however, this has not been evaluated for peppermint or spearmint.)

Prior to planting, growers should consider soil fumigation to manage pests such as soil arthropods, diseases, nematodes, and weeds. Although soil fumigation is expensive, it may be cost effective on fields destined for rootstock production.

To prepare the seed bed, fields are often plowed after fumigation, disked at least twice, then harrowed. To control erosion, planting directly into uncultivated ground is becoming more commonplace.

Foundation fields are planted in early spring or late fall, either mechanically or by hand, with about 10,000 tip cuttings per acre. Spacing is typically 30 in. between rows and 1 foot within rows, but varies depending on equipment and grower preference. Hilling the beds with soil as the plants grow during the season results in greater stolon production.

3.3.2.3 Registered and Certified Rootstock

The second field generation of a certified mint program is called the registered planting and is obtained by expanding the stolons from the foundation plantation. The final two certification categories, certified one and two, are obtained by expanding the stolons of the registered and certified plantation, respectively.

Mint stolons are usually harvested from the foundation field in late fall or early spring using a modified potato digger. To avoid root desiccation, roots should be harvested close to planting time, and fall-planted mint usually results in better growth the following season. However, if fields are planted too early in the fall before the onset of dormancy, root reserves may be depleted as the plant expends energy in vegetative growth, making the plant more susceptible to winter-related injuries.

Root stolons are planted with either a modified manure spreader or a custom mint planter (Lacy et al., 1981). Spacing between rows varies from 20 to 36 in., depending on grower preference and equipment. A good rootstock expansion ratio is to plant at least 10 new acres of mint for each acre of rootstock.

Growers of mint rootstock should be aware of possible stolon decline syndromes caused by root-rotting soil pathogens whose activity is exacerbated by soil insects and nematodes. According to Dr. Jack Horner, Emeritus Plant Pathologist, Oregon State University, mint stolons of established plantations in western Oregon usually die back to the crown each winter, in part due to nematodes and pathogens.

To manage soil erosion and prevent wind from damaging young mint shoots, cover crops are essential on new mint plantations. In western U.S., cereals are often seeded on top of newly planted mint fields, then killed with herbicides at about the one-foot stage. In the Midwest, willow windbreaks are often planted around field perimeters (Lacy et al., 1981).

Because rootstock costs decrease with each expansion, growers who purchase certified roots often expand them on their own farm for one or more generations beyond certified two. Caution must be exercised, however; the older the rootstock and the further removed it is from the nuclear generation, the greater the chance of vigor loss and pest infestation.

3.3.2.4 Commercial Mint Planting

Planting a commercial mint field is similar to planting a rootstock field except no acreage expansion occurs. After the first year of production, the mint stolons grow between the rows and the field becomes an established stand of meadow mint. The exception is furrow-irrigated fields where furrows must be maintained yearly.

Increasingly, mint growers are planting thicker plantations with more stolons per row. Thicker plantings tend to fill in more quickly and yield higher during the first and second year of production. The previous strategy was to plant fewer roots and allow the stand to fill in at a more leisurely pace, often after 3 years or more. This strategy is largely ineffective today because most mint plantations are kept in production for a shorter period of time to avoid disease, weed, and insect pressure. The average life of a mint field today is 3 to 4 years

compared with 5 to 20 years earlier. The exception is Native spearmint, which often maintains its vigor even after 10 or more years of production.

3.3.3 Mint Growth and Development

An understanding of mint growth and development is important for timing agricultural practices, including fertilization, pest control, and harvest. The key developmental stages for mint are the dormant stage, the vegetative growth stage, and the reproductive stage. However, unlike crops such as wheat (Cook and Veseth, 1991), peppermint and spearmint have few well-defined growth stages or thermal accumulation models that predict critical developmental events.

3.3.3.1 Dormant Stage

Peppermint and spearmint are perennial species thought to overwinter in a dormant state that begins with the onset of cold temperatures in the fall. Understanding the nature of mint dormancy is important for implementing agricultural practices such as the application of herbicides, many of which can only be applied safely to dormant mint.

The concept of mint dormancy is clearer in growing regions such as central Oregon and Montana where temperatures are coldest. In these regions, mint foliage is usually burned off by frost as well as extended periods of below-freezing temperatures. Growers in more temperate regions like western Oregon must exercise caution; in these areas, mint may never enter a state of true dormancy and can remain susceptible to herbicide injury all year long.

3.3.3.2 Vegetative Stage

There are two vegetative growth stages for peppermint and spearmint. The first is the period between winter dormancy and the reproductive stage in summer. The second stage occurs after harvest and continues until the onset of winter dormancy. Vegetative growth following harvest is important for building root reserves that increase winter survival (Mitchell et al., 1998). Plant characteristics that can be measured during the vegetative stages include plant height, number of nodes, distance between nodes, and number of leaves and lateral branches.

Agricultural practices that encourage smaller leaves and increased leaf retention can result in higher oil yields and better peppermint oil quality (Loomis, 1978). Research by Loomis and Croteau (1979) showed that smaller peppermint leaves produce an equivalent amount of oil as larger leaves, but that larger leaves cause excessive shading, which often resulted in fewer leaves per plant.

Peppermint and spearmint normally grow by producing leaves at the growing tip while losing leaves from the lower nodes. A healthy mint plant has 7 to 12 pairs of leaves during the season, with new leaves retained for about 40 days (Talkington, 1983). If the plant is under severe water stress, more leaf loss occurs. More leaves are found on plants that produce lateral branching off the main stem, which often occurs after the cessation of apical dominance.

3.3.3.3 Reproductive Stage

Peppermint and spearmint are functionally sterile, so the term reproductive stage refers to the onset of bud and flower formation. This stage is critical for timing peppermint harvest because oil composition varies greatly during this period (Hee and Jackson, 1973).

In contrast, the quality of spearmint oil does not vary as much during the reproductive stage. In addition to high temperature, stress factors such as inadequate water or fertility can induce the early formation of mint buds and flowers.

3.3.4 Fertilization

The importance of adequate nutrition to a growing mint plantation cannot be overemphasized. Accordingly, the U.S. Mint Industry has supported abundant research on this topic, especially on peppermint, resulting in the identification of specific nutrient requirements for the crop (Huettig, 1969; Hee, 1975; Jackson et al., 1983; Hart, 1990; Mitchell, 1995, 1996).

Although sufficient nutrition is essential, growers must avoid the overuse of fertilizers. Applying excessive nutrients is not only costly but can also contaminate water supplies and the soil environment or even harm the mint plant itself (Hart, 1990; Mitchell, 1996). Though research results on peppermint nutrients are often applied to spearmint, this may not be the best approach. Additional research on the nutrient needs of spearmint is desirable, and the same may be said for different mint varieties of each species.

3.3.4.1 Nitrogen

For optimum vegetative growth and leaf development, a mint plantation requires an adequate supply of nitrogen (N) throughout the growing season. Production and retention of healthy leaves is important because this is where the majority of oil glands are found (Loomis, 1978).

Studies from Oregon, Washington, Idaho, and Montana show that 250 lb N/acre (276 kg/ha) is all that is needed for a peppermint crop that is harvested only once per season and that is not produced through meristematic tissue culture (Wescott and Welty, 1992; Christensen et al., 1996; Mitchell, 1996). Meristemed peppermint may require 350 lb N/acre (388 kg/ha) during the growing season (Crowe et al., 1995; Mitchell, 1996).

Applying only the required amount of nitrogen is important since excessive levels can actually reduce peppermint oil yields. Studies by Wescott and Welty (1993) showed a 25% reduction in peppermint oil when nitrogen rates were increased from 240 lb/acre (266 kg/ha) to either 480 or 600 lb/acre (532 or 633 kg/ha) on a single-cut crop. More research is needed to assess the nutritional needs of double-cut peppermint.

Optimum mint nutrition also requires proper amounts of irrigation. Too much water can leach nitrogen below the root zone, making it unavailable to the crop during the growing season (Hee, 1975; Mitchell, 1996). Determining the actual zone of nitrogen availability to mint is not clear because these species may not be as shallow rooted as previously thought. Wescott and Welty (1993) observed healthy peppermint feeder roots growing 3 ft below the soil surface. The authors hypothesize that deep feeder roots may enhance the uptake of both water and nutrients, although more research is needed to determine their density and importance to commercial mint production.

Nitrogen is best applied before a mint plantation becomes nutrient deficient. In the spring, little nitrogen is available to the plant because winter rains leach all remaining nitrogen from the previous season to well below the root zone (Mitchell, 1996). Studies from western and northeastern Oregon on single-cut peppermint demonstrate that the optimal time to apply nitrogen is during May and early June, the time of maximum plant uptake (Hart et al., 1995; Cook et al., 1999; Sullivan et al., 1999). Although applications of nitrogen during July and August delayed peppermint maturity for a brief period, they did not increase hay or oil yields (Mitchell, 1996).

How nutrients are applied affects mint production as well. Fertigating even amounts of nitrogen throughout the growing season may undersupply the crop during June and

oversupply it during July. Higher rates of nitrogen may be needed in rill-irrigated mint fields because lack of overhead sprinkler irrigation makes it more difficult to move fertilizers into the zone of root uptake.

To ensure that the appropriate amount of nitrogen and other nutrients are applied, the mint crop should be monitored on a regular basis throughout the growing season. Several methods have been evaluated for estimating nitrate levels in mint. The traditional method is to submit mint stems for laboratory analysis. Peppermint plantations with adequate levels of nitrogen show stem nitrate levels between 4000 and 8000 ppm (Huettig, 1969; Mitchell, 1995), but others suggest that despite adequate nitrogen applications, stem analysis may show that nitrate levels decrease somewhat as harvest time approaches (Brown, 1982).

Two quicker ways to determine nitrogen levels in mint plantations have been evaluated at the field level: the CARDY meter measures nitrate levels in mint sap pressed from mint stems; the SPAD meter measures leaf reflectance related to chlorophyll levels, an indirect measure of nitrates in leaves. Results suggest that the traditional laboratory stem nitrate analysis is the most accurate (Mitchell, 1995), although the SPAD meter correlated well with laboratory methods in other studies (Simon and Alkire, 1993; Wescott and Welty, 1993).

3.3.4.2 Potassium, Phosphorous, Sulfur, and Micronutrients (Manganese, Zinc, Iron, Copper, Boron)

In addition to nitrogen, mint requires several other nutrients and micronutrients to achieve optimal growth and oil yield:

- Potassium (K)—This nutrient can be rapidly depleted from the soil during mint growth. Although most clay soils are high in potassium, this source is not readily available to the mint crop (Mitchell, 1996).
- Potash (K_2O)—Depending on the outcome of soil tests, peppermint requires 60 to 200 lb of potash per acre (66 to 222 kg/ha) each season (Jackson et al., 1983).
- Phosphorous (P)—An important nutrient for autumn root growth (Jackson et al., 1983), phosphorous must be applied prior to need because of its rapid uptake by the mint plant. Soil tests are also useful for determining a plantation's phosphorous need (Mitchell, 1996).
- Sulfur (S)—While mint requires about 30 lb of supplemental sulfur per acre (ca. 34 kg/ha) per season, the actual amount varies depending on factors such as soil type and the amount of sulfur in irrigation water. Soils derived from pumice and basalt tend to be sulfur-deficient, whereas soils originating from sedimentary rock usually have adequate amounts (Mitchell, 1996). As with potassium and phosphorus, sulfur requirements for mint can be estimated by soil testing.

In addition to the major plant nutrients, mint requires small quantities of several micronutrients to produce a viable crop. Fortunately, naturally occurring levels of micronutrients in mint plants are quite high when compared with other plants (Jackson et al., 1983), and supplemental applications of manganese (Mg), zinc (Zn), iron (Fe), copper (Cu), and boron (B) have not proven to enhance commercial mint production (Mitchell, 1996). Furthermore, applying even slightly excessive amounts of elements such as boron or molybdenum can cause severe phytotoxicity (Hart, 1990).

3.3.5 Soil Amendments That Adjust Soil pH

Lime is another soil amendment frequently applied to mint plantations that require lower pH. The optimal pH level for mint production is about 6.5. Contributors to high pH levels include

fertilizers containing nitrates, elemental sulfur and ammonium sulfate, and abundant rainfall in growing regions such as western Oregon. After conducting soil tests to determine pH levels, lime is usually applied in the fall or winter. Although products such as gypsum are available to raise soil pH, they are expensive and rarely used on mint fields.

3.3.6 Irrigation

Because of intense competition for water resources from urban and recreational users, more efficient irrigation methods are needed to conserve valuable water and help manage the cost of mint production. Increasingly, mint growers are relying on soil moisture sensors and evapotranspiration models to assist in determining optimal distribution and frequency of irrigation for their crop.

3.3.6.1 Distribution and Frequency of Irrigation

Both peppermint and spearmint demand an abundant supply of water to produce a viable crop: about 50 to 60 in. (127 to 152 cm) of water per season or 1 to 2 in. (2.5 to 5.1 cm) per week, depending on temperature, soil type, and type of irrigation method used (Green, 1963; Lacy et al., 1981). For example, sandy soils dry out faster and require more water more frequently than silt loam soils. Regions with cool, moist spring weather, such as western Oregon, typically require less irrigation than warmer growing regions like eastern Oregon. In general, mint requires less moisture during cool spring temperatures (Mitchell, 1997), although there is ongoing debate about whether or not limited moisture stress in spring is beneficial to mint production.

Although moisture stress is associated with lower oil yields and higher menthofuran levels (Clark and Menary, 1980b; Charles et al., 1990), a certain degree of moisture stress may be beneficial provided it occurs at the proper time. Loomis and Burbott (1977) suggested that moderate levels of moisture stress early in the season may improve yield because moisture stress produces smaller leaves, which are more likely to be retained by the plant because of decreased shade within the canopy. On the other hand, Charles et al. (1990) showed in studies with hydroponically grown peppermint that increasing moisture stress reduced oil yield in young plants, suggesting that moisture stress early in the season has a detrimental effect. Studies from the Midwest show that withholding water in June and July resulted in significant losses in oil yield, while regular irrigation during the growing season increased average oil yields by as much as 38% and 57%, for partial and normal irrigation regimes, respectively (Simon et al., 1992).

Overirrigating a mint plantation also can result in reduced oil yield through oxygen deprivation to mint roots, a higher incidence of root rot, increased leaf drop, and leaching of nutrients to below the root zone (Horner, 1955; Croteau, 1977; Mitchell, 1997). High humidity caused by overirrigation can also affect the quality of peppermint oil by increasing menthofuran levels, especially during summer heat (Loomis and Burbott, 1977).

After harvest, the mint crop must be adequately irrigated until the onset of fall rains to promote a vigorous root system that will carry the plants through the winter. Postharvest irrigation studies from central Oregon showed a yield increase of 12 lb of oil per acre (13.2 kg/ha) when peppermint plots were irrigated 10 times after harvest compared with only three (Mitchell, 1997).

3.3.6.2 Time of Day

Although preliminary studies by Loomis and Croteau (1979) indicate that nighttime irrigation increases peppermint oil yield while decreasing menthofuran levels compared with

daytime irrigation, no differences were detected in a follow-up experiment by the same authors; however, unusual weather during the growing season may have affected their results.

It would seem that irrigating at night would avoid the combination of high temperature and high humidity that appears to reduce oil quality and yield (Loomis and Burbott, 1977). In addition, night irrigation would result in a cooler mint canopy, which is also thought to improve oil quality (Burbott and Loomis, 1967; Clark and Menary, 1980a). However, irrigating only at night may not be practical because of high moisture demands that occur during periods of intense summer heat.

3.3.6.3 Methods of Irrigating Mint

The irrigation method and equipment used in mint production should be cost effective and capable of satisfying moisture needs during the hottest part of the growing season. Mint growers in the U.S. irrigate their crop by a variety of means, including gravity and sprinkler systems, whereas some mint growers use no supplemental surface irrigation at all.

3.3.6.3.1 No Supplemental Surface Irrigation

Mint growers farming the Midwest's muck soils often rely solely on rainfall to meet their irrigation needs. Under dry conditions, growers may subirrigate by closing the gates of drainage ditches to allow the water table to rise (Lacy et al., 1981). Although withholding supplemental surface irrigation can lower costs, there is an increased chance of unacceptable moisture stress under hot, dry conditions. When compared with mint fields receiving no supplemental surface irrigation, studies by Simon et al. (1991) showed that regular surface irrigation supplied to mint grown on Midwestern muck soils increased oil yields by 87% and 55% for first-year fields and established fields, respectively.

3.3.6.3.2 Drip Irrigation

Although drip irrigation is an efficient use of water, this method has not been widely adopted in mint agriculture because of its high cost. Cost is even more pronounced because mint growers are rotating out of mint sooner, so drip irrigation systems must be amortized over a shorter time period. If a drip system could be established and remain in place during the production of several different crops, these other crops could share in the overall expense. However, one difficulty in this approach is finding an optimal depth to place the drip line. Because of mint's shallow-root system, the drip line must be placed close to the soil surface to ensure that the plant has adequate moisture, which may interfere with field preparation for other crops.

3.3.6.3.3 Gravity Irrigation

Until about the 1980s, the most common method of irrigating mint fields in the arid regions of Washington, Idaho, and Oregon was by the rill method (also called furrow or gravity irrigation). Here, fields are leveled with a slight slope before planting. Furrows, also called rills, are spaced about 30 in. (ca. 76 cm) apart, then cut parallel to the slope with a rotary corrugator. Water is supplied to the rill by placing siphon tubes from the irrigation ditch into individual rills.

Peppermint and spearmint grown under rill irrigation have achieved the best oil yields, probably because they do not suffer from the drawbacks associated with sprinkler irrigation (see discussion later). Drawbacks to rill irrigation include poor water efficiency and phytotoxicity that results because salts accumulate on ridge tops between furrows. Although rainfall can alleviate some salt problems, severe salt injury may result if rains push salts into the root zone and not beyond it (Maloy and Skotland, 1969). The inability to apply agricultural chemicals through the irrigation system is another drawback with rill fields.

Furrow-irrigated peppermint fields also typically experience higher levels of moisture stress (Loomis and Burbott, 1977), resulting in higher levels of menthofuran and pulegone in the oil.

Fertilizing rill-irrigated mint fields is another challenge. When dry fertilizer formulations are applied, it may not be possible, without timely rainfall, to incorporate the material that falls on the ridges. Adding liquid fertilizer to the irrigation ditch and assuming equal distribution of nutrients along the length of the rill may not be realistic. Mostly because of high water and labor requirements, few new rill fields are being developed today. According to a recent Washington State University mint grower survey, only 27% of mint growers in Washington State currently use gravity irrigation, whereas 73% of mint growers use some type of sprinkler irrigation system (Ferguson and Walsh, 2002).

3.3.6.3.4 Sprinkler Irrigation Methods

More mint acres are irrigated by overhead sprinkler systems than by any other method. Sprinkler irrigation is even used on muck soils in the Midwest to minimize soil erosion and frost damage (Lacy et al., 1981) and to reduce damage to mint plants caused by wind-blown soil (Green, 1963).

Overhead sprinkler systems in use today include hand lines, wheel lines, center pivots, and linear track systems. About 57% of Washington mint growers now use center-pivot sprinkler irrigation systems (Ferguson and Walsh, 2002). Although expensive, center-pivot systems can irrigate large acreage without the labor necessary to move hand lines or wheel lines. Center pivots, and to a lesser extent linear-track systems, are also ideal for chemigating fertilizers and pesticides.

There are negative aspects of sprinkler irrigation to mint production, including reduced oil yields (Croteau, 1977). Lower oil yields may occur because water droplets physically damage leaves and oil glands (Bullis et al., 1948) and increase membrane permeability resulting in oil evaporation (Croteau, 1977). Sprinkler-irrigated mint typically has larger leaves compared with mint that is furrow irrigated, resulting in greater leaf loss due to shading (Loomis, 1978).

Despite the disadvantage of lower yields, sprinkler irrigation appears to improve oil quality (Nelson et al., 1971). Lower menthofuran in sprinkler-irrigated peppermint oil appears to be correlated with reduced moisture stress.

3.3.7 Integrated Pest Management

Mint fields are home to many living organisms, most of which are not harmful to the mint crop. In fact, most of these residents are beneficial, providing nutrient recycling, biological control, and food to beneficial arthropods. Unfortunately, there are several arthropods, diseases, mollusks, nematodes, and weeds that do cause economic damage to peppermint and spearmint and must be managed.

To address these issues, the U.S. Mint Industry has invested heavily in the development of an integrated pest management (IPM) approach to pest control, defined as the selection of cost-effective pest-control tactics that are least harmful to nontarget species and the outside environment (Morris and Lundy, 1995).

IPM tactics commonly used in mint include referencing pest populations to economic thresholds, crop monitoring, and a variety of cultural, biological, and chemical controls. These tactics work to varying degrees of effectiveness depending on the pest involved.

3.3.7.1 Crop Monitoring and Economic Thresholds

Regular crop monitoring is one of the best ways to manage overall costs of mint production. By checking fields on a regular basis, the grower can correctly determine fertilizer, irrigation,

and pest management needs; for example, by referencing pest population levels to economic thresholds, unnecessary pesticide applications are avoided.

The level at which a pest causes economic damage is called the economic injury level (EIL). Unlike other crops where cosmetic appearance is important, mint is grown for its oil, and higher pest levels can be tolerated provided economic damage does not occur. Equally important is the action threshold (AT), the point at which control intervention should occur to prevent pest levels from reaching the EIL. Pest presence alone does not mean control measures are necessary. In fact, low pest levels can be beneficial because they provide food for natural enemies. Although they are difficult to define exactly because of variability in mint oil prices, plant vigor, environmental conditions, and multiple pest infestations, EILs have been established on mint for many of the arthropod pests (Berry and Fisher, 1993) and for the lesion nematode (Ingham and Merrifield, 1996).

3.3.7.2 Cultural Control

One of the most effective ways of preventing pest damage is to avoid introducing pests in the first place. Many arthropods, diseases, and weeds are introduced in rootstock and associated soil. Pest infestations can be avoided by planting clean rootstock, and rotating to a nonhost crop can disrupt the life cycle of many established mint pests. Crop rotation also can prolong the life of pesticides labeled for use on mint by switching to another crop where different pesticides having different modes of action can be used. Tillage is another effective cultural practice for rejuvenating mint stands and controlling several pest species (Talkington and Berry, 1986; Pike et al., 1988). Unfortunately, tillage also increases the severity and spread of Verticillium wilt, one of the major limiting factors to U.S. mint production.

3.3.7.3 Biological Control

Biological control is most effective when natural enemies reduce or maintain pest populations below damaging levels. There are many kinds of natural enemies that serve as biological control agents in mint including bacteria, fungi, insects, mites, nematodes, and viruses. Some of these agents are commercially available as biological insecticides.

Although biological control plays a role in the management of most pests and weeds, the best understood implementation in mint is in the control of arthropod pests. Because mint is a perennial crop, arthropods' natural enemies have adequate time to colonize and become established. The lush growth and high humidity resulting from frequent irrigation also provide an excellent habitat for many natural enemies. Luckily, arthropods rarely spread diseases in mint; however, the mint's low tolerance for insect disease vectors in other crops often leads to frequent pesticide applications that can be disruptive to biological control.

3.3.7.4 Pesticide Strategy

Pesticides include insecticides, herbicides, fungicides, and nematicides. Because cultural and biological controls may provide only partial control in some situations, pesticides continue to be an indispensable part of mint IPM. In fact, the philosophy of IPM is very compatible with pesticide use (Croft, 1990). One advantage of pesticides is that they can quickly reduce pest populations. Natural enemies usually work more slowly and may be unable to control rapidly increasing pest populations before economic damage occurs. If a pesticide treatment is required, selective pesticides should be considered first if they can be used effectively and economically. Selective pesticides are those that are more toxic to the pest than to its natural enemies or other nontarget species. Effectiveness and crop safety of individual pesticides depend on many factors including regional variation in climate, soil types, agricultural

practices, crop vigor, and the use of adjuvants. For these reasons, the pesticide label should be carefully consulted prior to use.

3.3.7.5 Arthropods

The arthropods are a group of species that includes insects, mites, and symphylans. Most arthropods are not harmful to the mint crop and may actually be beneficial, for example, by feeding on mint pests. There are several species, however, known to be very harmful to mint plantations. In the following discussion, arthropod pests are categorized as primarily soil dwelling or those living on mint foliage.

3.3.7.5.1 Arthropod Pests of the Soil Environment

Cranefly. Adult craneflies superficially resemble large mosquitoes, and their thick-skinned larvae are called "leather jackets." Two introduced species, *Tipula paludosa* and *T. oleracea*, are reported to feed on a variety of crops including mint. *T. paludosa* was identified in the U.S. in the 1960s; *T. oleracea* was first detected in 1999 (Rao et al., 2002). As with all craneflies, adults of both species disperse by flight. Eggs are laid in the soil and hatch soon after rainfall or irrigation. *T. paludosa* has one generation per year whereas *T. oleracea* has two generations per year, one in spring and another in fall. There are probably other cranefly species that affect mint, but this needs to be determined.

Mint Injury, Crop Monitoring, and Management. Cranefly larvae damage mint plantations by feeding on mint roots, crowns, and shoots. In laboratory feeding studies, Rao et al. (2002) compared feeding by *T. oleracea* on peppermint and lettuce. Results showed that 68% of the larvae reached the fifth instar on lettuce while only 22% reached this stage on peppermint. The authors suggest this species may not pose a problem to mint by feeding on foliage, but the effects of root feeding must be evaluated.

Monitoring for craneflies may be done at the same time and using similar procedures as for soil cutworms and mint root borer (MRB), although further study is required to evaluate the effectiveness of this approach. Signs of freshly cut mint shoots lying on the soil surface may also indicate the presence of leatherjackets. No AT has yet been determined for cranefly.

Although natural enemies are known to feed on native cranefly species (Rao et al., 2002), their effect on cranefly levels in mint is currently not known. The insecticide chlorpyrifos [1.0 lb active ingredient (ai)/acre] (1.1 kg/ha) may be effective when applied in either fall or spring through chemigation, or by broadcast applications followed by immediate incorporation with overhead sprinkler irrigation (Fisher et al., 2004).

Soil Cutworms. Soil cutworms are the larval stages of several noctuid moths that survive the winter as eggs, larvae, or pupae. Cutworms disperse mostly by flight which, depending on the species, can occur anytime from spring through fall. Most cutworms that inhabit mint soils have a wide host range, able to live at least part of their lives on other plant species (Berry, 1978; Berry and Fisher, 1993). The most common species in mint plantations include the redbacked cutworm, actually a complex of at least six species in the genus *Euxoa,* which occur east of the Cascade Mountains (Berry and Fisher, 1993). Several *Euxoa,* species are capable of dispersing over long distances, often from nonagricultural ecosystems such as forests (Miller, personal communication). Another species, the black or greasy cutworm (*Agrotis epsilon*), is found primarily in mint-growing regions west of the Cascades.

Some cutworm species that are foliage-feeding pests in summer are also found in the soil environment. For example, the variegated cutworm (*Peridroma saucia*) and spotted cutworm (*Xesia c-nigram*) often overwinter in mint soils as larvae and feed on new mint growth in spring.

Mint Injury, Crop Monitoring, and ATs. Cutworm larvae cause damage by feeding on mint roots and new shoots, often resulting in irregular bare patches in mint plantations in early

spring. As with cranefly, freshly cut mint shoots lying on the soil surface are a possible sign of cutworm activity.

Soil sampling for larvae occurs from March through early June. One square-foot (930 sq. cm) soil samples are dug to a depth of 2 to 4 in. (5 to 10 cm), sifted through screens, and the larvae counted. A minimum of 10 samples should be taken per field, but sequential sampling plans may reduce the number of samples required (Danielson and Berry, 1978). Fields should be sampled more than once because different cutworm species may be present in the soil at different times during spring and fall. Higher cutworm levels are often observed in mint fields with an abundance of debris on the soil surface, but the relationship between soil debris and cutworm levels is unknown.

The AT for newly established mint fields is 1.5 larvae per sq. ft. Depending on vigor, two to six larvae per square foot may be tolerated on established stands (Berry and Fisher, 1993; Fisher et al., 2004).

Management Tactics. Biological control is often very effective against soil cutworms. Generalist predators feed on cutworm larvae and eggs, whereas parasitoids reduced *Euxoa* larvae by up to 80% in central Oregon (Berry and Fisher, 1993).

The insecticides acephate (1.0 lb ai/acre) (1.1 kg/ha) and chlorpyrifos (1.0 to 2.0 lb ai/acre) (1.1 to 2.2 kg/ha) are effective against soil cutworms, although chlorpyrifos is more effective than acephate during cool, spring temperatures. The application rate of chlorpyrifos depends on which larval stage is present. If larvae are less than 0.75 in. (1.9 cm), 1.0 lb ai/acre is effective; for larger larvae, a rate of 2.0 lb ai/acre is needed (Fisher et al., 2004). Broadcast insecticide applications should be followed by a minimum of 0.5 in. (1.3 cm) of overhead irrigation.

Mint Flea Beetle. The adult MFB, *Longitarsus ferrugineus*, is a small, tan beetle with enlarged hind legs used for jumping. This species is found throughout the U.S. but its host range may be restricted to mint. MFB overwinters as eggs in the soil which, depending on temperature, hatch from April through May (Vessels, 1984; Morris, 1989). After hatching, the small, slender larvae feed within peppermint rhizomes. In May and June they exit the rhizomes to pupate. Adult beetles emerge in about 3 weeks and feed on mint leaves for 2 to 3 weeks before laying eggs. The exact number of eggs laid per female is not known. There is one generation per year.

MFB disperses from one field to another on farm equipment and in rootstock. Although most adult flea beetles cannot fly over long distances, some adult females may actually have fully developed wings, enabling them to disperse over a wider area through flight.

Mint Injury, Crop Monitoring, and ATs. MFB larvae feed within mint rhizomes, killing them directly and providing entrance wounds to root-rotting organisms. Symptoms of MFB injury are observed from mid-April through June and include variable sized patches of purplish, stunted, dead, or drying mint. Although feeding by adult MFB results in small round holes on mint leaves, damage is seldom seen.

Sampling for immature stages of MFB is laborious and consists of taking 1 sq. ft soil samples, sifting the soil through screens, then counting the number of larvae, pupae, and teneral adults. In addition, larvae contained in rhizomes from soil samples can be processed in Tulgren-Berlese funnels (Morris, 1989). When sampling for larvae from late April through the beginning of May, only rhizomes need to be checked. Both rhizomes and soil must be checked for immature stages from mid-May through June. The AT for immature MFB is about 0.5/sq. ft (Berry and Fisher, 1993).

Sweep-net sampling for adult MFB should begin in early July in western U.S. (Morris, 1989; Berry and Fisher, 1993), and in mid-July to early August in the Midwest (Lacy et al., 1981; Vessels, 1984). Sweep-net sampling is most effective in early morning when temperatures are cool. Although night sampling for adults may be effective, this strategy has not been evaluated. The AT for adult MFBs is five per sweep (Lacy et al., 1981).

Management Tactics. Planting pest-free rootstock and rotating to a nonmint crop in 3 to 5 years are both effective against MFB. Tillage is often not effective. In fact, the worst MFB outbreaks observed by the author were in disked and harrowed peppermint fields. Perhaps tillage distributes and protects the eggs while disrupting naturally occurring soil predators, although the impact of generalist soil predators on MFB immatures is not known.

Applications of the entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis heliothidis* were effective against larvae in small plot studies (Morris, 1989). Applications of oxamyl (1.0 lb ai/acre) (1.1 kg/ha) in spring can reduce populations of larvae by about 50% (Morris, 1989). Subsequent tests, however, showed control to vary from 15% to 60%, not sufficient to reduce MFBs to below damaging levels (Morris, unpublished data).

Targeting the adult is another alternative. In the western growing regions, insecticides are applied against adult MFB in early July before they begin laying eggs. Adults often disperse to the thickest vegetation, making pesticide coverage difficult. In this situation, methomyl (0.68 to 0.9 lb ai/acre) (0.75 to 1.0 kg/ha) is usually more effective than malathion (1.0 lb ai/acre). In the Midwest, adults may not begin laying eggs until after harvest, and postharvest malathion applications are effective because less foliage allows for more thorough pesticide coverage.

Mint Root Borer. The MRB (*Fumibotys fumalis*) was first identified as an agricultural pest in 1971 when it was found feeding on peppermint in western Oregon (Berry, 1974). MRB is now found at high levels in peppermint and Scotch spearmint throughout western Oregon, south-central Washington, and western Idaho (Pike et al., 1988). More recently, MRB has been identified on peppermint in central Oregon, northern California, and LaGrande, Oregon. Although MRB is found at low levels on Native spearmint in Washington State, it does not appear to injure the crop, but Native spearmint could act as a reservoir host.

MRB overwinters as prepupae in silk-lined earthen cells called hibernacula. Pupation occurs in spring. In western Oregon, adult moths emerge from June through August with peak emergence in mid-July (Berry, 1974). Soon after emergence, the females mate and in about one week begin laying eggs on mint foliage. The eggs hatch and the larvae drop to the soil surface on silken threads where they burrow into mint rhizomes (Pike et al., 1988). As larvae grow, they completely hollow out the rhizome then migrate through the soil in search of another rhizome. Larvae exit the rhizomes in late August through September to construct their hibernacula. There is one generation per year.

Although MRB can disperse as hibernacula on mint rootstock, the potential for long-distance dispersal of the adult is not well understood. When disturbed during the day, MRB moths fly only short distances. At night, however, they appear capable of dispersing much greater distances. More research is needed to determine the true dispersal capability of MRB.

Mint Injury, Crop Monitoring, and ATs. MRB injury occurs when larvae tunnel through rhizomes, resulting in variably sized patches of unhealthy or dead mint. Tunneling not only reduces oil yield and overwintering survival, it also provides entrance to root-rotting organisms and renders mint fields more susceptible to cultural practices such as propane flaming (Pike et al., 1988).

Although pheromone traps are useful for determining the presence of MRB adults, they are not well correlated to populations of larvae in the field (Takeyasu, 1994). Traversing mint fields by foot and counting the number of moths that fly up from the canopy is another method of sampling for adults that may better correlate with levels of larvae (Takeyasu, 1994). Correct identification is important when sampling for adult MRB because they can easily be confused with other species such as the false celery leaftier (FCLT) and the orange mint moth. The orange mint moth is actually considered a beneficial because it feeds on terminal mint buds, promoting lateral shoot growth (Pike et al., 1986, 1987).

The best time to sample for early-stage larvae is from early to mid-August. Samples consist of rhizomes collected from 1 sq. ft soil samples and processed in Tulgren-Berlese funnels (Takeyasu, 1994). Sampling prior to harvest is tedious because foliage must be removed before placing rhizomes in funnels, but has the advantage in that populations can be estimated prior to significant injury. Because they move through the soil, larger larvae and hibernacula are sampled from mid-August to September by digging 1 sq. ft soil samples and sifting the soil through screens. Rhizomes contained in the sample should also be checked for any remaining larvae as well as hibernacula, which can be attached to roots and rhizomes.

The AT for MRB is two to three larvae per square foot (Berry and Fisher, 1993). This level may underestimate the potential for injury the following season because, as in treating MFB adults, control measures are applied after the majority of damage has occurred. How many larvae will result the following season by allowing three larvae per square foot to remain untreated during the current year is not known.

Management Tactics. Preventing the introduction of MRB on infested rootstock and rotating to a nonmint crop in 3 to 5 years are effective control techniques. The host range and ability of the adult to disperse need further investigation to determine how far new fields should be located from reservoir populations. Encouraging a thick, vigorous root system is another important practice because such stands can withstand greater injury. Tillage and splitting centers of ridges between rills in gravity-irrigated fields are also effective practices against MRB (Pike and Glazer, 1982), although cleaning old rills and throwing the debris on top of old ridges are not effective (Morris, unpublished data).

Generalist soil predators feed on MRB larvae as they move between rhizomes but their impact on MRB populations is unknown. Although parasitoids were observed to emerge from several specimens of larvae and prepupae, the parasitism rate appeared low (Takeyasu, personal communication). Applications of the entomopathogenic nematode *Steinernema carpocapsae* at two to three billion infective juveniles (IJs) per acre were very effective against all larval instars (Berry and Crowe, 1996), including the smallest larvae concealed within the rhizomes (Takeyasu, 1994). Nematodes have an advantage over conventional pesticides in that applications can be made prior to harvest and before significant injury occurs. Takeyasu (personal communication) suggests a possible way of effectively using lower number of nematodes under high MRB densities. In this scenario, smaller quantities are applied that infect some larvae. These larvae in turn act as nematode incubators, generating more nematodes to infect other larvae.

Chlorpyrifos is currently the only insecticide labeled for use against MRB. Postharvest chemigation of chlorpyrifos (2.0 lb ai/acre) (2.2 kg/ha) is more effective than broadcast application methods (Quebbeman et al., 2002). The difference in efficacy among application methods is probably related to the low solubility of chlorpyrifos and its tendency to tie up on soil organic matter (Pike and Getzin, 1981; Berry et al., 1991). Controlling adults with insecticides is another approach that has been suggested. However, because adults emerge over a long period of time, multiple insecticide applications may be required, increasing the chance of harming nontarget species.

Mint Stem Borer. The MSB, *Pseudobaris nigrina*, is a small, black weevil that occurs throughout much of the eastern U.S. and Canada (O'Brien and Wibmer, 1982). MSB was first described as a potential mint pest in Idaho and eastern Oregon by Baird et al. (1987). Why MSB has not been found infesting mint in other mint-growing regions is not known.

The host range of MSB is thought to consist mainly of species in the mint family (Kissinger, 1963), although it has been observed on plant species besides mint, including goldenrod (*Solidago canadensis*) and rabbit brush (*Chrysothamus nauseosus*). There is no evidence, however, that it can reproduce on either of these species (Maury, personal communication). MSB has also been reported on kochia, but this must be independently verified.

The life cycle of MSB is described by Baruni (1979). MSB overwinters as an adult, inside and outside of mint fields. Adult dispersal occurs from mid-May to mid-June. Eggs are laid on the soil surface near mint stems. Following egg hatch, larvae bore into stems, develop, then exit the stems to pupate. In addition to adult flight, MSB can disperse as larvae in contaminated rootstock. In fact, it may have been introduced into Idaho on infested rootstock from the eastern U.S. (Baird et al., 1990).

Mint Injury, Crop Monitoring, and Management. Plants infested with MSB larvae may appear wilted, yellowish, and lodged (Berry and Fisher, 1993). Research by Baird et al. (1987) and Maury (personal communication) have yet to identify MSB populations capable of causing economic damage to peppermint. Isolating MSB injury from other causes such as Verticillium wilts and MRB is difficult.

Monitoring for MSB adults is conducted from mid-May to mid-June using a sweep net. Another way to sample for adults is to place wooden lathes coated with sticker throughout the field at or just above canopy level. Proper identification of the adult is important because other similar looking species may be confused with MSB. Monitoring for larvae can be done in several ways. One way is to dig plants from several areas of the field, split open the stems and check for signs of MSB. Larvae also may be collected by processing plant material in Tulgren–Burlese funnels (Morris, personal experience). As for cranefly, no AT for MSB has yet been determined.

Although adult MSB disperses by flight, avoiding the planting of infested rootstock is probably a good management strategy. Other effective tactics include planting healthy and vigorous rootstock, reducing plant stress by controlling other pests, and following a 3- to 5-year rotation. Although no pesticide is currently labeled for use on MSB, treating for other mint pests that occur at the same time as MSB may provide some control, for example, treating cutworms in spring with acephate.

Root Weevils. Root weevil adults are called "snout beetles" because their mouthparts are located at the end of a long snout. RWs are located throughout the U.S. and have a wide host range, including rhododendron, blueberry, strawberry, and mint (Berry, 1978). In addition to peppermint, Native and Scotch spearmint also can harbor high RW levels. The strawberry root weevil (SRW), *Otiorhynchus ovatus*, is the most common weevil species infesting mint. Other commonly encountered species include the black vine weevil (*O. sulcatus*), rough SRW (*O. rugosostriatus*), and the obscure root weevil (*Sciopithes obscurus*) (Berry and Fisher, 1993). Other unidentified species are occasionally found.

Most RWs overwinter in mint as larvae, but some SRW adults overwinter and lay their eggs the following spring (Cacka, 1982). The RW adults important to mint are female, which produce fertile eggs without mating. Depending on accumulated heat units, pupae develop in late April to early May; adults emerge in mid-May through June. Adult females lay eggs around the bases of mint plants about 2 weeks after emergence.

Mint Injury, Crop Monitoring, and ATs. RW larvae feed on mint roots and rhizomes, resulting in variably sized patches of bare mint. Sampling for larvae is done by digging 1 sq. ft soil samples to a depth of 4 in. and sifting the soil through screens. A sequential sampling plan has been developed for SRW in peppermint, and the author suggests a minimum of 25 soil samples be taken for each field (Cacka, 1982). Intervention may be necessary if a total of 5.5 larvae, pupae, and adults are found per square foot. No treatment is necessary if larval numbers are below 2.7/sq. ft. If numbers are between these two levels, sampling should continue until a decision is reached. Sampling for RW adults is accomplished by sweeping foliage after sunset when they are most active (Fisher et al., 2004). An AT has not been established for RW adults.

Management Tactics. Because adult RWs do not fly, planting clean rootstock and a timely rotation of 3 to 5 years are effective control measures. Rotation should be to crops that are

poor RW hosts, such as alfalfa and corn (Berry, 1978). Resident natural enemies can reduce RW abundance; for example, Cacka (1982) found that carabid beetles (*Pterostichus vulgaris*) are very effective at reducing SRW populations. Applications of the entomopathogenic nematode *Steinernema carpocapsae* at three billion IJs per acre can kill RW larvae in late summer or early fall, but commercial applications often provided inconsistent control (Morris, personal observation).

Night applications of acephate (1.0 lb ai/acre) (1.1 kg/ha), applied when the majority of adults have emerged from the soil but before they begin laying eggs, is an effective RW control strategy (Fisher et al., 2004). Two other insecticides, thiamethoxam (0.023 to 0.124 lb ai/acre) (0.25 to 133 g/ha) and indoxacarb (0.1 lb ai/acre) (110 g/ha), also are effective against SRW adults (Quebbeman et al., 2001).

Symphylans. Symphylans have a wide host range, and some species in the family *Scutigerellidae* can consume up to 20 times their body weight in vegetable matter each day (Edwards, 1990). The garden symphylan (*Scutigerella immaculate*) is a major peppermint pest in western Oregon. Although the author has observed symphylans in arid growing regions such as eastern Washington, they were rarely seen, perhaps because they prefer loam soils and require 100% relative humidity for long-term survival (Edwards, 1990).

Symphylan eggs are laid in early spring or fall and hatch in 8 to 28 days depending on temperature. Newly hatched young have six pairs of legs, adults have 12; one new pair of legs is added at each successive molt. Development takes from 2 to 5 months at temperatures of 77°F (25°C) and 50°F (10°C), respectively (Berry and Robinson, 1974). There are one to two generations per year. Symphylans disperse on rootstock or on soil debris carried by floodwaters.

Mint Injury, Crop Monitoring, and ATs. Symphylans feed on roots, causing dead or unhealthy patches in mint plantations. The best time to sample for infestation is from March through September. Sampling must be done during warm-temperature periods because cold, dry, or hot conditions cause symphylans to migrate deep into the soil. In regularly irrigated mint fields, the highest populations are often observed in late summer. Populations are estimated by counting the number of individuals found in 1 sq. ft soil samples dug to a depth of 10 to 12 in. (25.4 to 30.5 cm). Proper identification is important because several other small soil arthropods may resemble symphylans. An AT of four to five symphylans per square foot of soil is suggested (Berry and Fisher, 1993).

Management Tactics. Planting symphylan-free rootstock and avoiding planting in fields suspected of harboring them, for instance woodlots and pastures, are effective management tactics against this pest. Resident natural enemies may also help keep symphylan populations in check. The predator mite, *Pergamasus quisquiliarum*, was observed to consume 12 symphylans per generation, suggesting that biological control by this species is possible (Berry, 1973).

Preplant soil fumigation is another symphylan management technique. Because symphylans can reside deep in the soil profile, seed beds should be prepared before fumigation to a depth of at least 18 in. (ca. 46 cm) (Morrison, 1957; Berry and Robinson, 1974). If the field contains a hard pan 6 in. (ca. 15 cm) below the soil surface, fumigation may provide poor results.

Once the mint crop is established, contact insecticides are needed to reduce symphylan levels that exceed the AT. Broadcast applications of chlorpyrifos (2.0 lb ai/acre) (2.2 kg/ha) followed by overhead sprinkler irrigation provide inconsistent control, especially in fields with high levels of organic matter and carbon, often observed in fields that are regularly flamed but not tilled. Similar results were observed for fonofos (2.0 lb ai/acre), another insecticide having low solubility. Ethoprop (3.0 and 6.0 lb ai/acre) (3.3 and 6.6 kg/ha) applied in the fall reduced symphylan levels by more than 95% throughout the following growing season.

More consistent and timely results are observed in mint when insecticides are applied as either granular formulations or through the irrigation system. Spring chemigation of chlorpyrifos (2.0 lb ai/acre) reduced symphylan levels by 77% during the growing season and by 81% the following season (Morris and Takeyasu, 1998). Granular chlorpyrifos applied in the spring (Lorsban 15G at 2.0 lb ai/acre) reduced symphylan levels by 82% (Morris and Takeyasu, 1998).

Wireworms. Wireworms are called "click beetles" because the adults often "snap" when placed on their backs. The most common wireworm species in the western U.S. are the Pacific coast wireworm (*Limonus canus*), sugar beet wireworm (*L. californicus*), and Great Basin wireworm (*Ctenicera pruinina*). These species have a wide host range including small grains, potato, corn, onion, beans, carrots, and grass seed (Berry, 1978).

Wireworms overwinter as larvae or young adults (Berry, 1978). After mating, eggs are laid 1 to 6 in. below the soil surface and hatch 3 to 4 weeks later. Larvae feed for 2 to 5 years before pupating; in the northwestern U.S., most wireworm species take 3 years to complete their development.

Mint Injury, Crop Monitoring, and Management. Wireworms feed on roots and rhizomes and are especially problematic in nursery plantings of rooted tip cuttings. Symptoms of wireworm injury include stunted and wilted mint that is reddish in color.

Sampling mint fields for wireworm before planting is very important because control measures applied after planting are not very effective. Because wireworms can reside deep in the soil, samples should be taken to a depth of 18 in. (ca. 46 cm) with a 6.75-in. (ca. 17 cm) posthole auger, then sifted through screens.

Avoid planting new mint fields where high wireworm populations are likely to be encountered, for example, pastures or fallowed land. In such situations, preplant soil fumigation or incorporation of effective insecticides prior to planting is recommended. Biological control is generally not effective because wireworms have few natural enemies (Berry, 1978).

3.3.7.5.2 Pests of Mint Foliage

Aphids. Aphids are insects that extract nutrients from plant sap and excrete their waste as a sugary substance called honeydew. The mint aphid, *Ovatus crataegarius*, is found in commercial peppermint and spearmint throughout the western U.S. (Berry and Fisher, 1993). Other aphid species such as the green peach aphid probably migrate into mint fields as well, especially in potato-growing regions such as south-central Washington and Idaho.

Mint aphids overwinter as adults on mint roots and stems beneath the soil surface (Berry and Fisher, 1993). In the spring, adult females give rise to mobile nymphs. A generation can be completed in as little as 7 to 10 days during the summer, and 12 to 15 generations are produced each year. Aphids disperse by flight and on rootstock.

Mint Injury, Crop Monitoring, AT, and Management. Aphid injury is characterized by leaf loss, reduced plant vigor, and excessive honeydew, which interferes with harvesting operations. Fortunately, aphids have not been shown to vector mint diseases.

Sampling for aphids can be done along with spider mites by counting the number of aphids per leaf. Most aphid injury occurs in rill-irrigated mint fields, although high populations are occasionally seen in fields that are sprinkler irrigated. An AT for aphids has not been experimentally determined, but an average of five to ten per leaf is a reasonable estimate.

Natural enemies are often effective at maintaining aphids below damaging levels, including ladybeetles, lacewings, syrphid flies, and parasites. The insecticides malathion (1.0 lb ai/acre) (1.1 kg/ha), acephate (1.0 lb ai/acre), and oxydematon-methyl (0.75 lb ai/acre) (829g/ha) are all very effective at controlling aphids in mint.

Foliar Cutworms. Although many cutworm species feed on mint foliage, only five are commonly encountered at damaging levels: the alfalfa looper (*Autographa californicus*),

bertha armyworm (*Mamestra configurata*), cabbage looper (*Tricoplusia ni*), spotted cutworm (*Xesia C-nigram*), and variegated cutworm (*Peridroma saucia*) (Lacy et al., 1981; Berry and Fisher, 1993). Another species less frequently encountered on mint is the western yellow-striped armyworm (*Spodoptera praefica*). Most of these species have a wide host range and a large geographical distribution (Berry, 1978).

Cutworms overwinter either as eggs, larvae, or pupae. Adult moths lay their eggs mostly on mint foliage. Variegated cutworms lay clusters of 200 to 500 eggs, whereas alfalfa and cabbage loopers lay single eggs. Eggs hatch in 4 to 7 days. Variegated cutworms take 4 to 6 weeks to pupate whereas loopers can develop from egg to adult in as little as 30 days. Loopers can produce up to three generations per year. The life cycle of the bertha armyworm is similar to the variegated cutworm except that it overwinters as a pupa (Berry and Fisher, 1993).

Mint Injury, Crop Monitoring, and ATs. Foliar cutworms injure mint by feeding on mint leaves. Pheromone and black-light traps are used to collect adult moths and estimate when sampling for larvae should begin. Fields should be checked on a regular basis because larvae can be found anytime during the growing season. Small larvae are best sampled from foliage with a sweep net. If 0.6 larvae are collected per 180° sweep, intervention is necessary.

Searching the soil surface is effective for estimating populations of more mature larvae. Shake the equivalent of 4 sq. ft of foliage to dislodge cutworms, part the foliage and count the number of cutworms on the soil surface. A minimum of 10 sites should be sampled for each field. Sequential sampling methods have been established that may reduce the number of samples required (Coop, 1987). The AT for ground search counts is about 1.3 larvae per sq. ft (Berry and Fisher, 1993). Larvae collected for all cutworm species can be combined for referencing to the AT.

Management Tactics. Agricultural practices that encourage a healthy mint stand can compensate for some cutworm injury, and healthy mint is also a good environment for natural enemies. Parasitic wasps are very effective at controlling loopers and variegated cutworm, but are less effective against the bertha armyworm. A naturally occurring nuclear polyhedrosis virus is also very effective against loopers. The effectiveness of generalist predators such as ladybeetles and syrphid flies is not well understood, but they may be important for reducing cutworm eggs and small larvae (Miller, personal communication).

The insecticides acephate (1.0 lb ai/acre) (1.1 kg/ha) and methomyl (0.9 lb ai/acre) (409 g/ha) are effective against most cutworms and loopers. Such insecticides should be used sparingly, however, because they can be harmful to beneficial natural enemies. An alternative approach is to use insecticides that are more selective, such as insect growth regulators. In one study, tebufenozide (0.065 to 0.125 lb ai/acre) (72 to 138 g/ha) was very effective against bertha armyworm in central Oregon (Morris and Takeyasu, 1996). In another study, tebufenozide (0.125 to 0.25 lb ai/acre) (138 to 277 g/ha), methoxyfenozide (0.25 lb ai/acre), and spinosad (0.167 lb ai/acre) (185 g/ha) reduced a mixed population of bertha armyworm and spotted cutworm by 99%, 100%, and 68%, respectively (Quebbeman et al., 2000). Formulations of *Bacillus thuringiensis* (BT) have not performed consistently against cutworms in mint, even when applied against small larvae.

False Celery Leaftier. The FCLT, *Udea profundalis*, is commonly observed feeding on mint foliage during spring and summer. Although this species is usually not considered a pest (Berry and Fisher, 1993), some peppermint fields in eastern Oregon sustained damage in 2003. High FCLT populations have also been observed on *Mentha citrata* (Schilperoort, personal communication).

FCLT overwinters as a pupa in and around mint fields. Adults emerge in the spring and lay their eggs on the underside of leaves (Berry and Fisher, 1993). After hatching, the larvae feed on foliage for about 2 to 3 weeks before pupating. Pupae are formed inside silken cocoons

within webbed mint leaves; adults emerge in about 2 weeks. There are up to three generations per year.

FCLT injures mint by feeding on leaves. Monitoring for FCLT larvae can be accomplished with either a sweep net or ground searches along with foliar cutworms. An AT has not been experimentally determined, but FCLT can be combined with cutworms for a conservative estimate. The insecticides acephate, malathion, and tebufenozide are all effective against this species (Takeyasu, personal communication).

Grasshoppers. Grasshoppers overwinter in the soil as eggs within pods, each pod containing 20 to 100 eggs. Nymphs emerge from April through June and feed for 40 to 60 days before reaching adulthood. There is usually one generation per year.

Several species in the genus *Melanopus* injure mint by feeding on foliage, mostly in arid growing regions. Sweep-net techniques such as those used for cutworms and MFBs are also effective for monitoring grasshoppers.

The AT for grasshoppers is six to eight per square yard (Berry and Fisher, 1993). When populations exceed this level, they are effectively managed with malathion (1.0 lb ai/acre) (1.1 kg/ha) or acephate (1.0 lb ai/acre). The selective insecticides spinosad (0.156 lb ai/acre) (173 g/ha) and thiamethoxam (0.47 lb ai/acre) (520 g/ha) also provided encouraging results, reducing populations by more than 90% in two separate studies (Quebbeman et al., 2000, 2001).

Spider Mites. Spider mites are a frequent problem on mint plantations in the western U.S. (Morris et al., 1999) and are an occasional problem in the Midwest (Wyman and Rice-Mahr, 1992). The most common species found in mint is the twospotted mite, *Tetranychus urticae*, which also feeds on a variety of other crops, including corn, hops, potato, and strawberry (Helle and Sabelis, 1985).

Spider mites disperse to and from mint fields mostly on wind currents. They overwinter in mint fields as reddish-colored females in soil duff and debris and on the underside of mint leaves not killed by subfreezing temperatures (Morris et al., 1996). Females begin laying eggs in the early spring, and a complete generation from egg to adult can take as little as 1 to 3 weeks depending on temperature (Helle and Sabelis, 1985).

Mint Injury, Crop Monitoring, and ATs. Spider mite injury to mint is caused by increased water stress (DeAngelis et al., 1982), decreased leaf weight (DeAngelis et al., 1983a) and reduced photosynthesis (DeAngelis et al., 1983b). In addition to reducing oil yield, spider mite feeding can also affect mint oil quality (DeAngelis et al., 1983c). Although spider mite injury is known to increase as temperatures rise, unseasonably low temperatures can increase spider mite damage as well. For instance, when temperatures dropped to 25°F (−3.9°C) one June in LaGrande, Oregon, peppermint plantations infested with spider mites exhibited a blackish, wilted appearance, resulting in severe defoliation (Morris, unpublished data).

Hollingsworth (1981) recommends the following sampling procedures for spider mites in mint plantations: for each field, sample a minimum of 15 sites per field. Select 15 plants from each site and three leaves from each plant: one from the top, one from the middle, and one from the bottom of the plant. Using a hand lens, count the number of spider mites per leaf. A binomial sampling plan may reduce effort by only counting leaves that have more than five spider mites (Berry and Fisher, 1993). Counting the number of predator mites can also affect management decisions. If the ratio of spider mites to predator mites is 10:1 or less, intervention may not be necessary. The AT for spider mites varies from five to ten mites per leaf depending on, among other things, temperature, moisture levels, and plant vigor.

Management Tactics. Several cultural practices affect the severity of spider mites in mint. Although tillage can reduce spider mites initially, this practice may only delay outbreaks until later in the season. Spring flaming for rust control can reduce spider mite levels, especially when fields are flamed twice in one week (Fairchild and Morris, 1989). However, fall flaming leads to spider mite outbreaks (Morris et al., 2000).

Biological control is often very effective against spider mites on mint. Spider mite injury is more common in peppermint and Scotch spearmint than in Native spearmint, perhaps because biological control is more effective in Native spearmint since fewer pesticides are used. Generalist predators can maintain spider mites at low levels. Specialist predators such as the phytoseiid *Neoseiulus fallacis*, shown by mint field surveys to be widely distributed in the western U.S. (Morris et al., 1999), are often effective at reining in increasing spider mite populations (Helle and Sabalis, 1985). Other phytoseiid species have been identified on mint (Morris et al., 1999; Walsh and Wight, 2002), but their role in regulating spider mite levels is not known. Inoculating strawberry fields (Coop and Croft, 1995) or mint fields (Morris, unpublished data) with low numbers of *N. fallacis* (1000 to 3000/acre) (ca. 2500 to 7500/ha) can be a cost-effective spider mite management tactic. But for reasons not always understood, such releases are frequently unsuccessful, pointing again to the importance of regular field monitoring.

The use of nonselective pesticides often reduces the effectiveness of predator mites and leads to spider mite outbreaks (Morris et al., 1996; Kaufman et al., 2000). The selectivity of moderately toxic pesticides can be improved by applying them in more selective ways. For example, timing the application of the herbicide oxyfluorfen during the winter months improves selectivity because predator mites are dormant and protected in soil duff and debris. Another way to improve selectivity is by altering the pesticide formulation or application method. Chemigation and granular formulations are generally less disruptive to predator mites than liquid formulations applied as a ground spray. For example, when bromoxnil was diluted through chemigation, predators were not harmed and weed control was equally effective (Morris and Takeyasu, 2000).

Should a miticide be necessary, selective ones such as propargite (1.6 to 2.0 lb ai/acre) (1.8 to 2.2 kg/ha) or bifenazate (0.5 to 0.75 lb ai/acre) (554 to 830 g/ha) are effective at lowering spider mites to levels where predators can reestablish control. If predator levels are too low, however, higher miticide rates and/or multiple applications may be necessary. The more frequently a miticide is applied, however, the more likely spider mites will become resistant to it. If multiple applications are needed, a good strategy is to switch to another miticide with a different mode of action.

Squirrley Mint. Although the condition known as "Squirrley mint" has been known for over 50 years, its cause was not determined until recently when Dr. Ralph Green, Emeritus Plant Pathologist, Purdue University, correlated this syndrome with a previously undescribed bud mite in the family Tarsonemidae. Researchers at Rutgers University described this new mite species as *Tarsonemus pipermenta*. For reasons yet unknown, Squirrley mint remains confined to the Midwest, where it continues to spread (Perry, personal communication).

T. pipermenta overwinters as an adult female in mint stolon buds. During the growing season, this species is most often found in terminal and lateral buds (Green, 2002b). Dispersal is thought to occur by movement on infested rootstock, although dispersal on wind currents and farm equipment cannot be ruled out.

Symptoms on peppermint include bunching of the top leaves and up to an 80% reduction in oil yield (Green, personal communication). Although several miticides appear effective against *T. pipermenta*, more research is needed before labels are pursued (Perry, personal communication).

3.3.7.5.3 Occasional and Potential Arthropod Pests

Several other arthropod species are occasional mint pests. Although the painted lady butterfly (*Vanessa cardui*) usually feeds on Canada thistle patches growing in mint fields, it will switch to feeding on mint when populations are high. Insecticides used to control cutworms are also effective against this species.

The larvae of several scarab beetle species are occasionally found feeding on mint roots and rhizomes, especially in plantations established from pasture or fallowed fields (Nelson, personal communication). Scarab larvae can take several years to develop and are difficult to control in established mint plantations. Twice in the last 20 years, larvae of the western spotted cucumber beetle (*Diabrotica undecimpunctata*) were observed feeding on peppermint rhizomes in western Oregon, but control measures were not evaluated. A small black mirid (*Halticus bractatus*) is known to be an occasional pest on Midwest mint.

Several unique arthropod pest species also have been encountered in peppermint grown outside the traditional regions surrounding the 40th parallel, including the pale striped flea beetle (*Systena blanda*) in Wyoming (Phillips, personal communication); whitefly (*Aleyrodidae*) in Arizona, Argentina, and India (Bolton, personal communication); and root aphids in Argentina.

3.3.7.6 Diseases

In addition to the threat posed by arthropod pests, peppermint and spearmint are subject to several debilitating diseases. The discussion on mint diseases begins with Verticillium wilt, a soil disease often considered the main limiting factor to economically viable peppermint and spearmint production in the U.S.

3.3.7.6.1 Verticillium Wilt

First identified on U.S. peppermint plantations in 1921 (Landing, 1969), Verticillium wilt remains a leading barrier to U.S. mint oil production despite extensive efforts to control it (Johnson and Cummings, 2000).

Based on the presence of microsclerotia (MS) (small, hardened survival structures) and unique features of its conidiophore, the Verticillium species of importance to mint is *Verticillium dahliae* and not *V. albo-atrum* (Heale, 2000). *V. dahliae* has a wide host range, infecting hundreds of different crops including cotton, potato, strawberry, and mint (Green, 1951; McCain et al., 1981; Heale, 2000). Many weed species are also suitable hosts for this pathogen (Green, 1951; McCain et al., 1981), and it may even survive for a limited time as a saprophyte on spent mint hay (Green, 1951).

There are many strains of *V. dahliae*, each varying in its aggressiveness toward different plant species and varieties, including peppermint and spearmint (Johnson and Cummings, 2000; Tjamos et al., 2000; Douhan and Johnson, 2001). Most *V. dahliae* strains isolated from crops such as potato and echinacea produce only mild symptoms on peppermint, and to date have not caused economic damage to this crop (Green, 1961; Crowe, 1998; Powelson et al., 1999; Douhan and Johnson, 2001). There may be exceptions, however. For instance, a strain of *V. dahliae* obtained from a California strawberry field with no history of mint production was highly aggressive to the resistant peppermint variety Murray Mitcham (Crowe, 1997). However, this strain produced only mild symptoms on Black Mitcham, a peppermint variety considered to be highly susceptible to Verticillium. There is also the chance that other nonmint strains, such as those found on potato, could change into more aggressive mint strains (Green, 1977). Paradoxically, mint oil yields may actually increase in peppermint grown in soils with subthreshold levels of mint-virulent strains of *V. dahliae* or higher levels of less virulent strains (Crowe, 1996, 1998), although managing this phenomenon is difficult to say the least.

V. dahliae overwinters in mint plantations as MS in the soil or as mycelium and conidia in living plant tissue (Crowe et al., 2000). Although MS were once believed to survive almost indefinitely in the soil, more recent information suggests that they must go through an infection cycle about every 3 to 5 years (Crowe, personal communication).

MS germinate and infect roots in response to stimuli such as mint-root exudates. If a systemic infection occurs, mycelium and conidia spread upward in the plant, partially blocking water supply to leaves. If mint-aggressive strains are involved, such infections often kill the plant. About one million MS are formed in dead mint stems (Horner, 1965), but the actual number may depend on the mint species or variety (Crowe, 1998). Verticillium can disperse on mint rootstock, in irrigation or floodwaters, on farm equipment and in windblown soil (Horner, 1955; Lacy et al., 1981; Tjamos et al., 2000).

Mint Injury. Economic injury occurs when Verticillium-infected mint stands produce less oil, are less able to survive the winter, and produce for a shorter period of time (Crowe et al., 2000). Because Verticillium may persist for many years under some field conditions, the amount of available land for future mint production also may decrease. Wilt symptoms include the diagnostic asymmetrical twisting and bunching of upper leaves, leaf chlorosis, plant necrosis, and death. Symptoms usually increase in severity as temperatures rise during the growing season, and a toxin produced by the fungus might also contribute to symptom expression (Horner, 1955; Heale, 2000).

An EIL of between 0.5 and 1.0 MS/gm of soil has been estimated for one mint-aggressive strain on Todd's Mitcham peppermint (Crowe et al., 2000). Although EILs have not been estimated for spearmint or the other peppermint varieties, their relative rankings with respect to wilt tolerance are discussed in the section on host-plant resistance. Several conditions may lower the EIL: for example, if more virulent mint strains are identified, if mint is grown in the presence of root lesion nematode (Faulkner et al., 1970; Johnson and Santo, 2001), or if other stresses occur such as application of certain herbicides (Santo and Wilson, 1990).

Soil Sampling, Strain Identification, and Crop Monitoring. Prior to planting mint, field soils should be sampled to gauge Verticillium levels and, if detected, determine if the strains involved are pathogenic to mint.

Once a mint field is established, visual monitoring techniques can help identify the presence of wilt symptoms and their severity. Such information assists mint growers in deciding when to rotate and whether a field should be used for rootstock. Another important aspect of monitoring is to track field history; such records are useful for planning future mint plantations.

Verticillium levels in soil are estimated by collecting samples with a soil probe or clam shovel, followed by plating the dried and pulverized soil onto selective culture media. Crowe et al. (2000) evaluated three such methods: the Anderson air sampler, wet sieving, and the Harris method; the latter is the most reliable. Although results of soil sampling can be quite variable, such information may prevent growers from planting into the most severely infected mint fields.

Determining the aggressiveness of a Verticillium strain usually involves a living-plant bioassay. Three such methods have been evaluated in mint. In the first method, rooted mint plants are dipped into aqueous suspensions of conidia, then planted into small pots. This method results in the fastest symptom expression, usually 2 to 3 weeks. The second method involves injecting conidia directly into mint stems. This method may be less reliable because more plants escape infection, and because resistance mechanisms located in mint root are bypassed (Mathre et al., 1999). However, such methods may be useful under some circumstances. For instance, Crowe (1991) showed that direct injection of Verticillium colonies from selective media into peppermint stems was effective at distinguishing between potato- and mint-aggressive strains. A third method involves inoculating MS into containers of established mint. Although this method more closely represents natural conditions, it takes longer for symptoms to appear.

Other Verticillium strain bioassays do not involve living plants. Douhan and Johnson (2001) have separated mint-aggressive and mint-benign strains by placing them into vegetative

compatibility groups (VCG). Theoretically, only closely related strains can fuse, or anastomose, to form heterokayons. More research is needed to determine whether or not virulent mint strains could be found in VCGs thought to contain only nonmint strains. In the future, perhaps genetic methods also may be used to distinguish among strains (Tjamos, 2000).

Management. Unless peppermint and spearmint varieties completely immune to Verticillium are developed, the best management approach is likely to be a combination of soil fumigation, cultural practices, and host plant resistance. Although conventional fungicides have not proven effective against wilt under field conditions, novel ones should be evaluated as they become available.

Prevention. Preventing the introduction of Verticillium into mint fields is a key management tactic (Horner, 1955; Green, 1963). Low to moderate wilt levels may be managed by preplant soil fumigation (MacSwan and Horner, 1965; Koepsell and Horner, 1975), although the high cost of this practice may only be justified in fields to be planted with rootstock.

Planting clean rootstock is essential because once wilt becomes established, inoculum levels may decline very slowly; the actual rate of decline may depend on the plant species grown, biological activity of the soil, and soil type. For instance, MS decline more slowly in sandy-loam soils than in silt-loam soils (Green, 1980). After planting, avoid introducing wilt by other means such as in contaminated water supplies or on farm equipment. Procedures used to sanitize equipment must be practical, however, or else they will not be adopted. The same can be said with regard to sanitizing footwear used by field workers.

Despite best efforts at avoidance, Verticillium is likely to occur in most peppermint and Scotch spearmint fields, especially the more times they were previously planted to mint or the longer they remain in production. Once Verticillium is identified in a mint plantation, practical methods can help contain the disease and reduce inoculum levels. One of the best ways to prevent spreading Verticillium throughout established mint fields is to avoid practices that involve tillage, which accelerates the release of MS from dead mint stems back into the soil (Crowe, 1996).

Propane flaming mint fields after harvest can effectively reduce the rate of inoculum increase (Horner and Dooley, 1965; McIntyre and Horner, 1973; Crowe, 1996), especially if wilt levels are low to begin with. A propane flamer traveling at one mile per hour (1.6 km/h) with a gas pressure of 35 psi (2.4 atmos or 241 kPa) nearly eliminates the fungus from peppermint stems. At 2.0 mph (3.2 km/h), wilt levels are reduced 80% to 89%, depending on whether or not the soil is wet or dry, respectively. The mortality of Verticillium in stems decreases rapidly at flamer speeds greater than 2.0 mph. Flaming is expensive, however. In addition, because this method results in unacceptable mint injury in cooler growing regions and increases the severity of spider mites, it may not be a practical management tool for the future.

Host Plant Resistance or Tolerance. The mechanism of Verticillium resistance or tolerance in mint may reside in the root system where the spread of infection from roots to the vascular system is reduced (Crowe, 1991; Mathre et al., 1999). Verticillium may produce less inoculum in resistant mint species and/or varieties (Crowe, 1998; Johnson and Douhan, 1997), increasing the time required to reach damaging levels.

Native spearmint is considered to be the most wilt-tolerant of commercial peppermint and spearmint species, but it is not immune. Even though symptoms were mild or absent, Crowe (1998) found that mint-aggressive wilt strains reduced native spearmint hay and oil yields by 10%. More importantly, another wilt strain isolated from California strawberry fields produced even greater symptoms on Native spearmint than did an aggressive peppermint strain (Crowe, 1998). Scotch spearmint is more susceptible to wilt than Native spearmint, and

Black Mitcham peppermint is considered the most susceptible of all commercial U.S. peppermint and spearmint cultivars (Johnson, 1991).

To increase Verticillium resistance in peppermint, Dr. Merrit Murray, an A.M. Todd geneticist, implemented a mutation-breeding program in the 1960s. This program led to the release of two wilt-resistant peppermint cultivars, Todd's Mitcham and Murray Mitcham (Murray, 1969; Murray and Todd, 1972), which allowed peppermint to be produced in regions where it otherwise could not grow.

When challenged with the same inoculum level, laboratory bioassays by Crowe (1999) showed similar wilt susceptibilities among Black, Murray, or Todd's Mitcham (Crowe, 1998). But more MS, followed by rapid stand decline, were observed after year two in field plots of Black Mitcham compared with Murray or Todd's Mitcham. Other studies confirm that the rate of inoculum increase is highest in plots of Black Mitcham compared with more wilt-resistant mints. Johnson and Douhan (1998) inoculated three different mints with Verticillium under greenhouse conditions, and in just 8 weeks Black Mitcham exhibited significantly more colony-forming units (CFU) per centimeter of stem than either Roberts Mitcham peppermint or Native spearmint: 1002, 542, and 0 CFUs/cm of stem, respectively. Another study from western Oregon showed a similar rapid trend in Black Mitcham decline; Gingrich and Mellbye (1999) observed that field plots of Black Mitcham exhibited significantly more wilt strikes during the first year of production than plots containing more wilt-resistant peppermint varieties.

Although Murray Mitcham and Todd's Mitcham allowed for continued peppermint production in areas with low to moderate wilt (Lacy et al., 1981), these varieties were not immune and higher levels of resistance were desired. To address this need, Roberts Mitcham, M83-7 and B90-9 were developed through another mutation-breeding program in the late 1970s and early 1980s. These cultivars were evaluated against Black Mitcham, Murray Mitcham, and Todd's Mitcham from 1994 through 2003 at university experiment stations throughout the major U.S. mint-growing regions.

In studies where Verticillium was not introduced, Black Mitcham almost always yielded more oil than the wilt-resistant cultivars; the exception was B90-9, which yielded as well or better than Black Mitcham. In studies where Vertcillium was introduced into field plots prior to planting, resistant cultivars usually yielded as well or better than Black Mitcham, especially after the first year of production. In most instances, significant differences were not detected among the wilt-resistant cultivars with respect to oil yield and wilt severity. Although variety B90-9 yielded significantly more oil than the other wilt-resistant peppermints in one study from central Oregon (Crowe, 2001) and in another study from the Midwest (Green, 2001), it did not outperform the other wilt-resistant cultivars in field experiments conducted in Washington, western Oregon, Montana, and Idaho. In fact, B90-9 was completely overcome by wilt during the second growing season in western Oregon (Mellbye and Gingrich, 2002) and after the third growing season in the Midwest (Green, 2002a).

Crop Rotation. Peppermint and Scotch spearmint should be rotated to another crop every 3 to 5 years even if the field continues to yield well and wilt symptoms are not detected. If such a program is followed, a field may be planted back to mint in 3 to 5 years (Koepsell and Horner, 1975). This may not be long enough, however, if initial inoculum levels are high or if crops are planted that serve as maintenance hosts for this disease. In such situations, Verticillium levels may remain constant or even increase (Lacy and Horner, 1966; Davis et al., 2000).

A nonmint crop was once thought to be a good rotation for reducing Verticillium if symptoms did not occur or if the fungus failed to colonize its vascular system. This may not be the case, however, because wilt levels are maintained or increased in the rhizosphere of some plant species considered to be Verticillium resistant or immuned (Lacy and Horner,

1966; Douhan and Johnson, 2001), including barley, buckwheat, oats, wheat, peas, and sugar beets (Davis et al., 2000). Good rotational crops to combat Verticillium may include alfalfa, broccoli, corn, onion, and Sudan grass because these crops do not support appreciable increases in Verticillium levels (Nelson, 1950; McCain et al., 1981; Davis et al., 2000). Broccoli is a poor Verticillium host and also an effective green manure, acting in a similar way to commercial fumigants. In studies where broccoli was grown prior to planting mint, levels of MS were reduced substantially but not low enough in one year to prevent mint injury (Powelson et al., 1999). Effective weed control is also important in Verticillium management because many weed species can harbor this disease (McCain et al., 1981).

Biological Control. Naturally occurring biological control can reduce Verticillium levels in the soil, but just how this occurs and which organisms are involved is not always well understood (Nelson, 1950; Tjamos et al., 2000). Several natural enemies show promise against *V. dahliae* under tightly controlled conditions, but efforts to use them effectively in commercial mint plantings have not been successful. When Green (personal communication) inoculated mint roots with *V. tricorpus* to cross-protect against *V. dahliae*, newly formed mint roots were not protected. Melouk and Horner (1975) showed that cross-protecting mint roots against *V. dahliae* by first inoculating with *V. nigrescens* provided good protection. However, cross-protection was not effective enough when roots were inoculated with *V. nigrescens* at the same time or after infection by *V. dahliae*.

3.3.7.6.2 Rust

Over 5000 species of rust infect many plant species worldwide (Agrios, 1997). Mint rust (*Puccinia menthae*) infects only plants in the mint family (Horner, 1955; Pschiedt and Ocamb, 2004), and 11 races have been identified that may affect peppermint and spearmint differently (Johnson, 1995). Rust isolates from Native spearmint are unable to infect peppermint, and peppermint isolates are unable to infect Native spearmint. Isolates from both Native spearmint and peppermint infect Scotch spearmint, although the peppermint isolates develop more slowly on Scotch spearmint than those from Native spearmint (Pschiedt and Ocamb, 2004).

Peppermint rust is prevalent throughout western Oregon, western Washington, and Montana, but occurs only occasionally and at very low levels in arid regions of Washington, Oregon, and Idaho. Peppermint rust does not survive well at temperatures above 90°F (32.2°C) (Horner, 1955). In more recent studies, Johnson and Douhan (2000) showed that temperatures at or above 82°F (27.8°C) increased the latent period of peppermint rust and prevented germination and growth of urideal spores. The authors believe these factors are responsible for the low incidence of peppermint rust in south-central Washington. For unknown reasons, peppermint rust is not a problem in the more humid Midwest (Lacy et al., 1981). Spearmint rust appears more tolerant of high temperatures and routinely occurs throughout all U.S. spearmint growing regions. Research into rust-resistant peppermint cultivars was conducted in the early 1950s, but for unknown reasons this work was discontinued (Horner, 1955; Roberts and Horner, 1981).

Mint rust overwinters mostly as a teliospore. However, in temperate regions such as western Oregon, rust can also overwinter as urideal spores on mint leaves during mild years when leaves are not killed by cold temperatures. There are several other spore stages that occur during the winter and early spring (Horner, 1955). A yellow spore stage is released from reddish blisters found on mint stems located near the soil surface. Yellow spores infect mint leaves and give rise to brown urideal spores, which cycle repeatedly throughout the growing season. Urideal spores are dispersed by wind and can infect mint fields some distance away.

Mint Injury and Management. Left unchecked, mint rust can cause complete defoliation (Horner, 1955; Lacy et al., 1981). Clean plowing to a depth of 3 to 4 in. (ca. 7.5 to 10 cm) is effective against all mint rusts because buried spores are not infective (Maloy and Skotland,

1969; Lacy et al., 1981). Propane flaming mature peppermint fields in spring is effective at controlling rust in western Oregon (Roberts and Horner, 1981), although flaming newly established fields can result in severe injury. Peppermint is flamed when the majority of reddish blisters are above the soil surface. In western Oregon this usually occurs about mid-April. Effective flamer speeds are 2.5 to 3.0 mph (4.0 to 4.8) at 35 to 40 psi (241 to 276 kPa). Mint growers in western Oregon often flame their fields twice in the spring, about 1 week apart. Spring flaming spearmint is considered to be ineffective because spearmint rust is systemic, leading to reinfection of new growth after flaming.

Some peppermint growers use desiccants to remove rust-infected mint foliage. Although desiccants such as phos-acid (17.5 gallons/acre) (ca. 160 L/ha) are effective at reducing rust outbreaks, spider mites are not controlled by this practice (Fairchild and Morris, 1989). When flaming or using desiccants, it is important to destroy volunteer mint growing around field margins to prevent reinfection.

The fungicides azoxystrobin (Quadris at 6.2 to 15.4 fl Oz/acre) (447 to 1082 mL/ha), myclobutanil (Rally 40W at 4.0 to 5.0 fl Oz/acre) (289 to 361 mL/ha), and propiconazole (Tilt 4.0 and 8.0EC fl Oz/acre) (289 and 577 mL/ha) are all effective at controlling mint rust. Control is less effective if fields are treated after severe outbreaks occur. Other fungicides also have been evaluated. Slabaugh et al. (2001) showed that the biofungicides Sonata WP and Seranade WP (*Bacillus subtilis*, strain QST 713) controlled peppermint rust in western Oregon.

3.3.7.6.3 Powdery Mildew

Powdery mildews (PM) are white to gray fungi that are among the most common plant diseases worldwide (Agrios, 1997). Although *Erysiphe cichoracearum* is thought to be the species infecting peppermint and spearmint, this needs to be verified (Grey et al., 1995). Correctly identifying which mildew species infect mint can influence management decisions; for example, which variety to plant and how it should be maintained. Long-range PM dispersal is by aerial spores whereas short-range dispersal may occur by splashing water droplets.

PM is known to increase mint leaf loss and reduce winter survival (Pschiedt and Ocamb, 2004). Scotch spearmint is the most susceptible commercial mint species, whereas Native spearmint is the least susceptible (Grey et al., 1996). Of the peppermints, Black Mitcham is the most susceptible to PM, while Murray Mitcham, Robert's Mitcham, Todd's Mitcham, and M83-7 are about equal in their susceptibility (Grey et al., 1995). Meristemed peppermint is more susceptible than nonmeristemed (Welty and Prestbye, 1994).

PM can be effectively managed in mint with fungicides. Crowe and Butler (1996) showed that sulfur (Microthiol at 5.0 lb product/acre) (5.54 kg/ha), propiconazole (Tilt 4.8EC at 6.0 fl Oz/acre) (433 mL/ha), mycobutanil (Rally 40W at 5.0 fl Oz/acre) (361 mL/ha), and chlorothalonil (Bravo 720 at 1.5 pints/acre) (ca. 1.75 L/ha) were all effective against powdery mildew on peppermint in central Oregon. Another fungicide, azoxystrobin (Quadris at 6.2 to 15.4 fl Oz/acre) (183 to 455 mL/ha), is also effective against PM (Pschiedt and Ocamb, 2004). The ability of fungicides to cure some PM outbreaks on peppermint suggests that an AT could be determined.

3.3.7.6.4 General Stolon Root Rot

Several fungi including *Fusarium solani* and *Rhizoctonia solani* injure mint through stem and stolon decay (Green, 1961; Pschiedt and Ocamb, 2004). Horner (1971) reported that another fungus, *Phoma strasseri*, attacked mint stems and rhizomes causing a blackened appearance. *Phoma* also infects Scotch spearmint grown under sprinkler irrigation in Washington (Johnson and Miliczky, 1990). The white mold fungus, *Sclerotina sclerotium*, was linked to

severe winter kill of Scotch spearmint in south-central Washington (Skotland, personal communication) and has been observed elsewhere at low levels on peppermint. Anthracnose or Leopard spot (*Sphaceloma menthae*) is another disease that occasionally occurs on peppermint in Indiana (Lacy et al., 1981).

Root rots are most active during winter and spring, especially if fields are overirrigated or if standing water is an issue. Reducing mechanical and pest-related injury can help manage these diseases (Horner, 1955). Using clean soil and sanitizing cutting implements used during greenhouse rootstock operations is a good way to avoid infection by *Phoma strasseri* (Horner, 1971). The fungicide azoxystrobin (Quadris at 6.2 to 15.4 fl Oz/acre) (183 to 455 mL/ha) has activity on *Rhizoctonia* (Pschiedt and Ocamb, 2004).

3.3.7.6.5 Viruses

The impatiens necrotic spot virus (INSV) and the tomato spotted wilt virus (TSWV) can damage peppermint under greenhouse conditions (Sether et al., 1991). Although symptoms may persist when infected mint is planted to the field, they soon disappear and the diseases may be self-eliminating (Allen, 1991). Mint propagators can reduce the threat of virus outbreaks by having plants virus tested, sanitizing equipment, and controlling thrips in the greenhouse (DeAngelis et al., 1994). Other potentially damaging viruses also may infect mint and require further evaluation.

3.3.7.7 Mollusks

The mollusks are a group of interesting organisms that include the clams, octopuses, snails, and slugs. The gray field slug, *Deroceras reticulatum*, is the most economically important species to agriculture in the Pacific Northwest (Fisher and Heim, 2004). Whether or not this is the predominant species infesting mint is not known.

Slugs are hermaphrodites so all individuals are capable of laying eggs. Most eggs are laid in late summer to early fall during humid conditions. The gray field slug may lay another clutch of eggs in spring. Most eggs hatch in about 2 weeks, but eggs laid in the fall may overwinter. Slugs feed mostly at night, remaining concealed during the day to avoid desiccation. Little feeding occurs during high or low temperature extremes.

Slugs feed on peppermint and spearmint in all major mint-growing regions. Their economic importance to mint is not well understood, but it may be more than realized. Because of frequent irrigation, mint fields provide an ideal environment for slugs, and very high numbers were detected while sampling at night for RWs with a sweep net (Morris, unpublished data). Slugs may be sampled by examining mint foliage at night with a flashlight or with a sweep net, although the latter can be quite messy. Slugs also may be sampled by placing a piece of plywood on the soil surface and counting the number of slugs found underneath (Fisher and Heim, 2004).

The effect of natural enemies on slug populations in mint is not known. When Berry and Crowe (1996) tested two species of entomopathogenic nematodes—*Heterorhabditis marelatus* and *Stienernema oregonensis*—against gray garden slug eggs and juveniles, applied rates of two- or four-billion IJs/acre (4.9 or 9.9 billion/ha) were ineffective. The commercial slug bait Deadline (metaldehyde 0.4 to 1.6 lb ai/acre) (467 to 1283 g/ha) is labeled for use on mint, but its economic benefits have not been evaluated.

3.3.7.8 Plant Parasitic Nematodes

Nematodes are a diverse group of unsegmented roundworms that include beneficial predators of arthropod pests (Dropkin, 1989) as well as several species that injure mint and must be managed. Nematodes feed on mint roots, reducing vigor, lowering oil yields, and reducing

winter survival (Santo and Wilson, 1991; Ingham and Merrifield, 1996). Nematodes also interact with other pests such as Verticillium wilt to cause even greater injury than would occur in the presence of either pest alone (Johnson and Santo, 2001). Damage caused by root-rotting pathogens such as *Rhizoctonia* and *Fusarium* is greatest when entrance wounds are provided by nematodes or soil arthropods (Horner, 1955).

The root lesion nematode *Pratylenchus penetrans* is considered to be the most important nematode pest of mint, capable of reducing foliage by 46%, root mass by 86% (Bergeson and Green, 1979), and oil yields by 95% (Pinkerton et al., 1988). Damage caused by *P. penetrans* is most severe when mint is grown in sandy soils (Pinkerton, 1983). This species is a migratory endoparasite that spends most of its life inside the roots of host plants including cereals, vegetables, and mint. Ingham and Merrifield (1996) show that for each incremental increase of 290 to 570 *P. penetrans* per quart of soil, or 45 to 165/g of fresh root, 1 lb (ca. 450 g) of oil is lost for stressed and vigorous peppermint, respectively. This injury level may need to be reduced to account for additional injury that may result during extremely cold temperatures (Santo and Wilson, 1991). Other nematode species known to injure mint include the needle nematode, *Longidorus sylphus* (Horner, 1955); pin nematode, *Paratylenchus* spp. (Ingham and Merrifield, 1996); Northern root knot nematode, *Meloidogyne hapla* (Santo et al., 1986); and stubby root nematode, *Trichodorus* spp. (Ingham and Merrifield, 1996).

3.3.7.8.1 Crop Monitoring

Nematode population levels are estimated from soil and mint roots by extraction methods selected based on biology and mobility (Dropkin, 1989). Sampling for root lesion nematodes is best done in spring or late summer while populations are at their peak (Ingham and Merrifield, 1996). *P. penetrans* is a migratory endoparasite, so it is important to sample mint roots; this species may go undetected with soil sampling alone. Other less damaging lesion species may be detected in soil but not in roots. Soil sampling for pin nematode in late summer is effective for estimating populations of this ectoparasite, and soil samples for two other ectoparasites, *Longidorus* and *Trichodorus*, may be taken in either early spring or late summer.

3.3.7.8.2 Cultural and Biological Control

Planting clean, vigorous rootstock, and sanitizing farm equipment are two important nematode management tactics. Because most nematode species that infect mint have a wide host range, avoiding them in new mint plantings is difficult. Rotating out of mint before plant vigor deteriorates is another effective management tactic.

Although many natural enemies feed on plant parasitic nematodes, their impact on nematode mint pests is not known. Commercial formulations of entomopathogenic nematodes have been evaluated against lesion nematode in mint. Ingham and Berry (1995) showed that a rate of 6 billion IJs/acre failed to reduce *P. penetrans* populations in one field study. In another study, Santo et al. (1999) showed that adding entomopathogenic nematodes 2 weeks prior to the addition of *P. penetrans* reduced lesion nematodes by more than 72%. The improved control was attributed to the lethal toxins released by entomopathogenic nematodes following their death. However, when entomopathogenic nematodes were applied at the same time as *P. penetrans*, populations were not reduced.

3.3.7.8.3 Pesticide Strategy

Preplant soil fumigation is an effective nematode control tactic. Applying low rates of fumigants such as metham-sodium to established mint fields should be avoided; however, because severe phytotoxicity can occur (Santo and Wilson, 1998). Oxamyl is currently the only nematicide labeled for use on mint in the U.S. Although oxamyl (1.0 to 3.0 lb ai/acre) (1.1 to 3.3 kg/ha) may reduce *P. penetrans* in sandy soils, it is much less effective against this

species when applied to heavier soil types (Bergeson and Green, 1979; Pinkerton et al., 1988). However, oxamyl may not work well against lesion nematode in the presence of Verticillium wilt. Oxamyl is very effective against the needle nematode (*Longidorus sylphus*) in western Oregon (Pinkerton and Jensen, 1983). Because oxamyl is expensive, more effort is needed to quantify its economic benefits to mint against a wider range of nematode pests and field conditions.

3.3.7.9 Weed Management

Weed management is among the most important aspects of commercial mint production (Green, 1961; Stanger, 1977; Lacy et al., 1981; Brewster and Appleby, 1985). Not only can weeds reduce mint oil yields by competing for light, water, nutrients, and space, they also can reduce mint oil quality (Green, 1961; Martin et al., 1976). Examples of important weeds that impart off odors to mint oil include groundsel (*Senecio vulgaris*), lambsquarters (*Chenopodium album*), kochia (*Kochia scoparia*), mustards (Brassicaceae), nightshades (Solanacea), prickley lettuce (*Latuca serriola*), redroot pigweed (*Amaranthus retroflexus*), Russian thistle (*Salsola iberica*), and salsify (*Tragopogon porrifolius*) (Colquhoun et al., 2001). Although weedy grass species impart off odors to mint oils less often than do broadleaf weeds, they can reduce the quality of mint oil color. Weeds also can negatively impact mint production by harboring mint pests. For example, many weed species are hosts to Verticillium wilt, possibly preventing the reduction of wilt inoculum to manageable levels (McCain et al., 1981).

3.3.7.9.1 Crop Monitoring and Cultural Control

Knowledge gained by monitoring weed germination, size, and species can assist farm managers in selecting the most appropriate weed control strategy. Monitoring mint fields for actively growing weeds can be done along with sampling for arthropods, plant nutrients, and nematodes.

Several cultural practices are effective at managing weeds in mint. Preventing the introduction of weeds in rootstock, farm equipment, and irrigation is especially necessary for noxious species that are difficult to control or not presently found in a particular geographical region, including Canada thistle (*Cirsium arvense*), creeping buttercup (*Ranunculus repens*), and field bindweed (*Convolvulus arvensis*).

Although tillage is effective against some weed species (Talkington and Berry, 1983), fewer acres are tilled today because of Verticillium wilt and soil erosion. Hand hoeing mint fields is also effective at removing the few weeds remaining after herbicide applications, but this practice alone is not practical for a conventional mint oil production program. An exception could be the organically produced mint oil where customers are willing to pay much more for the oil. Cost estimates for hand weeding a mint field may exceed $1500/acre, compared with under $200 for a judicious herbicide program.

Rotating out of mint every 3 to 5 years may allow for the use of different herbicides that more effectively control some problem weeds and may also slow the development of herbicide resistance. Several herbicide-resistant weed populations have been identified in mint including bromoxynil and terbacil-resistant groundsel, and terbacil-resistant pigweed and lambsquarters (Heap, personal communication; William et al., 2004). The highest incidence of herbicide resistance occurs in regions with a history of growing mint in the same field for 10 years or more.

3.3.7.9.2 Biological Control

Biological control is rarely effective at controlling weeds in mint. An interesting exception is the Colorado potato beetle (*Leptinotarsa decemlineata*), which occasionally eliminates volunteer potato in established mint fields. Another example is the painted lady butterfly. This

species is usually seen in mint fields feeding on Canada thistle; however, mint injury can occur during years when populations are very high.

The U.S. Mint Industry has sponsored research into biological control of field bindweed in mint. In this study, Kassim et al. (1998) released a bud mite (*Aceria malherbae*) and a noctuid moth (*Tyta luctuosa*) into mint plots in south-central Washington. Although both species established at low levels, they were not effective enough to control bindweed. Such a program may be effective, however, at reducing bindweed populations outside of mint fields, thereby reducing their dispersal into mint.

Another kind of biological weed control is the use of farm animals. Weeder geese, sheep and goats were used in mint for many years to control weeds before effective herbicides were labeled. However, the use of such animals is usually not cost effective in conventional mint plantings due to the labor required to fence the animals, move them from place to place, and prevent them from excessively feeding or trampling on mint.

3.3.7.9.3 Herbicide Strategy during the Dormant Season

Once mint enters dormancy, the preemergence herbicides clomazone, diuron, oxyflurfen, pendimethalin, trifluralin, and sulfentrazone may be applied safely to control germinating annual and perennial weeds (Colquhoun et al., 2001). Because herbicide efficacy and crop safety can depend on factors such as climate, agricultural practices, soil conditions, and stand vigor, the label should be consulted carefully for important restrictions and caveats. For example, to avoid mint injury, diuron should not be applied if soil organic matter is below one percent. To improve efficacy and crop safety, dormant-season herbicides are best applied from mid-December through early February, instead of late February through March. Because different herbicides vary in their weed-control spectrum, they are often tank mixed together. For example, clomazone is more effective against grassy weeds, whereas sulfentrazone is better against some broadleaf weeds. Extensive testing of tank-mix combinations should be conducted, however, to avoid harmful synergistic effects.

Two other herbicides, terbacil and paraquat, are used extensively during the dormant season. Terbacil, arguably the most important herbicide ever labeled for use on mint, can be applied safely to both dormant and actively growing mint. It controls many broadleaf and grassy weeds and has both pre- and postemergence activity. The major cause of control failures associated with terbacil is lack of moisture following application. Paraquat is usually tank mixed with preemergence herbicides to burn down weeds throughout the dormant season.

3.3.7.9.4 Herbicide Strategy during the Growing Season

A new weed flush occurs around the time mint breaks dormancy. Herbicides effective against broadleaf weeds in actively growing mint include bentazon, bromoxynil, clopyralid, pyridate, and terbacil. Broadleaf weeds usually are controlled more easily when they are small and actively growing. An exception is Canada thistle, where clopyralid should be applied at the 1-ft (ca. 30 cm) stage, while bentazon is more efficacious when applied at the flowering stage.

Herbicides that effectively control many grassy weeds in mint include clethodim, quizalofop, sethoxydim, and terbacil. Terbacil is often applied as a tank mix with other broadleaf and grass herbicides to enhance their activity and provide residual weed control. To avoid severe mint injury, applications of terbacil should not be applied within thirty days of the insecticide fonofos. Herbicides that are safe to use during the growing season are as safe or safer to use after final harvest in late summer and fall.

3.3.8 Harvest

When to harvest is one of the mint grower's most important decisions. Many factors influence harvest timing, including oil yield and quality, stand age, crop maturity, climate, length of the growing season, and whether the crop is to be harvested once or twice during the season.

Depending on temperature, wind speed, soil moisture, and other factors, irrigation should cease 1 to 5 days prior to harvest. The crop should not become overly dry prior to swathing or oil quality and crop vigor may suffer. Mint is first swathed into windrows and allowed to cure for 1 to 3 days. Following the curing process, windrows are chopped into 2-in. pieces with a forage chopper (Lacy et al., 1981). The windrow should not be too moist or too dry prior to chopping or poor oil extraction or leaf shatter may occur, respectively.

The ideal time to harvest mint is when oil yield and oil quality are at their peak. Because these two criteria rarely coincide, compromise is necessary. This is especially true for peppermint, whose oil quality varies considerably more than spearmint as harvest time approaches. For peppermint, desirable oil quality includes menthol levels above 50%, and menthofuran and pulegone levels below 4% and 2%, respectively. High-quality spearmint oil contains carvone above 60%.

Because maturation is delayed during the first year of production, new peppermint fields are often harvested toward the later part of August to early September for optimal yield. In new stands, harvesting at this relatively late date does not affect oil quality, as is the case for mature single-cut stands.

Mature peppermint stands to be single-cut are harvested at about 10% bloom, which occurs in early to mid-August. Although oil yields tend to increase as flower bloom reaches 50% or more (Hee and Jackson, 1973), menthofuran also increases, largely because peppermint flowers contain high levels of this compound. (Abundant flower bloom usually does not reduce spearmint oil quality.) Peppermint oil obtained by harvesting in late September or October is considered overmature. At this point, while menthol levels approach 60% and menthofuran has decreased, oil yields also drop dramatically. We love over-mature peppermint quality, but very low oil yields and other late-harvest perils make it impractically expensive to produce.

Mint fields harvested too late in the season suffer significant yield losses through oil catabolism (Croteau, 1988) and also can be damaged by fall rains. Late harvest also can lead to reduced oil quality because heavy dew accumulating on windrows imparts a musty odor to the oil. Finally, reduced winter survival may result from late harvest because root reserves are not replenished (Mitchell et al., 1998).

Although spearmint has long been harvested twice a season in regions with long growing seasons such as south-central Washington, more peppermint fields are also now double-cut to reduce cost. This practice has modified the concept of what constitutes desirable peppermint oil. Clark and Menary (1980b, 1984) observed significant changes in oil composition between the first and second cuts, with second cuts having higher levels of menthol, menthyl acetate, menthofuran, and limonene and lower levels of menthone and cineole. This second harvest may compensate somewhat for the immature oil qualities of the first cut. Studies on peppermint by Schneider (unpublished data) showed that harvesting the first cut in early July was preferable to harvesting in mid-July because higher oil yields were obtained in the second cut, and oil from the second harvest was higher in menthol. However, if temperatures approach or exceed 100°F (37.8°C) in mid-July, excessive heat stress can reduce regrowth following the first harvest, resulting in even lower oil yields during the second harvest. Winter survival is

also more precarious for a double-cut crop, especially when grown on sandy soils in colder growing regions.

3.4 SUMMARY AND CONCLUSIONS

Peppermint and spearmint growers in the U.S. are facing an uncertain future. Businesses that traditionally relied on U.S. peppermint and spearmint oils are presently under pressure to reduce their raw material costs. This urgency has been passed on to the U.S. farmer, who must now compete not only with foreign producers of these two crops, but also against lower cost formulations composed of less-expensive mint oils. This is a difficult task considering that production costs are increasing, especially those tied to petrochemicals, electric power, water, and labor.

Mint farmers in the U.S. cannot compete with peppermint and spearmint farmers from developing countries on a labor-cost basis alone. If the U.S. Mint Industry is to prosper, it must continue to implement safe and effective agricultural technologies, adopt sound business practices, and solve important production problems such as Verticillium wilt.

REFERENCES

Agrios, G.N. (1997). *Plant Pathology*, Academic Press, San Diego, 635 pp.

Allen, T.C. (1991). Research Report: Tomato Spotted Wilt Virus in Mint, Mint Industry Research Council Research Reports.

Baird, C.R., Bishop, G.W., and Baruni, M.A. (1987). Life history and distribution of *Pseudobaris nigrina* (Say) in Idaho (Coleoptera: Curculionidae), *Coleopterists Bull.*, 39, 221–225.

Baird, C.R., Bolz, D.G., and Bishop, G.W. (1990). The mint stem borer in Idaho, University of Idaho Current Information Series No. 808, 4 pp.

Baruni, M.A. (1979). The biology of *Pseudobaris nigrina* (Say) on mint in Idaho (Coleoptera: Curculionidae), M.S. Thesis, University of Idaho, Moscow.

Beckstrom-Sternberg, M. and Duke, J.A. (1996). *CRC Handbook of Medicinal Mints (Aromathematics): Phytochemicals and Biological Activities*, CRC Press, Boca Raton, 458 pp.

Bergeson, G.B. and Green Jr., R.J. (1979). Damage to cultivars of peppermint, *Mentha piperita*, by the lesion nematode *Pratylenchus penetrans* in Indiana, *Plant. Dis. Reptr.*, 63, 91–94.

Berry, R.E. (1973). Biology of the predaceous mite, *Pergamasus quisquiliarum*, on the garden symphylan, *Scutigerella immaculate*, in the laboratory, *Ann. Entomol. Soc. Am.*, 66, 1354–1356.

Berry, R.E. (1974). Biology of *Pyrausta fumalis* on peppermint in Oregon, *Ann. Entomol. Soc. Am.*, 67, 580–582.

Berry, R.E. (1978). *Insects and Mites of Economic Importance in the Northwest*, Oregon State University Bookstores Inc., Corvallis, OR, 189 pp.

Berry, R.E. and Crowe, F. (1996). Insect and slug control with entomopathogenic nematodes, Agricultural Research Foundation Report, Oregon State University, Corvallis.

Berry, R.E. and Fisher, G. (1993). A guide to peppermint insect and mite identification and management, PNW Bulletin 182, Oregon State University, Corvallis, 37 pp.

Berry, R.E. and Robinson, R.R. (1974). Biology and control of the garden symphylan, Oregon State University Extension Circular 845, 9 pp.

Berry, R.E., Takeyasu, J., and Morris, M. (1991). Biology and management of arthropods in peppermint, Agricultural Research Foundation Report, Oregon State University, Corvallis.

Biggs, R.J. and Leopold, A.C. (1955). The effects of temperature on peppermint, *Proc. Am. Soc. Hort. Sci.*, 66, 315–321.

Blumenthal, M. (2003). The *ABC Clinical Guide to Herbs*, American Botanical Council, Austin, TX, 480 pp.

Brewster, B.D. and Appleby, A.P. (1985). Repeated applications of herbicide tank mixes in peppermint, *West. Soc. Weed. Sci. Res. Prog. Rpt.*, 255 pp.

Brown, B. (1982). Nitrogen tissue test procedures for peppermint, Thirty-Third Annual Northwest Fertilizer Conference, Boise, ID, July 13–14, pp. 39–49.

Brown, D. (1995). *Encyclopedia of Herbs and Their Uses*, Dorling Kindersley, New York, 424 pp.

Bullis, D.E., Price, F.E., and Kirk, D.E. (1948). Relationship of Maturing and Weathering to Yield and Quality of Peppermint Oil, Station Bulletin 458, Oregon State College Agricultural Experiment Station, Corvallis, OR, 15 pp.

Burbott, A.J. and Loomis, W.D. (1967). Effects of light and temperature on the monoterpenes of peppermint, *Plant Physiol.*, 42, 20–28.

Cacka, J.F. (1982). Dispersion characteristics and economic threshold levels of strawberry root weevil in peppermint in Oregon, M.S. Thesis, Oregon State University, Corvallis, 74 pp.

Charles, D.J., Joly, R.J., and Simon, J.E. (1990). Effects of osmotic stress on the essential oil content and composition of peppermint, *Phytochemistry*, 29, 2837–2840.

Christensen, N.W., Hart, J., Melbye, M., and Gingrich, G. (1996). Optimal nitrogen and sulfur fertilization of peppermint grown in the Willamette Valley, Agricultural Research Foundation Report, Oregon State University, Corvallis.

Clark, R.J. and Menary, R.C. (1979). Effects of photoperiod on the yield and composition of peppermint oil, *J. Am. Soc. Hort. Sci.*, 104, 699–702.

Clark, R.J. and Menary, R.C. (1980a). Environmental effects on peppermint (*Mentha piperita* L.) I. Effect of day length, photon flux density, night temperature and day temperature on the yield and composition of peppermint oil, *Aust. J. Plant Physiol.*, 7, 685–692.

Clark, R.J. and Menary, R.C. (1980b). The effect of irrigation and nitrogen on the yield and composition of peppermint oil (*Mentha piperita* L.), *Aust. J. Agric. Res.*, 31, 489–498.

Clark, R.J. and Menary, R.C. (1984). The effect of two harvests per year on the yield and composition of Tasmanian peppermint oil (*Mentha piperita* L.), *J. Sci. Food Agric.*, 35, 1191–1195.

Colquhoun, J., Brewster, B., and Mallory-Smith, C. (2001). Weed management in mint, EM 8774, Oregon State University Extension Service, Corvallis.

Cook, R.J. and Veseth, J.R. (1991). *Wheat Health Management*, APS Press, 152 pp.

Cook, G., Hart, J., Christensen, N., Quebbeman, B., Kiemnec, G., and Diebel, K. (1999). Peppermint growth and nutrient uptake in plants in the Grande Ronde Valley, Agricultural Research Foundation Report, Oregon State University, Corvallis.

Coop, L.B. (1987). Phenology modeling to predict occurrence and treatment thresholds for variegated cutworm, *Peridroma saucia* Hubner, on peppermint, Ph.D. Thesis, Oregon State University, Corvallis, 154 pp.

Coop, L.B. and Croft, B.A. (1995). *Neoseiulus fallacis*: dispersal and biological control of *Tetranychus urticae* following minimal inoculations into a strawberry field, *Exp. Appl. Acarol.*, 19, 31–43.

Croft, B.A. (1990). *Arthropod Biological Control Agents and Pesticides*, Wiley Interscience, New York, 723 pp.

Croteau, R. (1977). Effect of irrigation method on essential oil yield and rate of oil evaporation in mint grown under controlled conditions, *Hort. Sci.*, 12, 563–565.

Croteau, R. (1988). Catabolism of monoterpenes in essential oil plants. In: Lawrence, B.M., Mookherjee, B.D., and Willis, B.J., Eds., *Flavors and Fragrances: A World Perspective*, Proceedings of the 10th International Congress of Essential Oils, Fragrances and Flavors, Washington, DC, U.S.A., November 16–20. Elsevier Science Publishers, Amsterdam, pp. 65–84.

Crowe, F. (1991). Verticillium wilt on mint, Agricultural Research Foundation Report, Oregon State University, Corvallis.

Crowe, F. (1996). Verticillium wilt on mint, Agricultural Research Foundation Report, Oregon State University, Corvallis.

Crowe, F. (1997). Do varietal specific isolates of *Verticillium dahliae* occur on mint, and do microsclerotia of mint isolates form in symptomless but infected stems of mint and non-mint plants? Agricultural Research Foundation Report, Oregon State University, Corvallis.

Crowe, F. (1998). Do varietal-specific isolates of *Verticillium dahliae* occur on mint? Agricultural Research Foundation Report, Oregon State University, Corvallis.

Crowe, F. (1999). Verticillium wilt on mint, Agricultural Research Foundation Report, Oregon State University, Corvallis.

Crowe, F. (2001). Peppermint variety trial, Central Oregon, Agricultural Research Foundation Report, Oregon State University, Corvallis.

Crowe, F. and Butler, M. (1996). Mint powdery mildew control in Central Oregon, Agricultural Research Foundation Report, Oregon State University, Corvallis.

Crowe, F. and Lommel, S. (1994). Evaluation of peppermint field performance from plants regenerated from meristem tip culture, and investigations of virus infection, Mint Industry Research Council Research Reports.

Crowe, F., Lommel, S., and Mitchell, A. (1995). Evaluation of peppermint field performance from plants generated from meristem tip culture, and investigations of virus infection, Agricultural Experiment Station Special Report, Oregon State University, Corvallis, 941, 7–18.

Crowe, F.J., Debons, J., and Farris, N. (2000). Peppermint performance and changes in inoculum density of *Verticillatum dahliae* associated with management practices, In: Tjamos, E.C., Rowe, R.C., Heale, J.B., and Fravel, D.R., Eds., *Advances in Verticillium Research and Disease Management*, The American Phytopathological Society, St. Paul, Minnesota, 357p.

Danielson, S.D. and Berry, R.E. (1978). Redbacked cutworm: Sequential sampling plans in peppermint, *J. Econ. Entomol.*, 71, 323–328.

Davis, J.R., Huisman, O.C., Sorensen, L.H., and Schneider, A.T. (2000). Field studies comparing the susceptibility of various crops to colonization by *Verticillium dahliae*. In: Tjamos, E.C., Rowe, R.C., Heale, J.B., and Fravel, D.R., Eds., Advances in Verticillium Research and Management, The American Phytopathological Society, St. Paul, MN, pp. 311–314.

DeAngelis, J.D., Larson, K.C., Berry, R.E., and Krantz, G.W. (1982). Effects of spider mite injury on transpiration and leaf water status in peppermint, *Environ. Entomol.*, 11, 975–978.

DeAngelis, J.D., Berry, R.E., and Krantz, G.W. (1983a). Evidence for spider mite Acari: Tetranychidae injury induced water deficits and osmotic adjustment in peppermint, *Environ. Entomol.*, 12, 336–339.

DeAngelis, J.D., Berry, R.E., and Krantz, G.W. (1983b). Photosynthesis, leaf conductance, and leaf chlorophyll content in spider mite-injured peppermint leaves, *Environ. Entomol.*, 12, 345–348.

DeAngelis, J.D., Marin, A.B., Berry, R.E., and Krantz, G.W. (1983c). Effects of spider mite Acari: Tetranychidae injury on essential oil metabolism in peppermint, *Environ. Entomol.*, 12, 522–527.

DeAngelis, J.D., Sethe, D.M., and Rossignol, P.A. (1994). Transmission of impatiens necrotic spot virus in peppermint by western flower thrips. *J. Econ. Entomol.*, 87, 197–201.

DeFrancisco, J. (2002). *Workshop on a Pest Management Strategic Plan for Pacific Northwest Mint Production*, Oregon State University, Corvallis.

Douhan, L.I. and Johnson, D.A. (2001). Vegetative compatibility and pathogenicity of *Verticillium dahliae* from spearmint and peppermint, *Plant Dis. Reptr.*, 85, 297–302.

Dropkin, V.H. (1989). *Introduction to Plant Nematology*, John Wiley and Sons, New York, 304 pp.

Edwards, C.A. (1990). Symphyla. In: Dindal, D.L. Ed., *Soil Biology Guide*, Wiley Interscience, New York, pp. 891–910.

Ellis, N.K., Fawcett, K.I., Gaylord, F.C., and Baldinger, L.H. (1941). A study of some factors affecting the yield and market value of peppermint oil, Purdue University Agricultural Experiment Station Bulletin No. 461, 27 pp.

Fairchild, E. and Morris, M.A. (1989). Twospotted spider mite control: Flaming vs Enquik, Agricultural Research Foundation Report, Oregon State University, Corvallis.

Faulkner, L.R., Bolander, W.J., and Skotland, C.B. (1970). Interaction of *Verticillium dahliae* and *Pratylenchus minyus* in Verticillium Wilt of Peppermint: influence of the Nematode as determined by a double root technique, *Phytopathology*, 60, 100–103.

Fenwick, A.L. and Ward, S.M. (2001). Use of random amplified polymorphic DNA markers for cultivar identification in mint, *Hort. Sci.*, 36, 761–764.

Ferguson, H. and Walsh, D. (2002). Pest management Practices in Washington State Mint: a 2002 survey, Washington State University Extension Bulletin, 1989E.

Fisher, G. and Heim, I. (2004). Slug control. In: Bragg, D. Ed., *Pacific Northwest Insect Management Handbook*, University Extension Services, Corvallis, OR, pp. 127–131.

Fisher, G., Gingrich, G., Melbye, M., and Morris, M. (2004). Mint insect pests. In: Bragg, D. Ed., *Pacific Northwest Insect Management Handbook*, University Extension Services, Corvallis, pp. 127–131.

Gingrich, G. and Mellbye, M. (1999). Evaluation of peppermint varieties and advanced selections in the Willamette Valley of western Oregon, Agricultural Research Foundation Report, Oregon State University, Corvallis.

Gobert, V., Moja, S., Colson, M., and Taberlet, P. (2002). Hybridization in the section Mentha (Lamiaceae) inferred from AFLP markers, *Am. J. Bot*., 89, 2017–2023.

Green, R.J. Jr. (1951). Studies on the host range of the Verticillium that causes wilt of *Mentha piperita* L, *Science*, 2930, 207–208.

Green, R.J. Jr. (1961). Stolon decay of commercial species of mint in Indiana, *Plant Dis. Reptr*., 45, 288.

Green, R.J. Jr. (1963). Mint *Farming, Agriculture Information Bulletin No*. 212. U.S. Department of Agriculture, Washington, D.C, 26 pp.

Green, R.J. Jr. (1977). Alteration of pathogenicity of *Verticillium dahliae* from *Mentha* sp. Under field conditions, *Plant Dis. Reptr*., 61, 373–374.

Green, R.J. Jr. (1980). Soil factors affecting survival of microsclerotia of *Verticillium dahliae*, *Phytopathology*, 70, 353–355.

Green, R.J. Jr. (2001). Field performance of selected MIRC advanced peppermint and spearmint lines in Indiana, Mint Industry Research Council Research Reports.

Green, R.J. Jr. (2002a). Field performance of selected MIRC advanced peppermint and spearmint lines in Indiana, Mint Industry Research Council Research Reports.

Green, R.J. Jr. (2002b). Squirrely peppermint—cause and control, Mint Industry Research Council Newsletter, Spring 2002.

Green, R.J. Jr., Sampson, R.W., and Fordyce, C. (1961). Verticillium wilt of potato in northern Indiana, *Plant Dis. Reptr*., 45, 1.

Grey, W.E., Johnson, M., and Mathre, D.E. (1995). Evaluation of standard mint varieties for disease reaction and latent infection period to mint rust and powdery mildew, Mint Industry Research Council Research Reports.

Grey, W.E., Johnson, M., and Mathre, D.E. (1996). Evaluation of standard mint varieties for disease reaction and latent infection period to mint rust and powdery mildew, Mint Industry Research Council Research Reports.

Hart, J. (1990). Fertilizer guide: fertilizer and lime materials, Field Guild 52, Oregon State University Extension Service.

Hart, J., Melbye, M., gingrich, G., Marx, E., and Kauffman, M. (1995). Biomass and nitrogen accumulation in peppermint, Agricultural Research Foundation Report, Oregon State University, Corvallis.

Heale, J.B. (2000). Diversification and speciation in Verticillium—An Overview. In: Tjamos, E.C., Rowe, R.C., Heale, J.B., and Fravel, D.R., Eds., *Advances in Verticillium Research and Management*, The American Phytopathological Society, St. Paul, MN, pp. 1–44.

Hee, S.M. (1975). The effect of nitrogen fertilization on peppermint oil quality and content, M.S. Thesis, Oregon State University, Corvallis, OR.

Hee, S.M. and Jackson, T.L. (1973). The effect of nitrogen fertilization and state of maturity on the yield and quality of peppermint oil, Proceedings of the Oregon Essential Oil Growers Leaque Abstracts, 29–41.

Helle, W. and Sabelis, M.W. (1985). *Spider mites: Their Biology, Natural Enemies and Control*, World crop pests, vol. 1A. Elsevier, Amsterdam.

Hollingsworth, C.S. (1981). Twospotted spider mite, *Tetranychus urticae* Koch, in Oregon peppermint, sampling and economic injury, Ph.D. Thesis, Oregon State University, Corvallis, 172 pp.

Horner, C.E. (1955). Control of peppermint diseases. Station Bulletin 547, Agricultural Experiment Station, Oregon State University.

Horner, C.E. (1965). Control of mint rust by propane gas flaming and contact herbicide, *Plant Dis. Reptr.*, 5, 5.

Horner, C.E. (1971). Rhizome and stem rot of peppermint caused by *Phoma strasseri*, *Plant Dis. Reptr*., 55, 814.

Horner, E.E. and Dooley, H. (1965). Propane flaming kills *Verticillium dahliae* in peppermint stubble, *Plant Dis. Reptr*., 49, 581–582.

Huettig, M.A. (1969). The effect of fertilizer treatments on oil content and nutrient concentration of peppermint in Western Oregon, M.S. Thesis, Oregon State University, Corvallis.

Ingham, R.E. and Berry, R.E. (1995). Determining the impact of BioVector, Mocap, and Vydate on the root-lesion nematode and soil microarthropod population in peppermint, Agricultural Research Foundation Report, Oregon State University, Corvallis.

Ingham, R.E. and Merrifield, K. (1996). A guide to nematode biology and management in mint. Integrated Plant Protection Center Publication 996, Oregon State University, Corvallis, 38 pp.

Jackson, T.L., Gardner, E.H., and Doerge, T.A. (1983). Fertilizer guide: peppermint (Western Oregon-West of Cascades), Oregon State University Extension Service, Corvallis, 2 pp.

Johnson, D.A. (1991). Management of mint diseases, Mint Industry Research Council Research Reports.

Johnson, D.A. (1995). Races of *Puccinia menthae* in the Pacific Northwest and interaction of latent period of mint infected with rust races, *Plant Dis. Reptr*., 79, 20–24.

Johnson, D.A. and Cummings, T.F. (2000). Evaluation of mint mutants, hybrids, and fertile clones for resistance to *Verticillium dahliae*, *Plant Dis. Reptr*., 84, 235–238.

Johnson, D.A. and Douhan, L. (1997). Management of mint diseases. Mint Industry Research Council Research Reports.

Johnson, D.A. and Douhan, L. (1998). Management of mint diseases. Mint Industry Research Council Research Reports.

Johnson, D.A. and Douhan, L. (2000). Management of mint diseases. Mint Industry Research Council Research Reports.

Johnson, D.A. and Miliczky, E. (1990). Management of mint diseases in Washington, Mint Industry Research Council Research Reports.

Johnson, D.A. and Santo, G.S. (2001). Development of wilt in mint in response to infection by two pathotypes of *Verticillium dahliae* and co-infection by *Pratylenchus penetrans*, *Plant Dis*., 85, 1189–1192.

Kassim, A., Baker, R.C., and Birch, L.M. (1998). Biological control of field bindweed, Mint Industry Research Council Research Reports.

Kaufman, D., Koskela, G., Morris, M., and Croft, B. (2000). Evaluation of the toxicity of pesticides to *Neoseiulus fallacis* in the laboratory and in field observations. In: Shenk, M. and Kogan, M., Eds., IPM in Oregon: Achievements and Future Directions. Special Report 1020, Integrated Plant Protection Center, Oregon State University, Corvallis, 168–172.

Kissinger, D.R. (1963). Notes on the habits of some North American Curculionidae, *Coleopterists Bull*., 517, 53–57.

Koepsell, P.A. and Horner, C.E. (1975). Cropping peppermint on soils infested with Verticillium wilt, Extension service FS bulletin 223, Oregon State University, 2 pp.

Lacy, M.L. and Horner, C.E. (1966). Behavior of *Verticillium dahliae* in the rhizosphere and on roots of plants susceptible, resistant, and immune to wilt, *Phytopathology*, 56, 427–430.

Lacy, M.L., Stephens, C.T., Green, R.J. Jr., and York, A.C. (1981). *Mint Production in the Midwestern United States*, North Central Regional Extension Publication 155, Cooperative Extension Service, Michigan State University, East Lansing, MI, 18 pp.

Landing, J.E. (1969). American Essence—A History of the Peppermint and Spearmint Industry in the United States, A.M. Todd Foundation, Kalamazoo, MI, 244 pp.

Langston, R. and Leopold, A.C. (1954). Photoperiodic responses of peppermint, *Proc. Am. Soc. Hort. Sci*., 63, 347–352.

Liu, J. and Blouin, M. (In preparation). Microsatellite markers in *Mentha* species: their isolation, characterization and transferability.

Lommel, S. and Trembay, D. (1997). Identification, characterization, and assessment of a Tricho-like virus in mint and its association with meristem-tip cultured material, Mint Industry Research Council Research Reports.

Loomis, W.D. (1978). Physiology of essential oil production in mint, Proceed, Oregon Essential Oil Growers League, 29, 23–24.

Loomis, W.D. and Burbott, A.J. (1977). Physiology of essential oil production in mint, Proceed, Oregon Essential Oil Growers League, 28, 37–51.

Loomis, W.D. and Croteau, R. (1979). Mint physiology and oil production, Proceed, Oregon Essential Oil Growers League, 30, 32–39.
MacSwan, I.C. and Horner, C.E. (1965). Suggestions for peppermint wilt control, Cooperative Extension Service FS Bulletin 93, Oregon State University, Corvallis, 2 pp.
Maloy, O.C. and Skotland, C.B. (1969). Diseases of mint, Extension circular 357. Washington State University Pullman, 6 pp.
Martin, J.H., Leonard, W.H., and Stamp, D.L. (1976). *Principles of Field Crop Production*, Macmillan Publishing Co., Inc. New York, NY, 1118 pp.
Mathre, E.E., Grey, W.E., and Reinhold-Johnston, M. (1999). Evaluation of commercial mint and mint germplasm for disease reaction to Verticillium wilt and investigations into the mechanisms of wilt resistance, Mint Industry Research Council Research Reports.
McCain, A.H., Raabe, R.D., and Whilhelm, S. (1981). Plants resistant or susceptible to Verticillium wilt, Division of Agricultural Sciences, University of California Leaflet 2703.
McIntyre, J.L. and Horner, C.E. (1973). Inactivation of *Verticillium dahliae* in peppermint stems by propane gas flaming, *Phytopathology*, 63, 172–175.
Mellbye, M. and Gingrich, G. (2002). Evaluation of peppermint varieties and advanced selections in the Willamette Valley of Western Oregon, Agricultural Research Foundation Report, Oregon State University, Corvallis.
Melouk, H.A. and Horner, C.E., (1975). Cross protection in mints by *Verticillium nigrescens* against *V. dahliae, Phytopathology*, 65, 767–769.
Mitchell, A. (1995). Nitrogen fertility management of peppermint. Final report to the Oregon Department of Agriculture, June, 9 pp.
Mitchell, A.R. (1996). Fertilizer Guide for Peppermint and Spearmint (East of the Cascades). Field Guide 69, Oregon State University Extension Service Publication, Corvallis.
Mitchell, A.R. (1997). Irrigating peppermint, EM 8662, Oregon State University Extension Service, Corvallis.
Mitchell, A.R., Rechel, E.A., and Dovel, R.L. (1998). Three methods for determining storage carbohydrate concentration in peppermint (*Mentha piperita*) rhizomes, *Hort. Sci.*, 33, 754–756.
Morris, M.A. (1989). Sampling methods, biology and management of the mint flea beetle in central Oregon peppermint, M.S. Thesis, Oregon State University, Corvallis, 87 pp.
Morris, M.A. (1998). Biological control of *Tetranychus urticae* (Koch) on peppermint by *Neoseiulus fallacies* (Garman); Density relationships, overwintering, habitat manipulation and pesticide effects, Ph.D. Thesis, Oregon State University, Corvallis, 79 pp.
Morris, M.A. and Lundy, R. (1995). Pesticide Environmental Stewardship Strategy for the U.S. Mint Oil Industry, Mint Industry Research Council, 34 pp.
Morris, M.A. and Takeyasu, J. (1996). Registration of insecticides and miticides that are compatible with biological control in mint, Agricultural Research Foundation Report, Oregon State University, Corvallis.
Morris, M.A. and Takeyasu, J. (1998). Control of the garden symphylan on peppermint in western Oregon, Agricultural Research Foundation Report, Oregon State University, Corvallis.
Morris, M.A. and Takeyasu, J. (2000). The importance of integrated pest management (IPM) for the successful use of predator mites to control spider mites in peppermint. In: Shenk, M. and Kogan, M., Eds., IPM in Oregon: Achievements and Future Directions, Special Report 1020, Integrated Plant Protection Center, Oregon State University, Corvallis, pp. 159–168.
Morris, M.A., Croft, B.A., and Berry, R.E. (1996). Overwintering and effects of autumn habitat manipulation and carbofuran on *Neoseiulus fallacis* and *Tetranychus urticae* in peppermint, *Exp. Appl. Acarol.*, 20, 249–258.
Morris, M.A., Berry, R.E., and Croft, B.A. (1999). Phytoseiid mites on peppermint and effectiveness of *Neoseiulus fallacis* to control *Tetranychus urticae* Acari: Phytoseiidae, Tetranychidae in arid growing regions, *J. Econ. Entomol.*, 92, 1072–1078.
Morris, M.A., Berry, R.E., and Croft, B.A. (2000). Fall flaming effects on densities, distributions, and dispersal of *Tetranychus urticae* and *Neoseiulus fallacis*, Acari: Tetranychidae, Phyytoseiidae on peppermint, *Environ. Entomol.*, 29, 95–100.

Morrison, H.E. (1957). Controlling symphylids, Agricultural Experiment Station Circular of Information 574, Oregon State College, 7 pp.

Murray, M.J. (1969). Successful use of irradiation breeding to obtain Verticillium-resistant strains of peppermint, *Mentha piperita* L. In: *Induced Mutations in Plants*. IAEA, Vienna, pp. 345–371.

Murray, M.J. and Todd, W.A. (1972). Registration of Todd's Mitcham peppermint, *Crop Sci.*, 12, 128.

Murray, M.J., Lincoln, D.E., and Marble, P.M. (1972). Oil composition of *Mentha aquatica* × *M. spicata* F1 hybrids in relation to the origin of x *M. piperita, Can. J. Genet. Cytol.*, 14, 13–29.

Nelson, R. (1950). Verticillium Wilt of Peppermint, Michigan State College, Agricultural Experiment Station, Tech Bull No. 221.

Nelson, C.E., Mortensen, M.A., and Early, R.E. (1971). Evaporative cooling of peppermint by sprinkling, Washington State Agricultural Experiment Station Bulletin 703.

Newall, C.A., Anderson, L.A., and Phillipson, J.D. (1996). *Herbal Medicines: A guide For Health-Care Professionals*, The Pharmaceutical Press, London, 296 pp.

Nielsen, K.F. (1974). Roots and root temperatures. In: Carson, E.W., Ed., *The Plant Root and Its Environment*, University Press Virginia, Charlottesville, VA, pp. 293–333.

O'Brien, C.W. and Wibmer, G.J. (1982). Annotated checklist of the weevils (Curculionidae sensu lato) of North American, Central America, and the West Indies (Coleoptera: Curculionidae), *Mem. Am. Entomol. Inst.*, No. 34.

Pike, K.S. and Getzin, L.W. (1981). Persistence and movement of chlorpyrifos in sprinkler-irrigated soil, *J. Econ. Entomol.*, 74, 385–388.

Pike, K.S. and Glazer, M. (1982). Strip rotary tillage: a management method for reducing *Fumibotys fumalis* (Lepidoptera: Pyralidae) in peppermint, *J. Econ. Entomol.*, 75, 1136–1139.

Pike, K.S., Campbell, C.L., and Allison, D.W. (1986). Orange mint moth: a beneficial insect in Washington's commercial mints, Washington State University, Pullman, XB0982, 8 pp.

Pike, K.S., Allison, D.W., and Campbell, C.L. (1987). Effects of *Pyrausta orphisalis*, (Lepidoptera: Pyralidae) on plant growth and oil yield in Scotch spearmint, native spearmint and peppermint, *J. Econ. Entomol.*, 80, 69–72.

Pike, K.S., Baird, C., and Berry, R.E. (1988). Mint root borer in the Pacific Northwest, Washington State University Extension Service, Pullman, PNW 322, 7 pp.

Pinkerton, J.N. (1983). Relationship of *Pratylenchus penetrans* population density and yield on peppermint, *Mentha piperita* L. Ph.D. Thesis, Oregon State University.

Pinkerton, J.N. and Jensen, H.J. (1983). Chemical control of *longidorus elongates* on peppermint with non-volatile nematicides, *Plant Dis. Reptr.*, 67, 201–203.

Pinkerton, J.N., Jensen, H.J., Newcomb, G.B., and Ingham, R.E. (1988). Management of *Pratylenchus penetrans* damage to peppermint with selected nematicides, *Plant Dis.*, 72, 167–170.

Powelson, M.L., Johnson, K.B., and Berlanger, I. (1999). Effect of broccoli residues on soil populations of Verticillium and response to mint to different Verticillium strains, Agricultural Research Foundation Report, Oregon State University, Corvallis.

Pschiedt, J.W. and Ocamb, C.M. (2004). *Pacific Northwest Plant Disease Management Handbook*, Oregon State University, Corvallis.

Quebbeman, B., Cook, D.G., Fisher, G., and Morris, M. (2000). Registration of new insecticides on mint, Agricultural Research Foundation Report, Oregon State University, Corvallis.

Quebbeman, B., Walenta, D., and Morris, M. (2001). Registration of new insecticides on mint, Agricultural Research Foundation Report, Oregon State University, Corvallis.

Quebbeman, B., Walenta, D., and Morris, M. (2002). Registration of new insecticides on mint, Agricultural Research Foundation Report, Oregon State University, Corvallis.

Rao, S., Fisher, G., and Royce, L. (2002). Evaluation of the impact of Crane flies on peppermint production in Oregon, Agricultural Research Foundation Report, Oregon State University, Corvallis.

Roberts, D.D. and Horner, C.E. (1981). Sources of resistance to *Puccinia menthae* in mint, *Plant Dis. Reptr.*, 65, 322–324.

Santo, G.S. and Wilson, J.H. (1990). Management of nematodes on mint, Mint Industry Research Council Research Reports.

Santo, G.S. and Wilson, J.H. (1991). Management of Nematodes on mint, Mint Industry Research Council Research Reports.
Santo, G.S. and Wilson, J.H. (1998). Management of *Pratylenchus penetrans* on mint, Mint Industry Research Council Research Reports.
Santo, G.S., Wilson, J.H., and Skotland, C.B. (1986). Nematode investigations on mint, Mint Industry Research Council Research Reports.
Santo, G.S., Johnson, D.A., Lacey, L.A., Baker, R.C., and Mojtahedi, H. (1999). Management of *Pratylenchus penetrans* on mint, Mint Industry Research Council Research Reports.
Sether, D.M., DeAngelis, J.D., and Rossignol, P.A. (1991). First report of tomato spotted wilt virus in peppermint, *Plant Dis. Reptr*., 75, 644.
Shasany, A.K., Srivastava, A., Bahl, J.R., Sharma, S., Kumar, S., and Khanuja, S.P.S. (2002). Genetic diversity assessment of *Mentha spicata* L. Germplasm through RAPD analysis, Plant Genetic Resources Newsletter 130, 1–5.
Simon, J. and Alkire, B.H. (1993). Nitrogen rate and form studies on Midwestern Spearmint and Peppermint, Mint Industry Research Council Research Reports.
Simon, J.E., Alkire, B.H., and Joly, B. (1991). Irrigation and water management for peppermint in the Midwest, Mint Industry Research Council Research Reports.
Simon, J., Joly, B., and Alkire, B.H. (1992). Irrigation, water stress and water management: Research on Midwestern peppermint, Mint Industry Research Council Research Reports.
Slabaugh, W.R., Calkin, J., and Cacka, J. (2001). Sonata, a novel biofungicide for rust control in peppermint, *Phytopathology*, 91(Suppl. 6), S82.
Stanger, C. (1977). Annual weed control in furrow-irrigated mint, Circular of information 658, Agricultural Research Station, Oregon State University, Corvallis, 19 pp.
Sullivan, D., Hart, J.M., and Christensen, N.W. (1999). Nitrogen uptake and utilization by Pacific Northwest Crops, Oregon State University, PNW 513.
Talkington, M.L. (1983). Effects of tillage on emergence of mint root borer, *Fumibotys fumalis* Guenee, and on other agronomic factors in peppermint, *Mentha piperita* L. M.S. Thesis, Oregon State University, Corvallis, 162 pp.
Talkington, M.L. and Berry, R.E. (1986). Influence of tillage in peppermint on *Fumibotys fumalis* Lepidoptera: Pyralidae, common groundsel, *Senecio vulgaris*, and soil chemical components, *J. Econ. Entomol*., 79, 1590–1594.
Takeyasu, J. (1994). Control of mint root borer, *Fumibotys fumalis*, with the entompathogenic nematode, *Steinernema carpocapsae*, M.S. Thesis, Oregon State University, 95 pp.
Tjamos, E.C., Rowe, J.B., Heale, R.C., and Fravel, D.R. (2000). Advances in Verticillium research and disease management, The American Phytopathological Society, St. Paul, MN, 357 pp.
Tucker, A.O. and Chambers, H.L. (2002). *Mentha canadensis*. (Lamiaceae): a relict amphidiploid from the Lower Tertiary, Taxon, 51, 703–718.
Tucker, A.O. and DeBaggio, T. (2000). The Big Book of Herbs: A Comprehensive Illustrated Reference to Herbs of Flavor and Fragrance, Interweave Press, 688 pp.
Vessels, K.J. (1984). Field sampling and lab rearing studies of the mint flea beetle, M.S. Thesis, Purdue University, 107 pp.
Walsh, E. and Wight, R. (2002). Economic and biorational screening of candidate insecticides and acaricides on mint, Mint Industry Research Council Research Reports.
Wang, N. and Reed, B.M. (2003). Development, detection, and elimination of *Verticillium dahliae* in mint shoot cultures, *Hort. Sci*., 38, 67–70.
Watson, V.K. and St. John, J.L. (1955). Relation of maturity and curing of peppermint hay to yield and composition of oil, *J. Agric. Food Chem*., 3, 1033–1038.
Welty, L.E. and Prestbye, L.S. (1994). Cultivar, management and crop rotation studies, Montana Peppermint Research Report, Montana State University, pp. 1–14.
Welty, L.E., Strang, L., and Sharp, G. (1997). Effect of freezing on survival of peppermint and spearmint rhizomes/stolons, Montana Mint Research Report, Montana State University, pp. 44–45.

Wescott, M.P. and Welty, L. (1992). Nitrogen and irrigation management for peppermint. Scheduling and rate effects on tissue nitrate, soil N behavior and oil yields, Montana Peppermint Research Report, Montana State University.

Wescott, M.P. and Welty, L. (1993). Nitrogen and irrigation management for peppermint. Scheduling and rate effects on tissue nitrate, soil N behavior and oil yields, Montana Peppermint Research Report, Montana State University.

William, R.D., Dailey, A.G., Ball, D., Colquhoun, J., Parker, R., Yenish, J.P., Miller, T.W., Morishita, D.W., and Hutchinson, P.J.S. (2004). *Pacific Northwest Weed Management Handbook*, Oregon State University, Corvallis, 430 pp.

Wyman, J.A. and Rice-Mahr, S.E. (1992). Insect and mite management on mint, Mint Industry Research Council Research Reports.

4 The Cultivation of Mints in India

S. Sushil Kumar, Shashi Pandey-Rai, and S.K. Rai

CONTENTS

4.1 INTRODUCTION

Many kinds of mints, *Mentha* species, grow wild in different parts of India and continue to be used widely as the traditional items of culinary or salad and herbal medicine. The commercial

FIGURE 4.1 A crop of cornmint in the middle of its growing phase, in a field in the Indo-Gangetic plains.

cultivation of mints as a source of essential oils is a relatively recent development (Figure 4.1). Because of the suitability of Indian agroclimates and soils for mint cultivation as an annual crop, India has become the major supplier of certain mint oil(s) to the international market.

Cornmint, *Mentha canadensis* (ex. *M. arvensis*), was the first industrial mint to be introduced into cultivation in India. In the early 1980s, the then Drug Research Laboratory located at Jammu found the available genetic resource(s) of cornmint to be highly adaptable to the semitemperate to subtropical agroclimate(s) of several selected sites in the Himalayan foothills (Terai) region. The Pantnagar field station of the Central Indian Medicinal Plants Organization (CIMPO), now called Central Institute of Medicinal and Aromatic Plants (CIMAP), with its headquarters at Lucknow (set up in 1963), was responsible for the initial work on the development of appropriate mint agrotechnology and spread of the same among the farmers of the Terai area (Gulati and Duhan, 1971).

Because of the R&D work done at the CIMAP field station at Pantnagar, with the cooperation of various agencies of the Government of India, and participation of industry, entrepreneurs, and farmers, mint cultivation got entrenched in the Himalayan Terai region by the 1970s. Later, CIMAP further intensified its R&D efforts in mint production and cornmint became a much-investigated plant in its laboratories in Pantnagar and Lucknow (Tyagi et al., 1992; Khanuja et al., 1998, 2000; Kukreja et al., 2000; Patra et al., 2000b, 2001c,d). Due to all-round progressive efforts, in the late 1990s, India was able to produce about 75% of the international requirement for cornmint oil. In the meantime, CIMAP had developed and introduced among farmers the cultivation protocols for other commercial mints, including *M. spicata, M. piperita, M. gracilis* (ex. *M. cardiaca*), and a clone of *M. spicata* (ex. *M. spicata* var. *viridis*) (Misra et al., 2000; Srivastava et al., 2002; Singh et al., 2002). However, cornmint remains the principal cultivated mint of India.

4.2 GEOGRAPHICAL AREAS OF MINT CULTIVATION

The geographical areas of India, where all the rice, wheat, potato, and sugarcane crops can be cultivated, are suitable for mint farming also (Figure 4.2). Much of this area falls in the

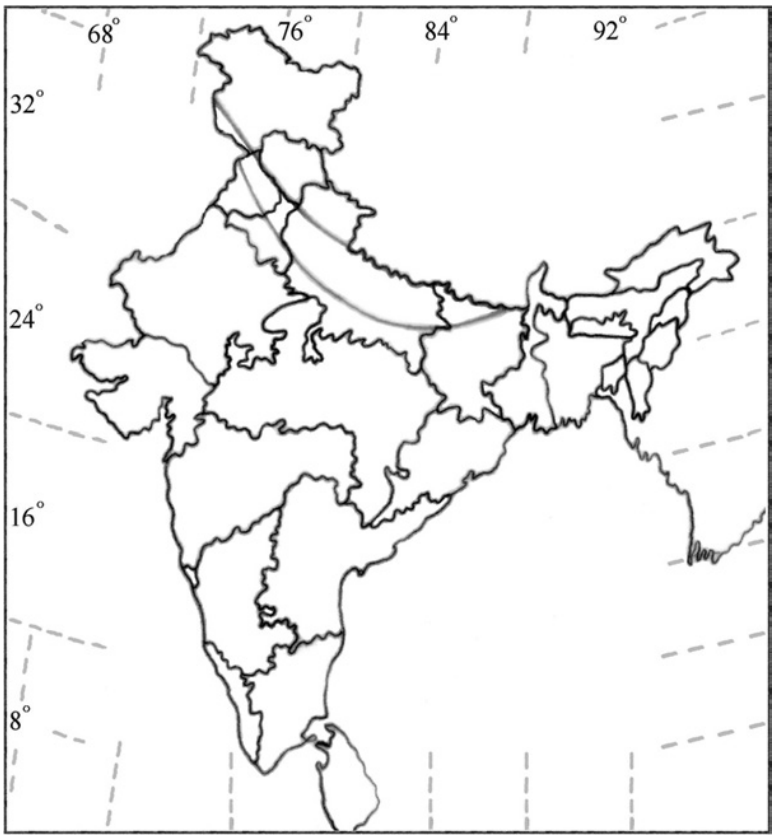


FIGURE 4.2 The geographical area in the Indo-Gangetic plains where mints are under intensive cultivation in India.

Indo-Gangetic plains, located immediately south of the Himalayas. This area is characterized by sandy, sandy-loam, loam, clay–loam, and clayey soils and a subtropical agroclimate. There is ample supply of irrigation water in the region. The winter season spans October or November to March or April; it is mild and temperatures vary from 2°C to 33°C. The summer season from April to June or July is harsh and temperatures reach up to 45°C. Southwest monsoon rains are received from July to September.

Cornmint is cultivated over 125,000 to 175,000 ha of land in the Indo-Gangetic plains region, principally in five pockets. In these centers of cultivation, the traders have set up an extensive network of oil collection centers of varying sizes for the purchase of cornmint oil from farmers, to sell to the local value-addition units and for exporting to the international market. The pockets of intensive mint cultivation are Hoshiarpur, Amritsar, Jalandhar, and Ludhiana districts in the state of Punjab; Ambala and Yamunanagar districts of the state of Haryana; Moradabad, Rampur, Bareilly, Badaun, and Basti districts of the northern region of the state of Uttar Pradesh; Nainital and Udham Singh Nagar districts of Uttaranchal; and Barabanki, Sitapur, and Lucknow districts of the central region of the state of Uttar Pradesh. Mint cultivation is likely to spread further in the Indo-Gangetic plains, in the eastern districts of Uttar Pradesh and in the state of Bihar in the future.

Some of the meteorological parameters of Udham Singh Nagar district at Pantnagar and Lucknow that represent the two relatively more important of the five pockets are given in Table 4.1. The pockets in Punjab and Haryana are relatively colder in winter. The agroclimate

TABLE 4.1
Average Weather Profiles of the Indian City of Lucknow Located at 26.5°N Latitude, 80.5°E Longitude, and 120 m above Mean Sea Level (MSL) and of Pantnagar Town in Udham Singh Nagar District Located at 29.0°N Latitude, 79.3°E Longitude, and 244 m MSL in the Indo-Gangetic Plains, in the 1990s

	Lucknow			Pantnagar		
	Temperature (°C)		Rainfall	Temperature (°C)		Rainfall
Month	Minimum	Maximum	(mm)	Minimum	Maximum	(mm)
January	8.3	23.7	18.4	6.3	20.6	62.1
February	11.2	26.2	13.0	8.2	22.8	30.0
March	15.5	32.8	2.2	11.5	28.7	2.3
April	19.8	38.5	6.1	15.8	35.1	13.5
May	26.3	41.4	5.9	27.2	39.4	14.0
June	28.0	39.6	137.1	25.8	38.0	88.3
July	26.8	35.2	158.0	25.4	32.8	368.7
August	25.8	32.9	184.4	24.7	31.8	499.3
September	24.4	34.5	99.2	23.1	31.6	126.2
October	18.3	34.6	2.2	17.2	32.2	10.3
November	12.0	26.5	5.2	10.7	28.0	2.2
December	9.1	26.6	5.1	7.0	23.5	4.0

of the pocket of which Rampur is a part has properties similar to that of Pantnagar. All the pockets receive rainfall $\geq$100 cm, well distributed through the year. Although the areas identified earlier are responsible for the production of the bulk of the Indian mint oils, experimental mint cultivation has begun in drier areas located south of the Indo-Gangetic plains. These include Bharatpur district of the state of Rajasthan, Hoshangabad district in Madhya Pradesh, and Hyderabad and Vishakapatnam in Andhra Pradesh.

The proportion of land allocated to the three species of mint widely cultivated in India appears to be cornmint, peppermint, and spearmint is in the ratio of 28:1.2:1. The cultivation of other species for their essential oils occurs on a much smaller scale; however, *M. spicata* (ex. *M. spicata* var. *viridis)* is cultivated on a large scale for use as an item of daily culinary usage.

4.3 GENETIC RESOURCES UNDER CULTIVATION

4.3.1 Cultivars

The genetic resources of cornmint that have been in cultivation at different times in India are listed in Table 4.2. The cultivars of the various other species of *Mentha* that are cultivated to varying extents in India are listed in Table 4.3. The salient features of the respective cultivars or types are also summarized in Table 4.2 and Table 4.3.

MAS-1 was the first cultivar of cornmint grown on a wide scale. However, the cultivar Shivalik, which was also extensively grown, remained popular for many years. Both these were introductions, whereas the new cultivars are a result of breeding programs initiated within the country. The overall objective of breeding new cultivars and introducing them in the field was to genetically diversify cornmint cultivation since it was noted that the mono-culture in the past led to widespread attacks of diseases and pests in the mint growing area. Hence, the breeding programs are variously enhancing the genetic variability and are using a

TABLE 4.2
Characteristics and Origins of Cornmint *(Mentha canadensis)* Cultivars Grown in India

Serial Number	Cultivar	Characteristics	Origin/References
1	MAS-1	Dwarf; bushy shoot; stem less hard, partially magenta-colored, 6.1-mm thick; leaf–stem ratio 1.2, leaves ovate-elliptic, thin, green fairly brittle, 6.1 cm^2 in size; leaf rust resistant and susceptible to leaf spot and powdery mildew diseases; less oil yielding with high menthol content (82% to 86%); suckers thin, white, 19-cm long, low in yield	Somatic variant of the MA-3 accession from Thailand (Anonymous, 1983; Kumar et al., 1999a, 2002a,b; Bahl et al., 2002a,b)
2	Kalka	Medium tall; bushy shoot, stem less hard; largely magenta-colored, 5.1-mm thick; leaf–stem ratio 1.1; leaves ovate-elliptic, thin, soft, green, 6 cm^2, 8 cm^2 in size; resistant to leaf rust, alternaria, and leaf blight diseases; tolerant to leaf spot and susceptible to powdery mildew diseases; less oil yielding with high menthol content (78% to 82%), suckers thin, milky white; 27-cm long, moderate in yield	Isolated from the seed progeny of MA-3 parent line (Anonymous, 1985b; Kumar et al., 1999a, 2000; Bahl et al., 2002b)
3	Shivalik	Tall; conical shoot; stem thick, woody, largely green, 9.7-mm thick; leaf–stem ratio 0.9; leaves ovate, thick rough, dark green, 7.7 cm^2 in size, susceptible to leaf rust, alternaria leaf blight, and leaf spot diseases and tolerant to powdery mildew; medium oil yielding with relatively low menthol content (70% to 74%); suckers thick, creamish white-top purplish tinged, 29-cm long, moderate in yield	Introduction from China (Kumar et al., 1997a,b, 1999a, 2000; Bahl et al., 2002b)
4	Gomti	Tall; conical shoot; stem thick, woody; largely magenta-colored, 9.1-mm thick; leaf–stem ratio 0.95; leaves ovate, thick, smooth, dark green, 7.8 cm^2 in size; susceptible to leaf rust, alternaria leaf blight, and leaf spot diseases and tolerant to powdery mildew; medium oil yielding with relatively low menthol content (70% to 74%); suckers thick, creamish white, 26-cm long, high in yield	A seed offspring of Shivalik (Kumar et al., 1999a, 2000; Bahl et al., 2002a,b)
5	Himalaya	Tall; pyramidal shoot; stem thick, woody, largely magenta-colored, 7.4-mm thick; leaf–stem ratio 0.95; leaves ovate, thick, smooth, dark green, 7.8-cm^2 in size; petioles long; resistant to leaf rust, alternaria, and leaf blight diseases and tolerant to leaf spot and powdery mildew; high oil yielding with medium menthol content (74% to 78%); suckers thick, creamish white, 26-cm long, high in yield	Hybrid of Gomti and Kalka (Kumar et al., 1997a,b, 1999a,d, 2000; Bahl et al., 2002b)
6	Kosi	Tall; dome-shaped bushy shoot; stem hard, largely green, 8.5-mm thick; leaf–stem ratio 1.2; leaves ovate, soft, thick, yellowish green, 7.2 cm^2 in size; petioles long; resistant to leaf rust, alternaria, and leaf blight diseases and tolerant to leaf spot and powdery mildew; resistance toward white fly *Bemesia tabaci*; high oil yielding with medium menthol content (74% to 78%); suckers of medium thickness, off-white, 31-cm long, high in yield	A seed offspring of Kalka (Kumar, 1998, Kumar et al., 1999a,c, 2000, 2002b; Bahl et al., 2002b)
7	Saksham	Tall; big shoot canopy; leaf 10.2 cm^2 in size, very long petiole; menthol content of essential oil high (~83%); other properties like Himalaya	Menthol-tolerant somaclone of Himalaya (Khanuja et al., 2001a)

continued

TABLE 4.2 (continued)
Characteristics and Origins of Cornmint *(Mentha canadensis)* Cultivars Grown in India

Serial Number	Cultivar	Characteristics	Origin/References
8	Sambhav	Tall; very big shoot canopy; leaf 10.9 cm^2 in size; resistant to the insect *S. obliqua*; other properties like Himalaya	*Spilarctia obliqua* insect-resistant somaclone of Himalaya (Khanuja et al., 2001c)
9	Damroo	Robust racemose inflorescence of axillary verticillasters, high seed set; high oil yielding; oil rich in menthol (78%); other properties similar to Shivalik	Highly fertile selection from among seed progeny of Shivalik (Patra et al., 2001b)

TABLE 4.3
Characteristics and Origins of the Cultivars of Various Other *Mentha* Species Grown in India

Serial Number	Cultivar	Characteristics	Origin/References
1	*M. piperita* cv. Kukrail	Medium tall; growth semi-erect; stem medium hard, amaranth-colored, 4.7-cm thick; leaf–stem ratio 1.2; leaves ovate, jungle green, veins amaranth-colored, 7.4 cm^2 in size; flowers not observed; essential oil content in shoot herb 0.5%, oil rich in menthol (35%) and menthone (39%), medium in yield	Mutant of a local strain (Anonymous, 1994; Bahl et al., 2000)
2	*M. piperita* cv. Pranjal	Medium tall; erect; stem red purple, 1.4 cm in girth; leaf–stem ratio 1.4; leaves yellow green, ovate, surface rough, 5.8 cm^2 in size; tolerant toward Bihar hairy caterpillar; essential oil content in shoot herb 0.8%; oil rich in menthol (52%) and menthone (14%); oil yield high	Mutant of an Indian accession (Dwivedi et al., 2001a)
3	*M. spicata* cv. MSS-5	Medium tall, erect; stem medium hard, largely green, lower portion magenta-colored, 4.6-mm thick; leaf–stem ratio 1.0; leaves elliptic ovate, green, 6.4 cm^2 in size; inflorescence raceme of verticillasters, upper cymes condensed and lower cymes lax, sessile; flowers white, medium in fertility; resistant to *Bemesia tabaci* white fly; essential oil content in shoot herb (0.55%); oil carvone rich (65%), medium in yield	Clonal selection in the accession MSS (Anonymous, 1985a; Bahl et al., 2000)

TABLE 4.3 (continued)
Characteristics and Origins of the Cultivars of Various Other *Mentha* Species Grown in India

Serial Number	Cultivar	Characteristics	Origin/References
4	*M. spicata* cv. Arka	Medium tall; erect, vigorous; stem hard, largely green, lower portion magenta-colored, 4.2-mm thick; leaf–stem ratio 1.1; leaves elliptic ovate, green, 6.8 cm^2 in size, similar to MSS-5, early flowering; *B. tabaci*-resistant; essential oil content in shoot herb (0.6%); oil carvone rich (68%), medium in yield	Clonal selection in the accession MSS-5 (Anonymous, 1993; Bahl et al., 2000)
5	*M. spicata* cv. Neera	Medium tall; growth semi-erect; stem medium hard, largely green, lower portion light magenta-colored, 4.1-mm thick; leaf–stem ratio 1.5; leaves elliptic, green, 4.5 cm^2 in size; inflorescence raceme of verticillasters, cymes condensed, sessile; flowers purplish white, low fertility; essential oil content in shoot herb (0.4%); oil carvone rich (58%); oil yield low; has unique odor and flavor	An Indian accession (Anonymous, 1993; Bahl et al., 2000)
6	*M. spicata* cv. Neerkalka	Medium tall; growth erect, vigorous; stem hard, hairy, largely green, purple pigmented at base, 5.5-mm thick; leaf–stem ratio 0.6; leaves ovate-elliptic, green, 6.9 cm^2 in size; inflorescence axillary verticillasters; flowers pinkish white, fertile; resistant to *Bemesia tabaci* white fly; essential oil content in shoot herb (0.89%); oil rich in carvone (72%) and limonene (10%); yield of oil medium	F_1 hybrid between *M. canadensis* cv. Kalka and *M. spicata* cv. Neera (Bahl et al., 2000; Patra et al., 2001c; Kumar et al., 2001a)
7	*M. gracilis* (as *M. cardiaca*)	Dwarf; growth semi-erect; stem soft, orchid purple, 3.8-mm thick; leaf–stem ratio 1.2; leaves dark green, crinkled, 11.8 cm^2 in size; inflorescence terminal condensed raceme of verticillasters, sessile; flowers white, sterile; resistant to *Bemesia tabaci* white fly; essential oil content in shoot herb (0.35%); oil carvone rich (65%); oil yield low	An Indian Himalayan accession (Anonymous, 1985a; Bahl et al., 2000)
8	*M. gracilis* cv. Pratik	Medium tall; growth semi-erect, wide canopy; stem semihard, dull green, quadrangular, 4-mm thick; leaf–stem ratio 1.1; leaves dull green, ovate, serrated, and rough, 10 cm^2 in size; essential oil content in shoot herb (0.8%) oil carvone rich (85%) with 8% limonene; medium in yield	Mutant of an Indian accession (Dwivedi et al., 2001b)

continued

TABLE 4.3 (continued)
Characteristics and Origins of the Cultivars of Various Other *Mentha* Species Grown in India

Serial Number	Cultivar	Characteristics	Origin/References
9	*M. aquatica* var. *citrata* (as *M. citrata)* cv. Kiran	Medium tall; grown erect, vigorous; stem hard, hairy, largely green, reddish tinge at nodes, 8.3-mm thick; leaf–stem ratio 1.5; leaves ovate; inflorescence axillary verticillasters, stalked; flowers white, sterile; resistant to *Bemesia tabaci* white fly; essential oil content in shoot herb (0.5%); oil carvone rich (64%), medium in yield	Clonal selection among progeny of accession MCA-1 (Anonymous, 1988; Bahl et al., 2000)
10	*M. spicata* var. *viridis* (as *M. viridis*) cv. Supriya	Medium tall; growth erect; stem medium hard, magenta-colored, 6-mm thick; leaf–stem ratio 1.2; leaves ovate, broad, green, petiole and midrib light magenta-colored. 19.5 cm^2 in size; inflorescence verticillasters, condensed, globose, sessile flowers, pinkish white, sterile; essential oil content in shoot herb (0.5%); is rich in linalool (48%) and linalyl acetate (38%); medium in yield	Mutant of MC-1 (Anonymous, 1987; Bahl et al., 2000)
11	*M. spicata* var. *viridis* cv. Ganga	Medium tall; semi-erect; stem medium hard, purplish; leaf–stem ratio 1.4; leaves elliptic, dark green, 15 cm^2 in size; inflorescence raceme of verticillasters, flowers pinkish white, fertile, essential oil content in shoot herb (0.5%); oil rich in piperitone (66%) has carvone (2.2%) and limonene (1.0%); yield of oil medium; oil highly effective against stored grain pests	Somaclonal variant of an Indian accession (Khanuja et al., 2001b)

variety of laboratory- and field-based approaches to identify better genotypes (Khanuja et al., 2001a,b,c; Kumar et al., 1997b, 1999c,d, 2002b; Patra et al., 2000b, 2001a,c,d). The selections are, therefore, well adapted to the local agroclimates. As the breeding populations could be screened against the local strains of diseases and pests, some of the new cultivars possess resistance or tolerance against several diseases or pests. Among the cultivars developed in recent years, the Kosi cultivar has found much favor with the farmers (Figure 4.3) (Srivastava et al., 2002).

4.3.2 Maintenance of Cultivar Purity

Cultivar purity is a requisite for stability in yield performance and quality of produce over seasons. Some of the genetic resources of mints used in cultivation are fertile and set seeds. The farmers are advised to maintain purity in their material by periodically refreshing their planting stocks by obtaining the breeder's stock from CIMAP. The gene bank established at CIMAP maintains the genetic resources of all mints including all cornmint cultivars in their vegetative state at the breeder's field nursery, as micropropagules in tissue cultures (Shasany et al., 1998; Kumar et al., 1999b; Bhat et al., 2001) and in the form of extracted DNAs (Khanuja et al., 2000). The field-maintained material is kept free of diseases and pests by use of microbiological,

FIGURE 4.3 A flowering top of the cornmint cultivar "Kosi" *Mentha canadensis*. This cultivar is highly popular among farmers in India.

entomological, and tissue culture procedures. The morphological, biochemical, and molecular markers have been defined for each cultivar. Cultivar-specific DNA fingerprints have been developed and these are used to ascertain the purity of farmers' planting materials.

4.4 PROPAGATION OF PLANTING MATERIAL

The planting stocks of the chosen cultivar(s) are multiplied by a farmer in nursery area(s) of his or her own field(s). Usually, a portion of the crop maintained from the previous season is used as the resource of mother plants for producing the fresh planting material. Depending on the choice of mint species or cultivar, one of the two kinds of propagules are mass-produced.

Suckers formed as underground stems on the mother plants in the winter season are the propagules used to field plant most of the cultivars. In species or cultivars that do not produce the suckers or runners or stolons, the propagules are the young regenerating shoots formed on the mother plants. In the Indo-Gangetic plain, which is presently the principal location for mint cultivation in the country, the preparations for the propagule production begin at the end of the monsoon season in August or September. The mother plants of ascertained purity

are taken out from the maintenance plots and transplanted in a nursery for the production of underground suckers or above-ground regenerating shoots, as desired according to the cultivar-wise innate properties (Kumar et al., 1997a,b).

4.4.1 Production of Suckers and Plantlets for the Early Mint Plantings

The nursery beds are prepared by applying 10 to 20 tonnes of farmyard manure (FYM) or 2 to 5 tonnes of wormy compost and 60 kg of N and 40 kg each of P and K per hectare of finely tilthed section of sandy loam or loam land. After the beds have been prepared, the mature and healthy mother plants are transplanted at a distance of 30 cm in rows that are themselves 30 cm apart. The nursery is given frequent irrigations and any stagnation of water is avoided. The plantlets or suckers are formed in ample measure by December. The suckers are underground stems that carry buds in the axils of the nodes. The buds borne on the suckers, quiescent during winter, regenerate shoots with the onset of summer. A piece of land measuring 200 or 500 m^2 is sufficient to produce suckers or young plantlets to cultivate a mint crop on about 1 ha of land. The crops of the nonsuckering cultivars, for which young plantlets regenerated on mother plants constitute the propagules, are usually planted in December. The crops of the cultivars, which form suckers, are planted from late December to early April using alternative planting procedures. The mint cultivars belonging to the species of *Mentha* other than *M. canadensis* are planted early between December and February in the winter season, preferably in late December.

The underground suckers formed on the mother plants are harvested during the period from December to early March before they start germinating *in situ* in the nursery. The suckers are harvested manually and can be used immediately (Figure 4.4). If not used on the day of harvest, the suckers are buried in shallow pits in the field for short-term storage. The suckers stored cold at 4°C to 8°C, retain their vigor and viability for several weeks (Bahl et al., 2002a). The cold-stored suckers are suitable for the late planting of mint crops. Before their use, the suckers are treated with contact fungicides such as Agallol, Tafason, or Captan to control any fungal infection. The suckers harvested from the nursery are suitable for direct sowing in the field. Planting of mint crops in the period from December to February is done in synchrony with the harvesting of suckers, so that freshly harvested suckers are used for planting. However, cold-stored suckers or nursery-raised plantlets produced from sucker pieces are also used as propagules for planting the late crops of mint in March or April.

4.4.2 Production of Plantlets for Late Mint Planting

To produce plantlets for 1 ha of main crop, nursery beds are prepared on 200 m^2 land of sandy loam or loam finely tilthed soil, mixed with FYM or wormy compost as mentioned earlier. About 100 kg of suckers are chopped to pieces of about 2.5 cm and broadcast on the beds. The sown sucker pieces are then covered with soil and mulching material and irrigated. This nursery is usually sown with pieces derived from fresh suckers in late February. Cold-stored suckers can also be used for seeding the nursery raised for plantlets. The nursery is irrigated and cared for regularly. The plantlets of two- to six-leaved stages are transplanted in March and April.

4.5 PLANTING OF MAIN CROP

4.5.1 Field Preparation

Mint crops are cultivated on soils of a wide range of properties; the pH and texture of the concerned soils vary from 6.5 to 9.0 and sandy loam to clay, respectively. However, the most economic yields are obtained on well-drained fields of loam soils that are rich in organic

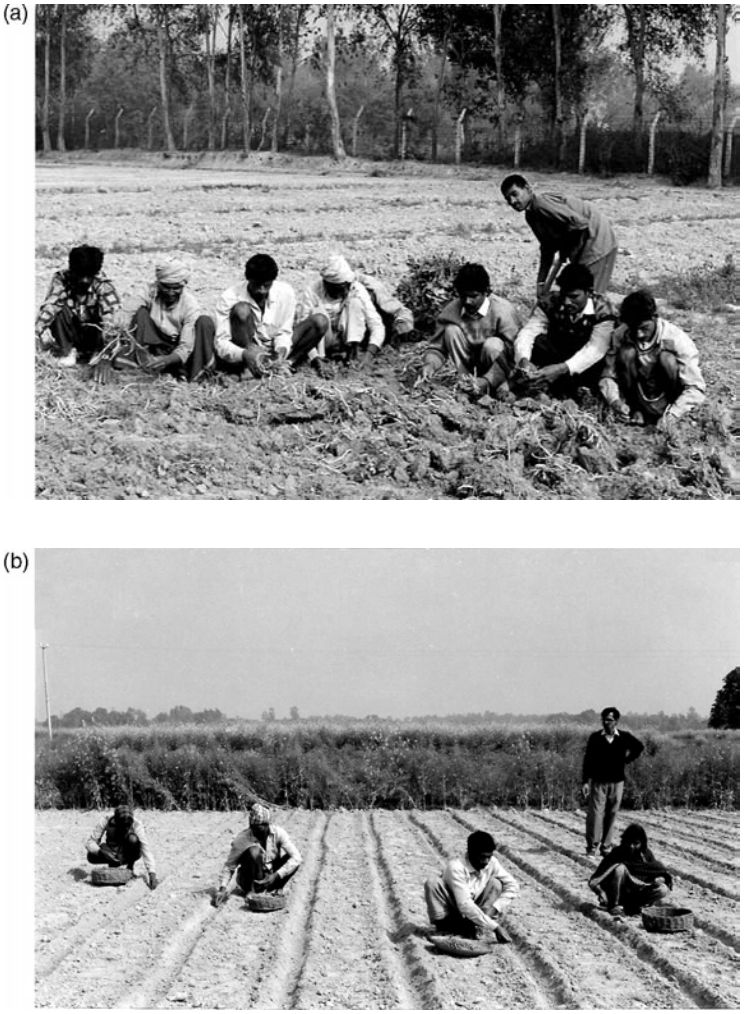


FIGURE 4.4 Mint planting time field operation in progress. (a) Farm workers are seen harvesting suckers from a nursery; (b) suckers are sown in furrows opened in a mint field.

matter and have pH in the range of 6.8 to 7.5. To prepare the land, the weeds are removed and a fine tilth is achieved by plowing with a soil-turning plow, followed by two or three cross-harrowings, and then by planking to flatten it. Any stubble and roots from the previous crop are also removed. The field is divided into beds to facilitate irrigation.

4.5.2 Planting Procedures

4.5.2.1 Sowing of Suckers

Suckers are sown in furrows opened about 5 to 8 cm deep. The furrows are opened at a distance of 25 to 75 cm from each other, depending on the cultivar to be grown and time of planting. Closer sowing is preferred under late planting conditions.

A tractor-driven harrow or manually operated local instrument called a *kotla* is used to open the furrows. Suckers are placed end to end in the furrows, which are thereafter closed

with soil and immediately irrigated. In the summer season, sowing is done in the mornings and evenings when temperatures are relatively lower.

4.5.2.2 Transplanting of Plantlets

Plantlets that have been produced on the mother plants or from sucker pieces in the nursery are transplanted in the main field line-wise. The lines are kept 25 to 75 cm apart depending on the cultivar and time of transplantation. The plantlets freshly dugout from the nursery are transplanted 10 cm apart in the already irrigated field. A light irrigation follows the transplanting operation. Transplanting of mint is completed by the third week of April at the latest (Ram and Kumar, 1998; Ram et al., 1998a,b).

4.6 SEASONAL AGRONOMY

4.6.1 Fertilizer Application

Manures and inorganic fertilizers are applied at the time of planting and subsequently at different stages of crop growth (Table 4.4). Full dose of manure(s), single doses of super phosphate and muriate of potash, and one-third or one-fourth dose of urea are applied at the time of planting. The manure is mixed well with the surface soil by harrowing, and a combination of inorganic fertilizers that is usually about 15 cm deep in between the rows of mint and covered with soil. Alternatively, manure and fertilizers are harrowed-in altogether before the beds are prepared. At this time, vesicular arbuscular mycorrhizal (VAM) fungi can also be added to this mixture to promote nutrition uptake by the crop (Gupta et al., 2002). Equal split doses of urea are top-dressed to the growing crop two or three more times, at 4 and 9 weeks from planting, after weeding operations and immediately after the first harvest (Kumar et al., 1997a,b). At the time of each application, the fertilizer urea is broadcast a few days after irrigation on the soil surface in between rows of mint; this manual

TABLE 4.4
Fertilization Schedules for 1 ha of Early- and Late-Planted Mint Crops

	Manure/Fertilizer and Rate of Application per Hectare			
		Following Weeding at		
Time of Crop Planting	At Bed Preparation	4 to 6 Weeks from Planting	8 to 10 Weeks from Planting	After First Harvest at 15 to 16 Weeks from Planting
Early	10 to 25 tonnes of farm yard manure/2 to 5 tonnes of wormy compost or 50 to 120 kg of urea + 40 kg K_2O + 50 kg P_2O_5[a]	50 to 70 kg urea	50 to 70 kg urea	50 to 70 kg urea
Late	10 to 25 tonnes of farm yard manure/2 to 5 tonnes of wormy compost or 50 to 120 kg of urea + 40 kg K_2O + 50 kg P_2O_5[a]	70 to 100 kg urea	70 to 100 kg urea	—

Source: From Gupta, P.P., Prasad, M.L., Ram, A.M., and Kumar, S., *Biores. Technol.*, 81, 77–79, 2002. With permission.

[a]Vaminoculum, if available, is also applied as part of manure (Gupta et al., 2002).

operation avoids contact of urea with the mint leaves. If available, the mulches of paddy straw, citronella, or other distillation wastes are applied at the rate of 5 to 10 tonnes/ha, as supplemental crop nutrition immediately after sprouting of suckers or establishment of plantlets (Patra et al., 1993; Ram and Kumar, 1997,1998; Patra et al., 1993, 2000a).

4.6.2 Irrigation and Drainage

To obtain a high yield of oil, the mint crop needs to be sufficiently fertilized and irrigated and saved from becoming waterlogged. The frequency of irrigation varies according to the local differences in the volume of winter and premonsoon rains. The mint fields are irrigated at an average interval of 2 to 3 weeks in the winter season, from December to March, and 10 to 15 days in the summer, from April to July, or at the time of the onset of monsoon rains. In the summer season, irrigation is carried out in the late evening or early morning. Operations such as weeding and first harvest are followed by irrigation. In problem lands prone to waterlogging, mints are cultivated on ridges. Mulching is used to reduce the frequency of irrigations.

4.6.3 Crop Protection

4.6.3.1 Weeds

Mint crops do not compete well with weeds. Therefore, weed infestation of mint fields must be controlled to minimize the possible losses in the yield of essential oil. A variety of weeds infest the mint fields. Location and field-wise differences are noted in the weed flora of the mint growing areas in India. There is a large overlap in the weed flora of the various locations of mint cultivation in the Indo-Gangetic plains. Some of the weed species noted in the mint fields of the area are listed in Table 4.5.

It has been observed that the fields cultivated with sugarcane or rice in the previous season are relatively free of weeds. The rice cultivation practice(s) tend to eliminate weeds, especially the dicot weeds. It has been further noticed that if a rice field that is used for mint was

TABLE 4.5
Lists of Weeds That Are Known to Occur Frequently in the Mint Fields of the Relatively Wetter (Terai) and Drier Subtropical Agroclimates of India

Class of Weeds	Wet (Terai) Lands	Dry Lands
Monocotyledonous	*Avena sativa, Cenchrus biflorus, Commelina benghalensis, Cyperus difformis, Cyperus rotundus, Dactyloctenium aegypticum, Digitaria ciliaris, Echinochloa crus-galli, Eleusine indica, Setaria glauca*	*Avena sativa, Cyperus rotundus, Digitaria ciliaris, Eleusine corocana, Eragrostis uniloides, Phalaris minor, Setaria tomentosa, Sorghum halepense*
Dicotyledonous	*Amaranthus spinosus, Amaranthus viridis, Cannabis sativa, Carthamus oxycantha, Chenopodium murale, Corchorus aestuas, Croton bonplandianum, Euphorbia dracunculoides, Gnaphalium pensylvanium, Lathurus aphaca, Melilotus alba, Oxalis richardiana, Parthenium hysterophorus, Phyllanthus amarus, Solanum violaceum, Striga angustifolia, Tribulus terrestris*	*Chenopodium album, Corchorus aestuas, Corchorus depresses, Croton bonplandianum, Euphorbia hirta, Fumaria indica, Gnaphalium polycaulon, Lathyrus sativa, Launea procumbens, Nicotiana rustica, Physalis minima, Rumex dentatus, Salvia plebei, Solanum nigrum, Sonchus oleraceus, Tribulus terrestris*

adjacent to a field used for wheat or potato or mustard cultivation, then the incidence of weeds is even further reduced.

The weeds in the mint fields must be removed at the earliest. The first weeding is done within 4 to 6 weeks of planting and the second 3 or 4 weeks thereafter. A handheld sickle, *khurpi*, or a mechanical hoe is used in these manual operations.

Mulching is also used to control the growth of weeds. Mulch is applied after the sprouting of suckers, establishment of plantlets, or after the first weeding. About 5 to 10 tonnes/ha of mulch is applied, as has been mentioned earlier in the section on "Irrigation and Drainage." Paddy straw, citronella, and other aromatic grass distillation wastes and sugarcane leaves are the locally available mulching materials. Besides contributing to the control of weeds, mulching is helpful in soil moisture conservation and in keeping the soil temperatures low. Some farmers spray the mint field with water suspensions of pre-emergence weedicides 2 or 3 days after the sowing of suckers. The herbicides used for the purpose include Pedimenthalin (1.0 kg/ha), Oxyflurofen (0.5 kg/ha), and Diuron (0.4 kg/ha). The backstrap diesel-run sprayer has been found to be a useful spraying equipment.

Although the combined use of chemical, mechanical or manual, and mulching methods allows the best management of weeds, small farmers prefer manual control of weeds in their mint fields.

4.6.3.2 Insect Pests

A variety of insects are found associated with mint crops in India. However, the damage caused by insects to mint crops is usually mild and easy to control. The seasonal (short duration annual) nature of mint crops, toxic nature of complex mixtures of terpenoids present in the essential oils accumulated by them, and their rotation with other crops are the reasons that seem to limit possibilities of heavy and widespread buildup of specific pest populations among mint fields. Some of the relatively important insect pests of mints and pesticides commonly used to control them in India are listed in Table 4.6 (Kumar et al., 1997a,b).

4.6.3.3 Nematodes

Sporadic occurrence of damage to mint crops by the associated root knot and root-lesion nematodes has been recorded in the Indo-Gangetic plains. The mint plants severely infected by the root knot nematode *Meloidogyne* species are stunted and bear chlorotic leaves and an aborted root system. The other kinds of nematodes that somewhat similarly affect the yield of mint crops are the root-lesion-causing *Pretylenchus* species. Treatment of the affected soils with neem oil seed cake or carbofuran and use of planting material from a clean nursery usually takes care of the danger to mint crops from the problems caused by nematodes. The heavy nature of the soils in the Indo-Gangetic plains also remediates nematode infestation.

4.6.3.4 Pathogens

Several diseases have appeared in their epiphytotic forms at different locations in different years in the Indo-Gangetic plains. Some of the major causal pathogens, disease symptoms produced by them, and control measures prescribed are listed in Table 4.7 (Kumar et al., 1997a,b; Singh et al., 2000; Kalra et al., 2001a,b, 2006).

Avoidance of pathogen(s) and selection of tolerant or resistant mint cultivars are the most important measures recommended to reduce any possible losses arising from diseases prevalent at the time in the areas of mint cultivation. As a disease avoidance measure, the suckers should be produced from disease-free mother plants and harvested suckers treated with some contact fungicide before planting. The use of plantlets sprayed with fungicide such as

TABLE 4.6
The Common Insect Pests of Mints in India

Serial Number	Name		Symptoms of Disease	Chemical(s) Used for Control
	Common	Zoological		
1	White grub	*Leucophalis coneophora*	Roots and leaves are eaten away	Chloropyrophos or Savidol treatment of soil
2	Red pumpkin beetle	*Aulocophora favicollis*	Leaves and buds are eaten away	Monocrotophos, Pyrethrin sprays
3	Hairy caterpillar	*Diacrisia oblique*	Leaves are damaged	Quinolphos sprays
4	Pod borer	*Heliothes armigera*	Leaves and buds are eaten away	Cipermetheline and Malathion sprays
5	Cutworm	*Agrotos flammatra*	Scission of plant in collar region	Soil treatment with Chloropyrophos
6	Termite	*Microtermis shasi, Odontotermis obesus*	Plants get cut at the base	Soil application of Phorate or Chloropyrophos at the time of planting of suckers or seedlings
7	White fly	*Bemesia tabaci*	Sucks the juice and spreads viruses	Phosphomidon sprays
8	Macho	*Macrosiphum pisi* and *Aphis* species	Sucks the juice and spreads viruses	Phosphomidon sprays
9	Mint leaf roller	*Syngamia abruptalis*	Rolling of leaves	Monocrotophos sprays

Propiconazole similarly prevents the transfer of pathogen(s) from one season to the next. The farmers are advised to procure disease-free suckers from CIMAP. They are also kept abreast of the newly released mint cultivars, which harbor disease- and pest-resistance properties. The farmers of a geographical location are expected to plant more than one cultivar of a species, possessing tolerance or resistance properties against various pathogens to prevent the spread of disease(s) over wide area(s).

4.7 HARVESTING

At harvest time, the mint field is maintained dry. The crop is harvested during bright sunny weather. Sharp sickles are used to cut the over-ground shoots about 2 to 5 cm above-ground level. The harvested herbage is spread on the ground to partially dry for about 1 day (Figure 4.5). The wilted, partially dry herbage is cheaper to distill. The yield of distilled oil decreases when the distillation of harvested herbage lying in the open is delayed beyond 36 h (Singh and Naqvi, 1996).

The mint crops planted with the use of suckers or plantlets in the period from December to February are harvested twice and those planted in March or April are harvested for herbage only once. The early-planted crop is first harvested after 100 to 120 days of growth in April or May. After the harvested crop has regrown, the second harvest is carried out between 60 and

TABLE 4.7
The Common Diseases and Pathogens of the Mint Crops Grown in India

Serial Number	Disease	Pathogen	Symptoms of Disease	Control Measure(s)[a]
1	Leaf spot	*Alternaria alternate*	Appearance of ringed and irregular dark brown spots on upper leaf surface that coalesce and cause defoliation	Spray of Propiconazole or Chlorothalonil
2	Rust	*Puccinia menthae*	Formation of dark brown postules on leaves followed by loss of leaves	Spray of Mancozeb or Propiconazole, use of suckers treated for 10 min at 45°C
3	Powdery mildew	*Erysiphe cichoracearum*	Whitish powder coating on leaves followed by their drying up	Wettable sulfur
4	Root and stolon rot	*Rhizoctonia bataticola/solani*	Necrotic yellowing of shoot followed by plant death	Use of healthy disease-free or Zineb-, Mancozdeb-, or Captan-treated suckers
5	Wilt	*Fusarium oxysporum*, and *Verticillium dahliae*	Etiolation and wilting of branches	Use of disease-free suckers or those heat treated at 47°C for 1 h
6	Stem rot	*Phoma strasseri*	Reddening of leaves, stunting and wilting of plant	Benomyl-soaked propagules
		Sclerotinia sclerotorium	Stolon gets decayed	Spray of Propiconazole, Chlorothalonil or Mancozeb on mother plants

[a]Use of cultivars that are known to be resistant to concerned pathogens and generally heat and treat suckers with a fungicide.

75 days following the first harvest. The late-planted mint crops are harvested in June or July. To realize the full yield potential of mint crop, premature or delayed harvesting is avoided. It is pivotal to determine the maturity of the mint plants before harvesting, to ensure optimum recovery of good quality essential oils. Since the cultivars differ in their growth and development properties, a reliable criterion to determine harvest maturity that is uniformly applicable to cultivars is as yet unavailable. To obtain the optimum yield of oil with good quality, the growers are advised to harvest the Kosi, Saksham, and Damroo crops after 12 to 15 weeks of planting, and the Sambhav, Shivalik, and Himalaya crops 15 to 17 weeks from planting.

4.8 LIFE TIME OF PLANTATION

Mints are well suited for the industrial farming of valuable molecules synthesized and accumulated in their herbage. Their biological properties allow adaptation of the life span of mint crops to the local agroclimate.

FIGURE 4.5 Harvesting of cornmint crop in progress. The harvested herbage is left in the field to partially dry before it is distilled the next day.

Mints are dicotyledonous species of the genus *Mentha* of family Lamiaceae. Generally, *Mentha* species are sexually fertile herbaceous perennials, which produce underground suckers and overground runners. Their populations grow through both seed dispersal and asexual multiplication. *Mentha* species display considerable morphological and biochemical diversity. Many of them are variously polyploid and allopolyploid hybrids. The properties given here are shared by the *Mentha* species to which the cultivated mints belong. Since the economic produce of most of the industrial mints is from the above-ground growth, the cultivated mints can be maintained and propagated asexually. Completion of the life cycle or extended perpetuation is not essential for the economic cultivation of mints for oil production. The commercial mint cultivation can, therefore, be adapted to the local conditions in semitemperate, subtropical, and tropical agroclimates.

In the subtropical agroclimates of the Indian subcontinent, mint crops can grow perennially, like in semitemperate and subtropical areas of other countries where mints are commercially farmed. In areas where rains are received intermittently and soils do not remain waterlogged and in drier areas where irrigation requirements are met by canal or tubewell water, mint crops can be maintained in the field for several years and herbage harvested three to six times in a year, without loss of regenerability. Perennial mint cropping is favored in areas where farmers are owners of large farms, cooperative farming is practiced, and cultivation of other crops in rotation with mint is uneconomical.

Several factors permit or dictate seasonal cultivation of mints in the Indo-Gangetic plains: (1) The high agricultural productivity of this area is related to ample rainwater received in the winter, summer, and monsoon seasons. Whereas light to heavy rains are received intermittently or sporadically in the winter and summer seasons, rains are frequent in the monsoon season when most fields can remain waterlogged for several days to weeks. The mint plants under such circumstances in the monsoon season lose vigor or viability. (2) The landholdings of the farmers are small. Their practice of diverse agriculture allows them to retain a part of

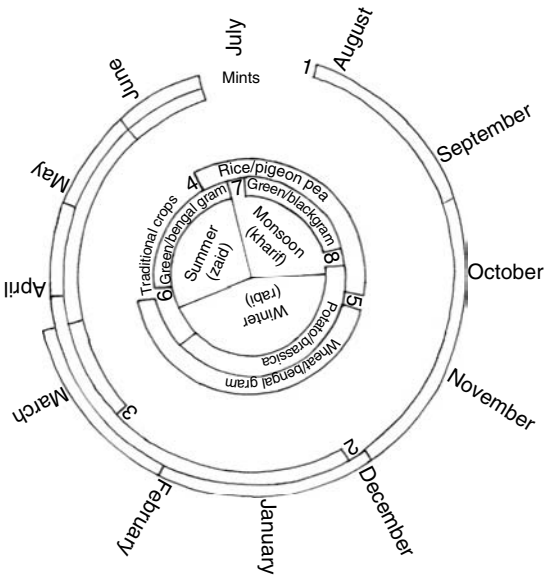


FIGURE 4.6 Crop sequences and mint life times in the Indo-Gangetic plains. (1) The mother plants of mints are transplanted into the nursery between late August and early October and the suckers or propagules formed are harvested from late December to late February. The nursery for raising plantlets from sucker pieces is planted in February or March. (2) The early crops of mint are planted by sowing of suckers or transplantation of propagules from late December to February. Herbage from such crops is first harvested in late April to May, the ratoon is allowed to regenerate, and the crop is harvested for the second time in June to July. (3) The late crop of mint is planted using cold-stored suckers or nursery-raised plantlets in late March to middle of April and the resulting crop is harvested in June or July. (4) Rice or paddy seedlings are transplanted in July and the crop is harvested in October. (5) Wheat or bengal gram crop is sown in October to December and harvested in late March to April (6,7). Green or black gram is sown in April, harvested in July, resown in July, and harvested in October. (8) Potato is planted and Brassica is sown in October. Vegetable potato is harvested in December. Brassica is harvested in January to March, depending on the species or cultivar used. Seed potato is harvested in March.

the food grains, vegetables, and spices for their own consumption. This also helps to ensure overall profitability and some degree of protection against wide fluctuations in the mint oil selling prices. (3) As the mint oil prices widely fluctuate and inputs in mint cultivation are larger than the resource exhaustive nature of crop growth, the farmers retain the option not to plant mint(s) in the next season.

The lifetimes of mint plantations vary widely in the Indo-Gangetic plains (Figure 4.6). Early-planted mint crops occupy fields from December or January or February to July or August or December to April or May or June depending on whether farmers decide to harvest their mint crops once or twice. The late-planted mint crops remain in the field from March or April to June or July. By varying the lifetimes of mint crops, the farmers of the Indo-Gangetic plains are able to contribute to food production and make larger profits by mint cultivation (Figure 4.6).

4.9 INTEGRATION OF MINT IN TRADITIONAL ROTATIONS

Mint is cultivated in some of the prime land of the Indo-Gangetic plains, which is one of the most agriculturally fertile areas of the world. The traditional crops of this area have been rice, pigeon pea, green and black gram, wheat, different types of Brassica, lentil, pea, chickpea, potato, and sugarcane. In the recent past, the rice–wheat rotation with cornmint has been

TABLE 4.8
Crop Rotations and a Cocultivation Involving Cornmint Practiced in the Indo-Gangetic Plains

Crop Rotation/Cocultivation	Relative Profit
Rice–wheat	1.0
Rice–cornmint	1.9
Maize/sorghum/bajra–cornmint	1.9
Rice–Brassica–cornmint	2.3
Rice–lentil/chickpea–cornmint	2.3
Rice–wheat–cornmint	2.4
Sugarcane + cornmint[a]	2.8
Rice–potato/other vegetable–cornmint	3.0

[a]Sugarcane is planted in early October or February in rows 90-cm apart. Three weeks later, two rows of cornmint are planted between two rows of sugarcane and irrigation is applied. *M. spicata* and *M. piperita* and *M. canadensis* can also be planted with October- and February-planted sugarcane, respectively. Mint crops cocultivated in October are harvested first in March and next in May and those cocultivated in February are harvested only once.

successfully practiced over a very large area in the Indo-Gangetic plains. The introduction of mint cultivation in the area has led to the development of certain new and profitable crop rotations (Table 4.8). Some of these rotations permit intensive farming of land and allow overall conservation of the soil fertility and irrigation water (Ram and Kumar, 1996; Kumar et al., 2001b, 2002a). CIMAP has recommended the following 2-year crop rotation: from July or August to October (*kharif* cropping season), rice; from November or December to March (*rabi* cropping season), chickpea; from March or April to June or July (*zaid* cropping season), cornmint; June or July to November or December, pigeon pea; from November or December to April, wheat; and from April to July or August, cornmint. Grain crop breeders are now selecting slightly early maturing genotypes, which will ease the tight-fitting rotational operations.

4.10 OIL DISTILLATION

The partially dried mint herbage is distilled by the farmers themselves in their fields, using small-sized water-cum-steam distillation units owned or hired by them. Alternatively, farmers use the services of local mint distillers who are equipped with large-sized steam distillation units (Kumar et al., 1997a). Usually, the distillation fee paid to the on-farm and off-farm distillers is in the form of 10% to 20% of the distilled oil.

4.10.1 Field Distillation

The field water-cum-steam distillation plants used by the farmers vary in size and efficiency. These are fabricated locally to distill the herbage in batches; a batch of about 5 to 7 quintals (500 to 700 kg) of mint herbage gets distilled in 6 to 8 h. The unit comprises four parts: furnace, distillation still, condenser, and receiver. In the low-cost version (Figure 4.7), mild and thin stainless steel is used to make the tank, which is provided with a perforated grid at the base. The tank is fully packed with herbage and is filled to a lower level with water. It is placed above a brick furnace. The spent mint herbage, other kinds of agrowaste, firewood,

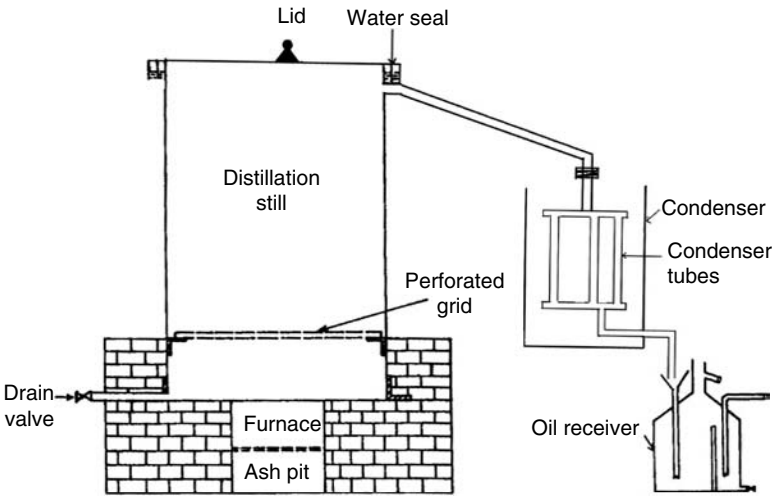


FIGURE 4.7 The design of the steam-cum-water distillation equipment fabricated locally in the mint villages for the distillation of mint herbage in small batches.

and straw are used as fuel. The tank is connected to the condenser through a vapor pipe. The receiver is placed below the condenser outlet. The condenser has a tubular structure suspended in a water tank. The vaporized mint oil and water vapor undergo condensation in the condenser, which is cooled by the water contained in its own tank. The condensate passes to the receiver in which the oil, which is lighter, floats on top of the water. The oil is drawn off and thus separated from the accompanying water in the receiver. In the entire distillation process of this kind, about 10% to 20% of the isolatable essential oil is lost. The kind of distillation equipment described here costs the farmers about Rupees (Rs) 15,000 to 20,000 (USD 300 to 400) only. Such equipment is used by the farmers who have small holdings (up to 2 ha) or are in the initial years of their mint cultivation enterprise.

The farmers with relatively larger landholdings or who have benefited from mint farming for several years prefer to install the economic and improved, but costlier, field distillation units, based on the design developed by CIMAP, Lucknow, India. In these distillation units, the total time of distillation is reduced to only 3 to 4 h and about 20% to 30% of fuel is saved along with minimal loss of the isolatable essential oil. This kind of distillation unit differs from the less expensive one described in the structure of the tank and the condenser (Figure 4.8). The tank is made of much thicker mild steel, which is sturdier, and hence lasts for many years. Besides, the tank has boiler (flue) tubes at the base that create a greater area for water heating and increase the rate of steam generation. The condenser is also more efficient because it has dozens of tubes and the condenser tank is cooled by running water. This type of equipment costs about Rs 50,000 (USD 1000).

The variants of the first and second kinds of distillation units are installed all over the Indian mint growing area in an approximate ratio of 1:2.8. Together, these kinds of distillation units may account for about 80% of the total of all the different kinds of units used in India.

4.10.2 Commercial Distillation

In areas of intensive cornmint cultivation, a larger-sized steam distillation equipment is used. These kinds of distillation plants are installed by the owners of large-size mint farms, cooperative societies, and business entrepreneurs. Of the total, about 20% of the mint oil

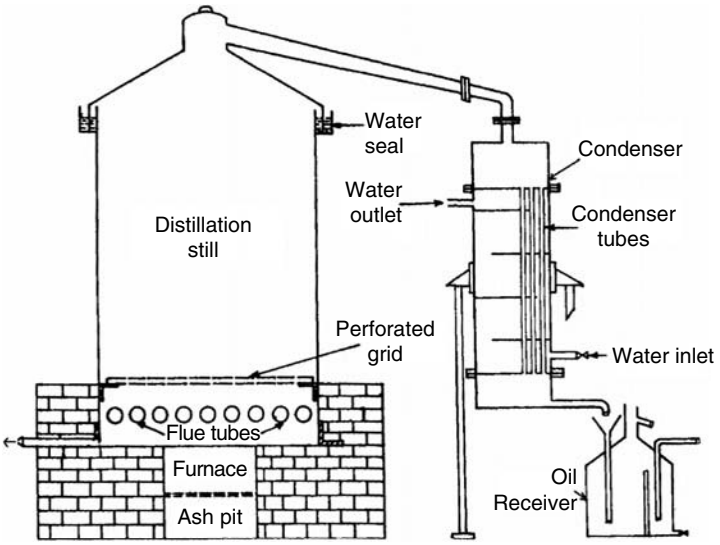


FIGURE 4.8 The structure of a high-efficiency distillation unit for batch-wise distillation of mint herbage designed by CIMAP, Lucknow, India.

distillation units are of this type. The plant consists of a boiler,and one or more (up to six) distillation tanks, each internally fitted with steam coil, besides a condenser and receiver for each of the tanks (Figure 4.9). The steam generated in the boiler passes through the steam coil in the herbage packed tank. This kind of equipment can process up to 30 quintals

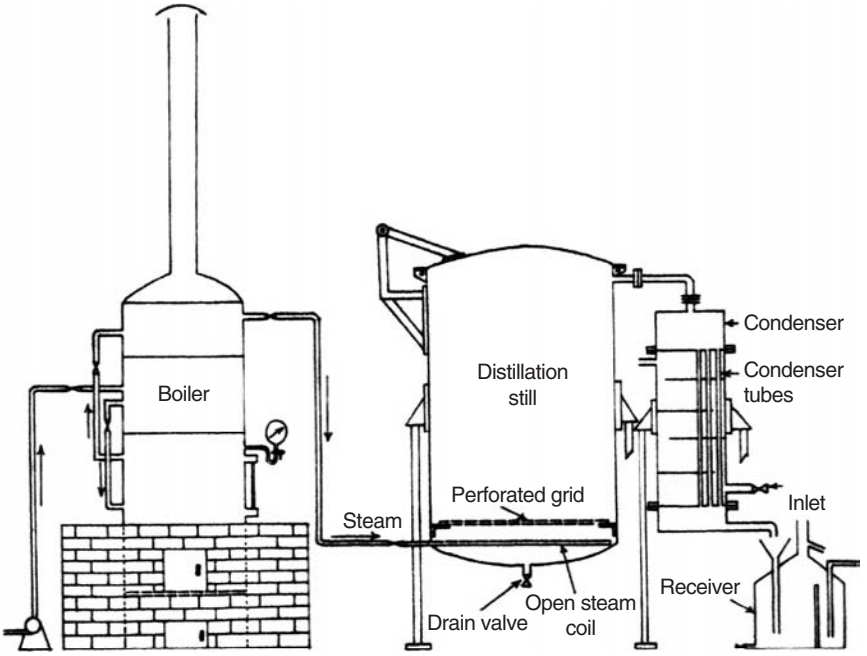


FIGURE 4.9 CIMAP design for the steam distillation of herbage in several tanks linked to a common boiler for use in larger-sized mint plantations.

FIGURE 4.10 A water-cum-steam distillation unit of the kind fabricated in Indian villages used in a mint field. The agrowaste to be used as fuel and dry herbage to be distilled are also seen.

(3000 kg) of herbage per batch, as six tanks are linked to the boiler for the simultaneous distillation in the tanks. The boiler operates on electricity. The cost of equipment suitable for a 10-ha farm comes to about Rs 500,000 (USD 10,000).

The large investment in the distillation equipment is a deterrent for the small farmers to take up mint cultivation. The local government agencies and the Spice Board have installed some distillation units for the cooperative use of the farmers. Figure 4.10 and Figure 4.11 are exemplary field views of the low-cost and large-sized distillation facilities used by the farming community in India. Figure 4.12 depicts a distillation unit for batch-wise distillation of herbage built according to the CIMAP design and installed in a village for cooperative use.

4.10.3 Oil Cleaning

To dry the mint oil, anhydrous sodium sulfate is added to the oil collected from the receivers and the mixture is left for 4 to 5 h and filtered thereafter. Such purified oil is fit for long-term storage. This treatment is useful as the Indian cornmint farmers have the tendency to store a substantial part of their oil in anticipation of obtaining more remunerative prices. The oil is stored in plastic, GI, stainless steel, and aluminum containers, depending on the quantity available and the period of storage.

4.11 ESSENTIAL OIL YIELDS

Industrial mints are cultivated by about 100,000 farmers in India. The large variation observed in the essential oil yields realized by the mint farming communities relates to the differences among their geographical locations, the nature of soils of their lands, the cultivar(s) used, the volumes of the inputs of manure, the fertilizer and irrigation water used, the

FIGURE 4.11 A field view of the larger-sized multiple tank unit installed in a big mint farm in India. This unit is for four tanks. Semidried mint herbage is unloaded from a tractor trolley. It is spread around to allow further loss of moisture.

FIGURE 4.12 Mint herbage distillation unit designed by CIMAP, Lucknow, installed for cooperative use in a village in Barabanki district of Uttar Pradesh in India.

TABLE 4.9
Modal Essential Oil Yields for the Mints Commonly Cultivated in India

Mentha Species	Cultivars	Time of Planting	Yields (kg/ha)
canadensis	Shivalik, Himalaya, Kosi, Sambhav, Saksham	Early	185
		Late	130
spicata	Neera, Neerkalka	Early	145
gracilis	MCAS-2, Pratik	Early	135
piperita	Kukrail, Pranjal	Early	100

stringency of the crop protection measures deployed, the planting and harvesting schedules selected, and the efficiency of distillation, among others. The yields averaged for a large number of farmers and demonstrations are shown in Table 4.9.

4.12 FUTURE PROSPECTS

It has been mentioned earlier that the agroclimate and soils of the Indo-Gangetic plains are conducive to mint cultivation. Here, all the different kinds of mint can be cropped between December or January and July. This period overlaps with the traditional winter or *rabi* (October or November to March or April) and summer or *zaid* (March or April to June or July) crop seasons of the area. The planting and harvesting time of cornmint can be varied within the January-to-July period such that the conventional food grain and vegetable crops could be grown before they are planted or after they have been harvested. This way, the overall effect of cornmint cultivation on food crop production is minimal. It has been observed that farmers gain substantial profit when they rotate mint with the traditional crops of the area. There are additional employment opportunities in the villages where cornmint is cultivated and in the nearby towns, relating to servicing and manufacture of distillation equipment, collection and trading of oils, and processing of oils into saleable products.

Presently, the total world market size of all kinds of mint oils appears to be about 30,000 tonnes and India meets about half of this requirement (Singh et al., 1999). In recent years, about 100,000 to 175,000 ha of land has been put under mint cultivation in the Indo-Gangetic plains (mostly cornmint), which is a small fraction of the area suitable for mint cultivation. The economic advantages associated with mint cultivation can be extended to a larger community of farmers, if the international demand for cornmint oil could be increased. This requires diversification in the technical uses of mint oils, based on their properties (Kumar, 1996).

At present, mint oils and their various major terpenoid fractions are investigated for their diverse biological activities. Among the variety of biological activities that have been revealed, the antioxidant, bioenhancing, phytotoxic, phytohormonal, antimicrobial, and pesticidal activities have opened possibilities for a variety of new technical uses of mint oils. At present, new biological activities are also discovered in the solvent extracts of mint herbage.

A study at CIMAP has revealed that mint oils have lethal effects on widely different bacteria and fungi (Agarwal et al., 2001a). Mint oils and their specific components are potent insecticides (Agarwal et al., 2001b). Identified terpenes of origin in mint oils promote or prevent meristematic growth in specific plant systems (Farooqui et al., 2001). Based on these findings, already three kinds of products have been developed and field-tested successfully.

The properties of the oil of the new spearmint cultivar, Ganga (ex. *M. spicata* var. *viridis*), have allowed the development of formulations that are usable like (a) hand and surface

disinfectants in homes, establishments, and hospitals (Khanuja et al., 2004) and (b) stored grain protectants (Singh et al., 2001; Tripathi et al., 2003a,b). The whole and dementholized cornmint oil obtained from Kosi and Himalaya cultivars have been found to safely retard the process of microbial hydrolysis of the soil-applied fertilizer urea to ammonium and nitrate and their subsequent loss. The results indicate that up to 50% of the fertilizer urea can be saved by coating it with mint oils. Large-scale applications of one or more of the technologies given here can increase the demand of mint oils exponentially (Patra et al., 2001a).

The functional genomics investigations on mints are opening possibilities of producing novel terpenes in mints. It appears possible to breed cultivars or hybrids of mints that will synthesize one or more of all the industrially important monoterpenes. Other kinds of secondary metabolites could also be farmed in the trichomes of the mint vegetation background (Kumar, 1998).

There is a need to strengthen R&D: (a) to breed improved cultivars or hybrids of mints for high yields of specific terpene(s) or secondary metabolite(s), easier planting, early maturity, tolerance to waterlogging, diverse genetic resistance to various diseases and pests, formation of thin and sturdy suckers that can withstand cold storage for extended time periods, and improved oil harvest index; (b) for comprehensive understanding of the biological activities of the conventional mint oil constituents to develop new technical uses of mint oils; and (c) to extend to industry and popularize the large-scale use of newly developed mint-oil-based products.

ACKNOWLEDGMENTS

S.K expresses grateful thanks to scientists of CIMAP, Lucknow, who shared with him the information available with them on mints; in this regard, he is especially thankful to N.K. Patra, A.K. Singh, and K.K. Agarwal. His grateful thanks are also due to the Council of Scientific and Industrial Research and Department of Biotechnology of the Government of India for supporting his work while he held the Emeritus Scientist position at the National Centre of Plant Genome Research (NCPGR) after his superannuation from directorship of CIMAP and to the Indian National Science Academy for presently supporting him as INSA senior scientist, and to the Director, NCPGR, for providing the facilities and encouraging discussions. The authors thank S. Chattopadhyay, J. Sen, and S. Bhatia for reading their manuscript.

REFERENCES

Agarwal, K.K., Khanuja, S.P.S., Ahmad, A., Santha Kumar, R.R., Gupta, V.K., and Kumar, S. (2001a). Antimicrobial activity profiles of the two enantiomers of limonene and carvone isolated from the oil of *Mentha spicata* and *Anthum sowa, Flav. Fragr. J.*, 17, 59–63.

Agarwal, K.K., Tripathi, A.K., Ahmad, A., Prajapati, V., Verma, N., and Kumar, S. (2001b). Toxicity of menthol and its derivatives against stored grain insects, *Insect Sci. Appl.*, 21, 229–235.

Anonymous (1983). Super-menthol strain of Japanese mint, CIMAP, *Newsletter*, 10, 17–18.

Anonymous. (1985a). CIMAP develops superior strains of HY-77 of *Mentha arvensis*, MSS-5 of *Mentha spicata* and MCAS-2 of *Mentha cardiaca*, CIMAP, *Newsletter*, 12, 1–3.

——— (1985b). CIMAP Hybrid-77, a new high yielding strain of Japanese mint. CIMAP, *Newsletter*, 12, 11–12.

Anonymous (1987). A high yielding superior strain of *Mentha viridis*—Supriya developed. CIMAP, *Newsletter*, 14, 2–3.

——— (1988). A high yielding variety of *Mentha eitrata*-Kiran developed, CIMAP Newsletter, 15, 1–2.

——— (1993). CIMAP develops two superior varieties of spearmint, CIMAP Newsletter, 20, 1.

——— (1994). CIMAP releases the variety kukrail of peppermint. CIMAP Newsletter, 21, 3–4.

Bahl, J.R., Bansal, R.P., Garg, S.N., Naqvi, A.A., Luthra, R., Kukreja, A.K., and Kumar, S. (2000). Quality evaluation of the essential oils of the prevalent cultivars of commercial mint species

Mentha arvensis, *spicata*, *piperita*, *cardiaca*, *citrata* and *viridis* cultivated in Indo-Gangetic plains, *J. Med. Arom. Plant Sci*., 22, 787–797.

Bahl, J.R., Bansal, R.P., and Kumar, S. (2002a). Direct planting of short duration summer crops using cold stored suckers in menthol mint *Mentha arvensis, J. Hortic. Sci. Biotech*., 77, 217–219.

Bahl, J.R., Singh, V., Bansal, R.P., Singh, A.K., and Kumar, S. (2002b). Evaluation of genotypes of industrially important mints for high productivity of sucker propagules under variable durations of cropping, *J. Hort. Sci. Biotech*., 77, 217–219.

Bhat, S., Gupta, S.K., Tuli, R., Khanuja, S.P.S., Sharma, S., Bagchi, G.D., Kumar, A., and Kumar, S. (2001). Photo-regulation of adventitious and axillary shoot proliferation in menthol mint *Mentha arvensis*, *Curr. Sci*., 80, 878–881.

Dwivedi, S., Singh, M., Singh, A.P., Singh, V., Naqvi, A.A., Khanuja, S.P.S., and Kumar, S. (2001a). Registration of new variety of *Mentha ×piperita*, *J. Med. Arom. Plant Sci*., 23, 99–101.

Dwivedi, A., Singh, M., Singh, A.P., Singh, V., Naqvi, A.A., Khanuja, S.P.S., and Kumar, S. (2001b). Registration of a new variety Pratik of *Mentha ×gracilis* cv. cardiaca, *J. Med. Arom. Plant Sci*., 23, 102–103.

Farooqui, A.H.A., Agarwal, K.K., Fatima, S., Ahmad, A., Sharma, S., and Kumar, S. (2001). Anti-sprouting agent for potato tuber and a method for producing the same. United States Patent, 6313073.

Gulati, B.C. and Duhan, S.P.S. (1971). Japanese mint in Nainital Terai of Uttar Pradesh, *Indian Perfum*., 15, 1–11.

Gupta, P.P., Prasad, M.L., Ram, A.M., and Kumar, S. (2002). Effect of vesicular–arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* on the essential oil yield related characters and nutrient acquisition in the crops of different cultivars of mint (*Mentha arvensis*) under field conditions, *Biores. Technol*., 81, 77–79.

Kalra, A., Singh, H.B., Patra, N.K., and Kumar, S. (2001a). Economic evaluation of fungicides for leaf blight (*Alternaria alternata*) control in transplanted crop of Shivalik menthol mint *Mentha arvensis*, *J. Ind. Agric. Sci*., 71, 460–462.

Kalra, A., Singh, H.B., Patra, N.K., Pandey, R., Shukla, R.S., and Kumar, S. (2001b). The effect of leaf spot, rust and powdery mildew on yield components of nine Japanese mint (*Mentha arvensis*) genotypes, *J. Hortic. Sci. Biotech*., 76, 546–548.

Kalra, A., Singh, H.B., Patra, N.K., Srivastava, R.K., and Kumar, S. (2006). Screening of menthol mint *Mentha arvensis* cultivars prevalently cultivated in Indo-Gangetic plains against leaf blight disease caused by *Alternaria alternata*. *Indian J. Agric. Sci*. (in press).

Khanuja, S.P.S., Shasany, A.K., Dhawan, S., and Kumar, S. (1998). Rapid procedure for isolating somaclones of altered genotypes in *Mentha arvensis*, *J. Med. Arom. Plant Sci*., 20, 359–361.

Khanuja, S.P.S., Shasany, A.K., Srivastava, A.K., and Kumar, S. (2000). Assessment of genetic relationship in *Mentha* species, *Euphytica*, 111, 121–125.

Khanuja, S.P.S., Kumar, S., Shasany, A.K., Dhawan, S., Darokar, M.P., Naqvi, A.A., Dhawan, O.P., Singh, A.K., Patra, N.K., Bahl, J.R., and Bansal, R.P. (2001a). A menthol tolerant variety Saksham of *Mentha arvensis* yielding high menthol, *J. Med. Arom. Plant Sci*., 23, 110–112.

Khanuja, S.P.S., Kumar, S., Shasany, A.K., Dhawan, S., Darokar, M.P., Tripathy, A.K., Satapathy, S., Santha Kumar, T.R., Gupta, V.K., Awasthi, S., Prajapati, V., Naqvi, A.A., Agarwal, K.K., Bahl, J.R., Singh, A.K., Ahmed, A., Bansal, R.P., Krishna, A., and Saikia, D. (2001b). Multi-utility plant Ganga of *Mentha spicata* var. *viridis*, *J. Med. Arom. Plant Sci*., 23, 113–116.

Khanuja, S.P.S., Shasany, A.K., Dhawan, S., Darokar, M.P., Satapathy, S., Santha Kumar, T.R., Saikia, D., Patra, N.K., Bahl, J.R., Tripathy, A.K., and Kumar, S. (2001c). Insect tolerant high yielding mint plant Sambhav of *Mentha arvensis, J. Med. Arom. Plant Sci*., 23, 117–121.

Khanuja, S.P.S., Darokar, M.P., Santha Kumar, T.R. Shasany, A.K. Ahmad, A., Aggarwal, K.K., Chaturvedi, P., Gupta, V.K., Krishna, A. Singh, A.K., Bahl, J.R., and Kumar, D. (2004). Composition and Process of preparing disinfectant comprising *Mentha spicata* variety *viridis* and their use. United States Patent, 676, 7876.

Kukreja, A.K., Dhawan, O.P., Ahuja, P.S., Sharma, S., and Kumar, S. (2000). Yield potential and stability behavior of *in vitro* derived somaclones of Japanese mint (*Mentha arvensis*) under different environments, *J. Genet. Breed*., 54, 109–115.

Kumar, S. (1996). Promise in bioprospection of plant species for pharmaceuticals and crop/food protectants, *J. Med. Arom. Plant Sci.*, 18, 255–256.

Kumar, S. (1998). Genomics and mediculture productivity, *J. Med. Arom. Plant Sci.*, 20, 347.

Kumar, S., Kahol, A.P., Patra, D.D., Ram, M., Singh, S., and Tyagi, B.R. (1997a). *Cultivation of Menthol Mint in India*. Central Institute of Medicinal and Aromatic Plants, Lucknow, India, pp. 1–36.

Kumar, S., Tyagi, B.R., Bhal, J.R., Khanuja, S.P.S., Shasany, A.K., Shukla, R.S., Sattar, A., Singh, D., Haseeb, A., Singh, V.P., Ram, P., Singh, K., Singh, S., Singh, S.P., Patra, N.K., Alam, M., Naqvi, A.A., Ram., M., Agarwal, K.K., and Singh, A.K. (1997b). Himalaya—a high menthol yielding hybrid clone of *Mentha arvensis*, *J. Med. Arom. Plant Sci.*, 19, 729–731.

Kumar, S., Bahl, J.R., Shukla, P., Singh, A.H., Ram, G., Bansal, R.P., and Sharma, S. (1999a). Screening of genotypes of menthol mint *Mentha arvensis* for high yields of herbage and essential oil under late cropping conditions of the sub-tropical Indo-Gangetic plains, *J. Hort. Sci. Biotech.*, 74, 680–684.

Kumar, S., Gupta, S.K., Bhat, S., and Tuli, R. (1999b). Tissue culture process for producing a large number of viable mint plants *in vitro* from internodal segments. United States Patent 5898001.

Kumar, S., Patra, N.K., Singh, H.P., Kalra, A., Singh, H.B., Ram. P., Singh, V.R., Mengi, N., Singh, V.P., Ram, M., Singh, K., Singh. A., Khanuja, S.P.S., Shasany, A.K., and Tanveer, H. (1999c). Kosi—an early variety requiring late planning of *Mentha arvensis*, *J. Med. Arom. Plant Sci.*, 21, 56–58.

Kumar, S., Tyagi, B.R., Singh, H., Singh, V., Ujagir, R., Khanuja, S.P.S., Shasany, A.K., Shukla, R.S., Sattar, A., Singh, V.P., Ram, P., Singh, K., Patra, N.K., Alam, M., Naqvi, A.A., Ram. M., Agarwal, K.K., and Singh, K. (1999d). Mint plant *Mentha arvensis*. Himalaya. United States Patent, 10935.

Kumar, S., Bahl, J.R., Bansal, R.P., Kukreja, A.K., Garg, S.N., Naqvi, A.A., Luthra, R., and Sharma, S. (2000). Profiles of the essential oils of Indian menthol mint *Mentha arvensis* cultivars at different stages of crop growth in northern plains, *J. Med. Arom. Plant Sci.*, 22, 774–786.

Kumar, S., Patra, N.K., Khanuja, S.P.S., Shasany, A.K., Kalra, A., Singh, H.B., Singh, H.P., Singh, V.R., Mengi, N., Tanveer, H., Naqvi, A.A., Singh, V.P., and Singh, K. (2001a). Hybrid mint plant named Neerkalka. United States Patent, PP12030.

Kumar, S., Srivastava, R.K., Singh, A.K., Kalra, A., Tomar, V.K.S., and Bansal, R.P. (2001b). Higher yields and profits from new crop rotations permitting integration of mediculture with agriculture in Indo-Gangetic plains, *Curr. Sci.*, 80, 563–566.

Kumar, S., Bahl, J.R., Bansal, R.P., Gupta, A.K., Singh, V., Ram, G., and Sharma, S. (2002a). High economic returns from companion and relay cropping of bread wheat and menthol mint in the winter–summer season in north Indian plains, *Ind. Crops Prod.*, 15, 103–114.

Kumar, S., Patra, N.K., Singh, H.P., Kalra, A., Singh, H.B., Ram, P., Singh, V.R., Mengi, N., Singh, V.P., Ram, M., Shukla, R.S., Singh, K., Singh, A., Khanuja, S.P.S., Shasany, A.K., Naqvi, A.A., Kumar, B., Singh, M., Rajput, D.K., Ram, P., Singh, J.P., Kumar, V., Tanveer, H., and Sharma, S.R. (2002b). Mint plant named Kosi. United States Patent, PP12426.

Misra, P.N., Hasan, S.A., and Kumar, S. (2000). *Cultivation of Aromatic Plants in India*. Lucknow, India, Central Institute of Medicinal and Aromatic Plants, pp. 331.

Patra, D.D., Ram, M., and Singh, D.V. (1993). Influence of straw mulching on fertilizer nitrogen use efficiency, moisture conservation and herb and essential oil yield in Japanese mint (*Mentha arvensis*), *Fert. Res.*, 34, 135–139.

Patra, D.D., Anwar, M., and Chand, S. (2000a). Integrated nutrient management and waste recycling for restoring fertility and productivity in Japanese mint and mustard sequence in Uttar Pradesh, India, *Agric. Ecosyst. Environ.*, 80, 267–275.

Patra, N.K., Tanveer, H., Tyagi, N.K., and Kumar, S. (2000b). Cytotaxonomical status and genetical and breeding perspectives of *Mentha, J. Med. Arom. Plant Sci.*, 22, 419–430.

Patra, D.D., Anwar, M., Chand, S., Chattopadhyay, A., Prasad, A., Pande, P., Kumar, A., Singh, S., Srivastava, R.K., Krishna, A., Singh, V., Tomar, V.K.S., Bansal, R.P., Singh, A.K., Bahl, J.R., and Kumar, S. (2001a). Use of mint essential oil as an agrichemical: control of N-loss in crop fields by using mint essential oil urea as fertilizer, *Curr. Sci.*, 81, 1526–1528.

Patra, N.K., Kumar, S., Khanuja, S.P.S., Shasany, A.K., Kalra, A., Ram, P., Singh, H.B., Singh, H.P., Singh, V.R., Kumar, B., Tanveer, H., Mengi, N., Rajput, D.K., Negi, M.S., Tyagi, N.K., Singh, V.P., Singh, J.P. and Naqvi, A.A. (2001b). Damroo—a seed producing menthol mint variety (*Mentha arvensis*), *J. Med. Arom. Plant Sci*., 23, 141–145.

Patra, N.K., Kumar, S., Khanuja, S.P.S., Shasany, A.K., Kalra, A., Singh, H.B., Singh, H.P., Tanveer, H., Singh, V.R., Kumar, B., Mengi, N., Negi, M.S., Singh, V.P., Singh., J.P., and Naqvi, A.A. (2001c). Neerkalka—an interspecific hybrid variety of *Mentha arvensis* and spearmint, *J. Med. Arom. Plant Sci*., 23, 129–132.

Patra, N.K., Tanveer, H., Khanuja, S.P.S., Shasany, A.K., Singh, H.P., Singh, V.R., and Kumar, S. (2001d). A unique interspecific hybrid spearmint clone with growth properties of *Mentha arvensis* L. and oil qualities of *M. spiciata*. *Theor. Appl. Genet*., 102, 471–476.

Ram, M. and Kumar, S. (1996). The production and economic potential of cropping sequences with medicinal and aromatic crops in a subtropical environment, *J. Herbs. Spices Med. Plants*, 4, 23–29.

Ram, M. and Kumar, S. (1997). Yield improvement in the regenerated and transplanted mint *Mentha arvensis* by recycling the organic wastes and manures, *Biores. Technol*., 59, 144–149.

Ram, M. and Kumar, S. (1998). Yield and resource optimization in late transplanted mint (*Mentha arvensis*) under subtropical conditions, *J. Agron. Sci*., 180, 109–112.

Ram, P., Patra, N.K., Kumar, B., Singh, H.B., and Kumar, S. (1998a). Transplanted mint as a suitable component for traditional crop rotation in northern India, *Tropic. Agric*., 75, 473–475.

Ram, P., Patra, N.K., Kumar, B., Singh, H.B. and Kumar, S. (1998b). Productivity and economic viability in early and late transplanted Japanese mint (*Mentha arvensis* L.), *Indian Perfum*., 42, 211–215.

Shasany, A.K., Khanuja, S.P.S., Dhawan, S., Yadav, U., Sharma, S., and Kumar, S. (1998). High regenerative nature of *Mentha arvensis*, *J. Biosci*., 23, 641–646.

Singh, A.K. and Naqvi, A.A. (1996). Post harvest storage to boost essential oil recovery in Cymbopogon and mint species, *Indian Perfum*., 40, 26–31.

Singh, H.B., Kalra, A., Patra, N.K., and Kumar, S. (1997). Stolon decay of menthol mint caused by *Sclerotinia sclerotiorum* (Abstract), New Delhi: International Conference on Integrated Plant Disease Management for Sustainable Agriculture, pp. 244.

Singh, A.K., Srivastava, R.K., and Kumar, S. (1999). Production and trade of menthol in India, *Fafai J*., April–June, 28–32.

Singh, H.B., Kalra, A., Patra, N.K., and Kumar, S. (2000). Stolon decay of menthol mint caused by *Sclerotinia Sclerotiorum* and its biological control New Delhi: Proceedings of the International Conference on Integrated Plant Disease Management for Sustainable Agriculture. Indian Phytopathological Society, 1, 386–388.

Singh, D., Mehta, S.S., Srivastava, A.K., and Kumar, S. (2001). A process for the preparation of pest repellant tablet. Indian Patent, 184–803.

Singh, A.K., Srivastava, R.K., Kalra, A., Bansal, R.P., Tomar, V.K.S., and Kumar, S. (2002). Characteristics of menthol mint *Mentha arvensis* industrial crop production in Indo-Gangetic plain, *Exp. Agric*., 39, 199–207.

Srivastava, R.K., Singh, A.K., Kalra, A., Tomar, V.K., Bansal, D.D., Chand, S., Naqvi, A.A., Sharma, S., and Kumar, S. (2002). Characterization of menthol mint *Mentha arvensis* cultivated on industrial scale in the Indo-Gangetic plains, *Ind. Crops Prod*., 15, 189–198.

Tripathi, A.K., Aggarwal, K.K., Prajapati, V. and Kumar, S. (2003a). A formulation useful as fumigant against stored grain insect pests. Indian Patent, 188–378.

Tripathi, A.K., Singh, A.K., and Kumar, S. (2003b). A herbal formulation useful as fumigant against stored grain insects. Indian Patent, 188–413.

Tyagi, B.R., Ahmad, T., and Bahl, J.R. (1992). Cytology, genetics and breeding of commercially important *Mentha* species, *Curr. Res. Med. Arom. Plant Sci*. (now *J. Med. Arom. Plant Sci.*), 14, 51–56.

5 Production of Mints and Mint Oil in China

Wennuan Liu and Brian M. Lawrence

CONTENTS

5.1 INTRODUCTION

China is one of the largest mint producers in the world. Historical records show that cornmint was first used as a traditional medicine to treat febrile diseases and heat stroke during the Song Dynasty (960 to 1279) (Zhao, 1991). Mint has been cultivated in China for a long time although it is difficult to trace the exact date when cornmint was first grown. However, it was reported that mint cultivation could be traced back to as early as the Ming Dynasty (1368 to 1644) in China (Dai, 1981). Since cornmint possesses aromatic cooling and germicidal properties and has been used to dispel rheumatic pains, it was mainly used as a Chinese herbal drug and as a component of mint tea, a refreshment used in the summer in ancient China.

Watts (1997) reported that the first large-scale production of menthol from cornmint oil in China commenced in Shanghai in 1923 and it was sold under the brand name "Buddha." A second factory for the production of "Polar Bear brand" was started 4 years later in Shanghai. Subsequent to that a number of other brands such as White Cat (1966), AE (1989), Snow Peak, Penguin, White Bear, Glacier, etc., have been introduced over the years. It is of interest to note that for the past 10 plus years the brand names for Chinese menthol are meaningless as the owner of the brand name can lease the use of it to any processor or middleman for a price.

Watts (1997) reported that there were seven major factories processing menthol from cornmint oil. This means that anyone of the seven major menthol processors could produce any brand of menthol so long as they purchase the rights to use the name. It also means that the brand could be produced from Chinese oil, purchased Indian oil, purchased Indian menthol powder, or a mixture of the above and sold for export as Chinese menthol. As a result, the cultivation of cornmint in China is not always directly related to the production of menthol in China.

5.2 THE ESSENTIAL OIL AND AROMA CHEMICAL INDUSTRY

From the 1970s to early 1980s the essential oil industry, which included the sale of cornmint oil and menthol, was under the control of the Ministry of Light Industry. Export of these products was facilitated through one of China's ten export corporations, namely, China National Native Produce and Animal By-Products and Import and Export Corporation. This corporation, which was based in Beijing, had 12 branch offices and a number of suboffices in different regions of China. Seven of these branch offices negotiated export sales of cornmint oil and menthol with foreign buyers (Manheimer, 1980).

Cultivation of cornmint for oil production and subsequent menthol isolation commenced initially around Shanghai. As menthol processing factories were established in Anhui, Jiangsu

and Henan, cornmint cultivation was established within a 200-km radius from the major cities in each of the major producing provinces: Hefei (Anhui), Nanjing (Jiangsu), and Zhengzhou (Henan). The cultivation areas are in the vicinity of Taihe, Liguan, and Bozhou (Anhui), Dongtai, Gaoyi, and Haiman (Jiangsu), and Shangqui and Yongchen (Henan) (Watts, 1997).

These state corporations were allocated money from Beijing to buy all the cornmint oil either from the co-operatives (communes) or from small producers. Money was borrowed from the local banks to buy the entire year's production without pressure to repay the loan. The oil was sent to the various processing factories and both unprocessed oil and menthol were stored in the Shanghai area. Forms and contracts with foreign buyers were allocated from Beijing generally on a long-term basis (Watts, 1997).

According to Zhao (1991), the largest cultivation area of cornmint was around the mouth of Yangtze River in the Nantong Prefective (Jiangsu). In 1991 ca. 10,000 ha were devoted to cornmint cultivation in this area. The area of cornmint cultivation was under government control. The co-operatives and small cornmint grown produced the crude cornmint oil in hundreds of small water distillation units of a similar design (Lawrence, 1995).

State Trading Corporations purchased the oil produced each year. More recently, the influence of Beijing has reduced resulting in a loss of credit lines by the various State Trading Corporations. As a result, these corporations no longer have the ability to buy all the crude cornmint oil. According to Watts (1997), they are no longer prime players in the cornmint oil and menthol market. They have been replaced by middlemen who buy oil directly from the producers and get it processed into menthol on a speculative basis. If the oil prices in India are less than the price they have to pay for Chinese oil, they buy both crude Indian cornmint oil and menthol powder and have them processed into menthol in China and offer it for both domestic and export sales as Chinese menthol. Consequently, the quantity of menthol being sold as Chinese menthol no longer reflects the quantity of crude cornmint oil produced in China as it once did.

Two other factors have influenced the production of crude cornmint oil in China and they are (a) the growers themselves can decide on the size of their cornmint cultivation and (b) domestic and export supply and demand for Chinese menthol. The former decision is somewhat based on the market economics of other crops such as rice, corn, cotton, barley, soybean, rapeseed, etc., and any government subsidies of fixed prices for specific crops. The latter decision is based on the quantities and prices quoted for cornmint oil by the middlemen.

5.3 MINTS GROWN IN CHINA

In addition to cornmint [ex. *M. canadensis* L. syn. *M. arvensis* L. f. *piperascens* Malinv. ex Holmes], spearmint (ex. *M. spicata* L. syn. *M. viridis* L. or *M.* ×*gracilis* Sole syn. *M. cardiaca* Gerarde ex. Baker), peppermint (ex. *M.* ×*piperita* L.), and Mentha citrata [ex. *M. aquatica* L. var. *citrata* (Ehrh.) Fresen] are currently grown commercially in China (Dai, 1981; Yang et al., 1991; Wang et al., 1992; Jian and Zhou, 1993; Zhang et al., 1995). In addition, wild-growing *M. canadensis* (as *M. haplocalyx* Briq.) is also grown commercially in China albeit to a very limited extent. It is known locally as "Rend Dan Cao" or "Fan He Cai" (Xu et al., 1990). It is cultivated mainly in Jiangsu, Henan, Anhui, and Jiangxi and it is sold specifically to the Chinese herb trade and is not used for oil production.

5.4 CORNMINT

Most mint cultivars grown in China were developed from local mint varieties of cornmint or the varieties of spearmint produced by various breeding and research institutes after 1957

(Wang et al., 1992). The cultivars of each of the mints used for oil production are examined here according to the specific mint.

The cultivars of cornmint grown in China are as follows.

5.4.1 Mint 271, Mint 148, and Mint 18

Mint 271, Mint 148, and Mint 18 were developed in 1963 through selection of naturally occurring mutants and plants segregated from local cornmint cultivars. With purple and thin stems, flat and ovate leaves, and dark-purple midrib, Mint 271 and Mint 148 produce fewer and thinner rhizomes, more thinner and longer stolons, and fewer fertile flowers than Mint 18. The yield of cornmint oil of these cultivars was about 105 kg/ha in the early 1960s. With about 83% menthol content, the oils from these cultivars possessed a pure menthol aroma. However, they were not resistant to drought. They were mainly cultivated in the vicinity of Shanghai and Jiangsu Province from 1963 to 1969. On the other hand, Mint 18, with a strong green stem and green midribs, produced more strong and thicker rhizomes and light purple fertile flowers. Being highly resistant to both drought and water-logging, this cultivar produced about 150 kg/ha mint oil, which contained about 81% menthol.

5.4.2 Mint 119

Mint 119 was developed from a local cultivar, "Crystal" in 1963. With a strong, thick, and green stem, white midrib, brown rhizomes, and light purple male sterile flowers, this cultivar, which is highly resistant to stress, grows very rapidly and can produce about 225 kg/ha cornmint oil. However, the menthol content in the oil is only 70%. With a grassy smell, both the quality and the aroma of the oil are low. Although it had been a popular cultivar, it is now not used for cornmint oil production because of its poor quality.

5.4.3 Mint 409

Mint 409 was developed from the progeny of Mint 271 in 1969. With a purple stem, thin and long rhizomes, grayish green ovate leaves, yellowish green midribs, and light purple fertile flowers, this cultivar yields about 150 kg/ha oil that contains around 80% menthol. Both the quality and the aroma of the oil were determined to be good. However, the yield of oil varied year by year because the stress resistance and the growth rate of this cultivar were not as good as those of some current mint cultivars.

5.4.4 Mint 68–7

Mint 68–7 is a hybrid developed from Mint 409 $\times$ C-119 in 1974. The stem color of this cultivar at the early stages is purple, which becomes brown to green from the bottom to the top during its later developmental stages. With purple stolons, thick rhizomes, and dark green ruffled leaves, white midribs, and a light purple fertile flower, it produced about 150 kg/ha oil that contained approximately 80% menthol. This cultivar was found to be resistant to stress, grew rapidly, and needed less management. However, the aroma of the oil was found to be poor and the level of some components in the oil failed to meet the requirements for cornmint oil export. As a result, this cultivar is no longer used for cornmint production in China.

5.4.5 Mint 73–8

Mint 73–8 was developed from the progeny of hybridization of Mint 409 in 1979. The color of the stem changes from purple to green from the bottom to the top. With brownish purple stolons, strong and thick rhizomes, shining oval leaves, and small light purple flowers, this

cultivar yields more than 150 kg/ha oil that contains roughly 80% menthol. Since it is highly resistant to stress, grows rapidly, and produces high-quality cornmint oil with good aroma, it was the main cultivar for production in China in the early 1990s. Today, it is still the most commonly grown cornmint cultivar in China.

5.4.6 Haixiang 1

Haixiang 1 was developed from Mint 68–7 and Mint 409 in 1976. The color of the stem changes from purple to green from the bottom to the top at the later developmental stages. With purple thick stolons, long rhizomes, long oval-shaped yellowish green leaves, light purple midribs, longer branches, and light purple fertile flowers, this cultivar yields about 150 kg/ha oil that contains more than 82% menthol. Although both the quality and the aroma of its oil are high, as Haixiang 1 is not resistant to lodging, which results in a variation of oil yield from year to year, it is not grown commercially.

5.4.7 Haixuan Mint

Haixuan Mint was developed from a propagating population of Haixiang 1 in 1984. The color of the stem is the same as Haixiang 1. With thin and long rhizomes, dark yellowish green oval leaves, light purple midribs, and light purple fertile flowers, this cultivar produced oil with the best quality in China in the early 1990s. Its oil contains about 84% menthol; however, its resistance to stress is poorer than that of mint 73–8. As a result, the yield of oil from this cultivar varies year to year. Consequently, it is not grown commercially.

5.4.8 Mint 80-A-53

Mint 80-A-53 was developed from the progeny of a mutant derived from grafting Haixiang 1 and Mint 40–9 in 1987. The color of the stem changes from purple to dark green from the bottom to the top at the later developmental stages. With strong thick rhizomes, long oval shining leaves, and light purple flowers, Mint 80-A-53 has continued to yield about 150 kg/ha oil that contains more than 80% menthol over the years (Wang et al., 1992; Zhou et al., 1993). Both the resistance to lodging and the growth rate of this cultivar are high, with the increasing planting acreage in the early 1990s.

5.5 SPEARMINT

Spearmint is also known as green mint in China. It was first introduced into China in 1950 presumably from Europe via Japan. The cultivars of spearmint grown in China include large-leaf spearmint, small-leaf spearmint, and Spearmint 73–2. The aroma of the oil from these cultivars is low. They are mainly cultivated in Jiangsu, Anhui, Jiangxi, Zhejiang, Henan, Sichuan, Guangdong and Guangxi Provinces. Ever since *M.* ×*gracilis* was introduced into China from the USA in 1980, the aroma of spearmint oil has improved significantly. The hectarage if this cultivar has been increased in areas mentioned above to replace the old cultivars. Spearmint oil is produced and mainly used in China with a small amount exported. The total yield of spearmint oil produced in China was about 800 metric tonnes in the early 1990s. The cultivars in question are as follows.

5.5.1 Spearmint 73–2

Spearmint 73–2 was developed from a selected progeny of Mint 409 in 1973 and passed the variety test in 1997. The color of the stem is green with single oblanceolate leaves paring in

the opposite positions on the stem. The flower is small, light purple in color, incompletely developed, and produces seeds at a very low rate. This cultivar grows very quickly, ripens early in Jiangsu and Shanghai, and yields 75 to 125 kg/ha oil that contains 60% L-carvone. However, the aroma of the oil is poor.

5.5.2 Spearmint 80–1

Spearmint 80–1 (Scotch spearmint) was introduced from United States in 1980. The color of the stem changes from purple to green from the bottom to the top. Trichomes develop on the surface of the nodes of the stem. The long oval leaves are flat and dark green in color. Raceme inflorescence produced seeds at a low rate. This cultivar is not resistant to lodging and yields about 75 to 79 kg/ha oil that contains more than 70% L-carvone. The quality and aroma of its oil were the best among the cultivars grown in China in the early 1990s. However, the yield of its oil fluctuates significantly year by year because of its susceptibility to lodging and diseases.

5.5.3 Spearmint 79–2

Spearmint 79–2 was developed from a population of ^{60}Co-γ mutagenized seeds of Jinzhou Spearmint (73–2) in 1987. The stem, which is green in color, produces many aerial roots at the base. The long, oval leaves produce trichomes on both sides of the leaves. Raceme inflorescence, with incompletely developed small light purple flowers, produces seeds at a low rate. With strong rhizomes and rapid growth and developmental rate, this cultivar yields about 105 kg/ha of oil that contains less than 60% L-carvone.

5.5.4 Spearmint 80-S-108

Spearmint 80-S-108 was developed from the progeny of Jinzhou Spearmint in 1984. The color of the stem is purple and its large, wide oval leaves are light green. Trichomes develop on the surfaces of both stems and leaves. Raceme inflorescence is composed of light purple fertile flowers. With strong growth and development, high resistance to stresses, and low rate of abscission, this cultivar produces about 150 kg/ha of oil that contains roughly 60% L-carvone. The quality and the aroma of the oil are not as good as that of Spearmint 80–1.

5.6 PEPPERMINT

Peppermint (*Mentha* ×*piperita* L.) is also known as European mint or Western mint in China. One of the best known peppermint cultivars is "Mitcham" mint from England. The main varieties of peppermint grown in China are *M. piperita* var. *officinalis* Sole and *M. piperita* var. *vulgaris* Sole. The quality of the mint oil from *M. piperita* var. *officinalis* is very high. Since the biomass and the pest resistance of this subspecies are low, its current acreage in China is limited.

The peppermint grown in China was first introduced from the former Soviet Union and Bulgaria in 1959. There are two types of most cultivated peppermints in China, green-stem peppermint (*M. piperita* var. *officinalis*) and purple-stem peppermint (*M. piperita* var. *vulgaris*). The former produces a high yield of mint oil with low quality. The quality of the oil from purple-stem peppermint is higher but the yield of the oil is lower. The components of the oil are mainly menthol, menthone, menthofuran, and menthyl acetate. Since the content of menthol in peppermint is lower than that in cornmint, it is not used for isolation of menthol. The oil of peppermint is often used directly to make flavors because of its pleasant taste and aroma. The major growing areas of peppermint in China include Jiangsu, Hebei, Zhejiang, Anhui, and Xinjiang, the acreage is much smaller than that in the USA, India, or even Italy.

5.7 *MENTHA CITRATA*

Mentha citrata is known as lemony mint in China. It was introduced into China from Egypt in 1960. It is mainly used domestically in cosmetics, soaps, and food products. The major growing areas include Jiangsu, Zhejiang, and Anhui, although the hectarage is small compared with that of cornmint and spearmint.

5.8 ENVIRONMENTAL REQUIREMENTS

In general, mints have a wide adaptability to different environments and high resistance to stresses. Since different species have evolved under different climates on the earth, the specific environmental conditions required for maximum growth for various species are different. The rest of this chapter is devoted to the discussion of cornmint, the most cultivated species in China. Cornmint grows rapidly under warm climate with high light intensity. Under optimum environmental conditions, it can grow indefinitely and be harvested several times in a year. In Jiangsu, Shanghai, Anhui, Henan, and Jiangxi, mints are usually harvested two times per year, while they are harvested three times per year in Fujian, four times in Guangdong, Guangxi, and Hainan, but only once a year in north and northeast of China.

The growth and development of cornmint plants and the quality of the oil are very sensitive to environmental factors. Precipitation, climate, and weather conditions have significant effects on the oil yield and composition of the oil (Wang et al., 1992).

5.9 TEMPERATURE

Temperature is one of the most important factors affecting the growth and development of cornmint plants. During the entire growing season, the accumulative temperatures for the first harvest are about 2000°C to 2300°C. Within a certain range of temperature, the rate of growth increases as the temperature rises, resulting in a shorter growing season for harvest. The cold resistance of cornmint is dependent on the tissue and the developmental stage of the plant. After planting, the rhizome can survive under 0°C to −20°C in the soil during winter. Shoot buds develop as soon as the soil temperature reaches above 0°C. The optimum temperature for the rapid growth of mint plants is 20°C to 30°C.

5.10 SUNLIGHT

As a long-day plant, cornmint grows better under high light intensity. Longer photoperiods promote flowering and accumulation of oil and menthol in the plant. During the entire growing season, as the light intensity increases, the number of leaves falling from the plant decreases and the content of oil per plant increases. This is especially true for the later stages of the growing season. Rain and lower light intensity during the later stages of growth and development are the main factors resulting in lower yield of oil.

5.11 WATER SUPPLY

With only a fibrous root system, multiple branches, and large leaves, cornmint needs liberal, well-distributed rainfall to provide adequate water supply during the early and middle stages of growth and development. Limited water supply during these stages will result in reduced vegetative growth and a decline in the yield of biomass. On the other hand, too much rainfall, poor soil drainage, and high relative humidity will promote disease development, resulting in

the abscission of the low and middle leaves. Dry weather and low soil moisture during the later stages, especially 10 to 20 days before harvest, are optimum conditions to ensure a high oil yield. On the other hand, too much rainfall during the later growth and developmental stages and during harvest will cause a low oil yield. After the first harvest, ample water supply is needed to support high percentage of regrowth for the next crop.

5.12 SOIL

Cornmint can generally be grown in a wide range of soils, from sandy to clay. Except very sandy, heavy clay, and high-salt soils, most types of soils can be used for cornmint production. The best types of soils for good growth are sandy and silt loams. The optimum soil pH range is 6 to 7.5. Also, it is very difficult to establish young plants when the salt content is equal to or higher than 0.1% as can be found in coastal areas.

5.13 PRODUCTION PRACTICES IN CHINA

Mints are marketed mainly in the form of mint oils. The ultimate purpose of cultivation is to produce maximum yield of oil with high quality. The yield of mint oil is determined by the biomass yield and the oil content of the mint plants. The yield of biomass is mainly dependent on the cultural practices, while oil content is mainly affected by the genotype as well as the growth rate and the morphology of the plant, the number and the size of the leaf, and the environment where the mint grows (Guo and Chan, 1993; Jian et al., 1995; Ren, 1995; Yin, 1995; Zuo et al., 1995; Wang, 1996; Wang and Yang, 1997; Liu, 1997, 2001; Zhu, 1997). It is, therefore, very important to employ the appropriate cultural methods during the entire grown seasons in addition to choosing the best cultivars suitable to the climate for mint production.

5.14 PLANTING

The season for planting cornmint in the field varies with the climates in different production areas. In general, it can be planted at any time except during the winter when there are frosts or the ground is frozen. For example, cornmint can be planted at any time of the year in Guangdong, southern Guangxi, and Hainan Provinces. In northern China, cornmint can be planted from April to October, while mints are usually planted from the end of October to the end of November in Jiangsu, Zhejiang, and Shanghai. It was reported that the yield and the menthol content of cornmint oil were affected by the planting date (Table 5.1, Wang et al., 1992). If cornmint is planted too early in the fall with a high temperature, most of the shoots will emerge from the soil. They may be killed by the frost and/or freezing temperatures in the winter. Although new shoots develop from the rhizome in the next spring, the vigor of the new shoots could be dramatically reduced because some of the nutrients in the rhizomes were used for development of the shoots before the winter. Occasionally, some of the rhizomes may lose the ability to produce new shoots, which will result in fewer plants per hectare. The more the shoots emerge from the soil and the stronger the shoots become before the winter, the weaker the shoots will be and fewer the plants per hectare in the next spring. On the other hand, if mints are planted too late, the growing season will be shortened, which also may result in a decrease in the yield of cornmint oil.

Although seeds can be used to produce seedlings, rhizomes and stolons are usually used for planting in cornmint production. It is essential that fresh rhizomes and stolons of good quality are used for planting. It was reported that the number of seedlings per hectare and the percentage of vigorous shoots can be significantly affected by the freshness of the

TABLE 5.1
The Effect of Planting Date on the Yield and Total Alcohol Content of Cornmint Oil

Cultivar	Planting Date	Harvesting Date	Yield of Oil (kg/ha)	Alcohol Content* (%)
68–7	10/12/1974	July 22, 1975	94.50	82.4
	10/27/1974		102.75	83.8
	01/20/1075		75.75	79.2
	10/10/1978		92.25	84.7
Haixiang 1	11/01/1978	July 9, 1979	99.75	85.9
	01/30/1979		93.75	81.6
	10/10/1984		108.00	85.7
Haixuan	11/01/1984	July 15, 1985	125.25	86.9
	01/10/1985		99.75	84.2

*As menthol.

rhizomes (Table 5.2, Wang et al., 1992). When there is a sufficient supply of rhizomes, the whole rhizome should be used for each plant to produce vigorous and uniform shoots, which are critical for a high yield of oil. When there is a limited supply of rhizomes, each rhizome can be cut into multiple segments. Each segment should have two to three internodes of the rhizomes or stolons.

During planting, a furrow with a width of 23 to 25 cm and a depth of 5 to 7 cm is first made by a plow. The whole rhizomes or segments of rhizomes are usually spread evenly in the furrow. The distance between two furrows is about 10 cm. Rhizomes should be placed and covered with moist soil in the field. The amount of rhizomes planted per hectare is in the range of 1125 to 1500 kg, depending on the quality of the soil, the freshness and quality of the rhizomes, and the method that is used for planting. In general, more rhizomes are used if the soil is poor, the quality of the rhizome is low, if there is an intercrop planted with cornmint, or if machinery is used for planting.

In the areas that receive a large amount of precipitation during the growing season, it is very important to establish a water drainage system in the field during planting because both cornmint and other mints are very sensitive to water-logging. Wang et al. (1992) reported that drainage had a significant effect on the yield of oil. The effects of water-logging include (1) limiting O_2 supply in the soil and inhibiting the development of the root system, which

TABLE 5.2
The Effect of Freshness of Cornmint Rhizomes on the Number of Seedlings per Hectare and the Percentage of Vigorous Shoots

Days after Rhizome Stored before Planting	Seedlings/ha*	% of Vigorous Shoots
1–2	652,500	80
3–6	591,000	62
12	444,000	45
20	211,500	25

*Planting at 1500 kg/ha.

results in reduced uptake of nutrients and limited growth of plants; (2) increasing the activities of anaerobic microbes that result in the production of a number of toxic compounds such as organic acids, hydrogen sulfide, and ferrous compounds, which may cause black and rotten roots, reduced root respiration, and "red" seedlings; (3) lodging, leaf rotting, development of diseases under high humidity that cause a decrease in the quality of the oil; (4) growth of more weeds under high humidity that affect not only the growth of the mint plants but also the quality of the oil by giving it undesirable aroma and color, especially when weeds are mixed with cornmint in the processing and isolation of the oil. The combination of all the aspects described here will result in a significant decrease in the yield and quality of the oil. The drainage systems usually consist of vertical and horizontal ditches. In a large field with sandy loam, a drainage system with horizontal ditches every 10 m and vertical ditches every 30 m is established during planting. Every drainage ditch should connect to the main drainage channels in the field.

After planting, the surface of the soil is usually compacted by a roller to firm the loose soil particles, preserve moisture, and protect the rhizomes from freezing during the winter. It was reported that shoots emerge about 1 week earlier in a rolled field in comparison to those in an unrolled field (Wang et al., 1992). In the areas with a sufficient water supply, irrigation before frosting and freezing can be used instead of rolling. The timing of irrigation is very important. If the field is irrigated too early when the temperature is still high, irrigation will promote the emergence of a large number of shoots from the soil, which could be killed in a cold winter. On the other hand, if the field is irrigated too late and free water cannot completely seep into the soil ice formed during the winter may damage the rhizomes and result in a reduced number of emerging shoots during the next spring. After irrigation, cultivation is needed to prevent salts from moving up to the surface of the soil.

5.15 FIELD MANAGEMENT PRACTICES

5.15.1 Population Density Adjustments

Plant population density affects the yields of biomass and oil. Although the population density is set when mints are planted in the field, unusual low temperatures in the winter and early spring could cause some plants to die and result in a lower population density than anticipated. It is, therefore, necessary to adjust the population density by transplanting young plants to the places where plants had died after the temperature in the spring stabilized at or above 12°C. When the temperature is below 10°C, the survival rate of the transplants is usually low. After the temperature rises above 25°C, the survival rate is also low, especially for large transplants. For example, in Jiangsu Province, transplanting to adjust the population density is usually carried out between 15 March and 15 May.

Another main factor affecting the population density is the purity of the cultivar. The population density decreases as the number of the off-type plants increases. Decrease in the purity of the commercial cultivars not only affects the yield but also reduces the quality of the oil. The decline in purity can be caused by a number of factors including segregating seedlings, mechanical mixtures, and somatic mutations. Most of the off-type mint plants reproduce rapidly and yield less oil with lower quality. Identification of the off-types is mainly based on the morphology of the plants. The following six types of plants are usually considered as off-types and removed before they reach the stage of eight leaves: (1) long internode type, (2) long petiole type, (3) thin lamia type, (4) lanceolate leaf type, (5) excess trichomes type, and (6) brittle leaf type. As soon as the off-types are generally removed from the field, in their place seedlings of the same cultivar should be transplanted to maintain the optimum population density.

5.15.2 Cultivation and Weed Management

There are a number of weed species that can be found growing in mint fields, especially in the area where aquatic–terrestrial rotation is not used. Weeds not only compete for the nutrients in the field, but also shade the lower part of mint plants. These usually cause abscission of mint leaves and result in a significant yield reduction in biomass and oil. The mixture of weeds and mint during harvest can also reduce the quality of oil. Therefore, weeds must be removed from the field before they impose a significant effect on the yield and the quality of the oil.

Weed control is usually carried out through cultivation starting in the early spring when daily average temperatures reach above 10°C. Cultivation not only kills the weeds in the field but also loosens the soil, reduces water evaporation, and mobilizes the nutrients available to mint plants. Weeds are also controlled by various herbicides in most mint production in China (Hu and Shao, 1990; Huang, 1992; Wang et al., 1992).

5.15.3 Topping

Topping is a culture practice to remove the shoot tip of the main stem to promote the growth and development of lateral shoots when the population density is low. Topping is not recommended for a normal population density without intercropping. The effects of topping under different population densities on the yield of cornmint oil were studied by Wang et al. (1992) and their results are presented in Table 5.3. When mints are interplanted with other crops, they should be topped as soon as the other crop is harvested.

5.15.4 Fertilization

Nutrients play an important role in the growth and development of mint plants (Yang and Guo, 1993; Le et al., 1996; Sun, 1997; Zhu et al., 2000). High yield and quality of mint oil from a specific cultivar are mainly achieved via production of a large amount of healthy mature leaves at harvest.

It is reported that leaves developed before mid-May in Jiangsu barely contribute to the final yield of oil (Wang et al., 1992). Rapid growth in the early stages usually results in thinner and taller plants under a normal population density. Since the plants start to overlap each other at very early stages of development, the light intensity is reduced and the humidity is increased under the canopy. These will foster lodging and disease, cause a significant loss of leaves from the lower part of the plants, and result in a large decline in the yield and quality of mint oil. Table 5.4 shows the effects of the same total amount of fertilizer applied at different developmental stages on the yield of biomass and oil (Wang et al., 1992). It is apparent that the application of appropriate amounts of fertilizer at specific developmental stages is critical to the yield of both biomass and oils.

TABLE 5.3
The Effects of Topping on the Yield of Cornmint Oil from cv. Mint 73–8

Population Density (plants/ha)	Treatment	First Harvest Yield of Oil (kg/ha)
597,000	Topping	94.65
	Nontopping	107.10
211,500	Topping	108.60
	Nontopping	90.15

TABLE 5.4
The Effects of Fertilizer Applied at Different Stages on the Length of Internodes and the Yield of Biomass and Cornmint Oil

Cultivar	Fertilizer (urea kg/ha)	Application Date	Biomass (kg/ha)	Oil (kg/ha)	Oil Content (%)	Internodes 1 to 5 (mm)
Mint 68–7	37.5	April 15				
	37.5	May 29	29,085	76.65	0.26	14.33
	150.0	June 10				
Mint 68–7*	37.5	March 22				
	112.5	April 20	27,862	111.45	0.40	11.41
	75.0	June 10				
Haixiang 1**	225.0	May 9	22,223	42.60	0.19	NT†
	225.0	May 28	22,583	63.45	0.28	NT
	225.0	June 9	23,546	77.70	0.33	NT

*579,000 plants/ha.
**528,000 plants/ha.
†Not tested.

It was reported (Wang et al., 1992) that increasing the amount of fertilizer (urea) from 127.5 to 277.5 kg/ha under a normal population density resulted in a significant decrease in the yield of oil (Table 5.5). Lodging apparently was the major contributor to the decrease in the yield of oil. Therefore, using fertilizers during planting is typically not recommended for the fertile field to avoid overgrowth in the early stages of development. In addition, the application of fertilizers during planting is also considered as a wasteful practice because it takes about 3 months for the plants to emerge from the soil in most of the production areas in China and most of the fertilizers usually leach out before they can be absorbed by the plants. For the fields with poor soil and deficient in nitrogen (N), potassium (K), and phosphate (P), an organic fertilizer is typically used during planting.

The amount, type, and time of fertilizer application depend on the growth of mint plants in the fields. At the early stages of development, they are small and grow slowly. The nutrients in the soil or from the organic fertilizers applied are sufficient to support mint plant growth. From the middle of May to the end of June, plants grow very rapidly in Jiangsu Province. Chemical fertilizers, including N, P, K, and micronutrients combined with organic fertilizers,

TABLE 5.5
The Effect of the Amount of Fertilizer on the Yield of Cornmint* Biomass and Oil

Fertilizer Urea** (kg/ha)	Mint Biomass (kg/ha)	Oil (kg/ha)	Oil Content (%)	Lodging
225	23,411	65.55	0.28	+++
150	25,572	79.35	0.31	++
75	26.334	118.65	0.45	Normal

+++ A lot of lodging.
++ Moderate amount of lodging.
*Cultivar Mint 68–7 at 465,000 plants/ha.
**Applied on May 28, in addition, 52.5 kg/ha urea applied on June 10.

are usually applied to promote healthy growth and branching of the mint plants. Urea at the rate of 105 to 120 kg/ha is usually recommended for this stage (Zhou, 1999). At the later stages of development, small amounts of chemical fertilizers are usually applied about 30 days before harvest to sustain the growth. Excessive amounts of chemical fertilizer or fertilizer applied too early will result in overgrowth, thereby affecting the yield of oil.

5.16 FIELD MANAGEMENT FOR SECOND HARVEST

The second crop of cornmint needs about 1600°C to 2000°C of accumulative temperature from emergence to flowering within 80 to 90 days during which time the average daily temperature, in general, decreases especially after the plants mature, in most of the production areas in China. Since the precipitation is usually limited and the daily temperature is high when the plants emerge from the soil, field management becomes especially important to ensure an adequate number of strong and healthy plants per hectare.

5.16.1 Removal of Above-Ground Stems to Promote Plant Emergence

After the first crop is harvested (usually around the end of July), the stems above the ground should be cut off as soon as possible. Any delay in removing the residuals of the stem will result in weak shoot development and thereby affect the yield of biomass and oil. New cornmint plants are usually produced from the rhizomes under the ground after the stems above the ground are removed. Since these plants have strong shoots and root systems, they are resistant to multiple stresses and grow rapidly in the field to establish a productive population for high biomass and oil yield. In most areas, removal of stems and stolons is accomplished simultaneously at the time of harvest. In addition to mechanical removal of the stem residuals, some herbicide is also used to kill both the stem tissues above the ground and the weeds after cornmint has been harvested (Fueng, 2000).

5.16.2 Covering the Field to Maintain Moisture

In the areas where precipitation is limited after the first crop is harvested, spreading a thin layer of soil from the drainage ditches after harvest in the field is usually carried out to maintain the moisture. This practice not only ensures that enough plants will emerge from the soil to form an optimum population, but it will also enhance the vigor of the shoot and root systems of the plants.

5.16.3 Controlling Weeds Strengthens Cornmint Plants

The period from the harvest of the first cornmint crop to the establishment of the plants of the second crop is also the season in which a number of weeds grow rapidly in the field. Therefore, it is critical to kill the weeds before they affect the emergence and growth of the plants. Depending upon the species of the weeds in the field, different types of herbicides can be applied after the first crop is harvested or at the 3-leaf stage (Hu and Shao, 1990; Huang, 1992; Wang et al., 1992).

5.16.4 Fertilizing at Appropriate Developmental Stages

Fertilization is more important to the second crop than the first crop, especially for the fields with poor soil or those lacking organic fertilizers. It was reported that the early application of fertilizers for maturation resulted in higher yield of oil for the same cultivar under the same amount of fertilizers (Table 5.6, Wang et al., 1992).

TABLE 5.6
The Effect of Fertilizer Application on the Yield of Cornmint* Biomass and Oil for the Second Crop

Amount of Fertilizer	Application Date	Mint Biomass (kg/ha)	Oil (kg/ha)
30 kg/ha urea	September 3	26,250	15.75
45 kg/ha urea	September 30		
75 kg/ha urea	September 3	15,000	45.0

*Cultivar: Mint 73–8 with 1200 kg/ha decomposed cotton seed meal plus 150 kg/ha urea.

5.16.5 Harvesting before the First Frost

The yield of cornmint oil is correlated well with the temperature at which it is harvested. In general, the plants reach maturity from mid- to the end of September in most production areas except for Guangdong, Guangxi, Hainan, Fujian, and Northeast of China. Therefore, the second crop should be harvested as soon as it reaches maturity. The effects of the date of harvest on the yield of biomass and oil are shown in Table 5.7 (Wang et al., 1992). Harvest after a frost will result in a dramatic loss in the yield of cornmint oil although the yield of biomass is usually not affected significantly.

5.17 CULTIVAR PURIFICATION AND REJUVENATION

After cultivation and vegetative proliferation for a number of years, the purity and vigor of most cultivars decline significantly, especially in the fields where the seeds from self-pollinated plants fall into the soil. Therefore, the yield and quality of the oil from the same cultivar will decrease dramatically without purification and rejuvenation (Zhao, 1990).

A field with fertile soil supplemented with about 30,000 kg/ha manure and 750 to 1200 kg/ha decomposed cotton seed meal is usually used to purify and rejuvenate a cultivar. In some areas, inorganic fertilizers of potassium, phosphate, and calcium are also applied to fields that are deficient in these elements. Sections of stolons, stems, or rhizomes from vigorous plants of the cultivar treated with 80 ppm methyl α-naphthyl acetate are chosen to be planted vertically into the soil with two to three internodes above the ground. The optimum population for purification and rejuvenation is about 420,000 plants per hectare.

TABLE 5.7
The Effects of the Date of Harvest on the Yield of Cornmint Biomass and Oil

First Harvest Date	Second Harvest Date	Temperature (°C)	Biomass (kg/ha)	Oil (kg/ha)
	September 26	19.1	18,690	78.75
	September 30	20.8	18,810	75.75
	October 3	18.1	18,735	67.05
July 16	October 9	24.0	18,765	77.40
	October 22	15.7	18,885	47.10
	November 3	11.3	18,960	31.50
	November 13	3 days frost	18,930	23.25

It has been reported that 1 ha can produce high quality rhizomes for 10 to 15 ha of cornmint production in the next season and at the same time increase the yield of oil by 10% to 15% (Gu, 2000).

5.18 INTERCROPPING CORNMINT WITH OTHER CROPS

Intercropping of cornmint with other crops is a common practice in China to increase the productivity of the land for agriculture (Wang et al., 1992; Le, 1993; Ye and Shi, 1993, Xue, 1999; Li and Lu, 2000).

However, more efforts are needed to prevent the mixing of other crop residues with the cornmint during harvest. Otherwise, the quality of the oil could be affected significantly, depending on the crop that is intercropped with the cornmint or any other mint.

5.18.1 Intercropping with Barley

The intercropping of cornmint with barley is mainly done in the Yanzhuo Region of Jiangsu Province. Both crops are usually planted at the same time. The row width of barley is expanded up to 23 cm; the distance between rows is about 100 cm where mint is planted normally. Table 5.8 shows the effect of intercropping with barley on the yield and quality of cornmint oil (Wang et al., 1992).

The cultivars of barley chosen to intercrop with cornmint should be resistant to lodging and semishort in plant height and mature earlier than monocropped barley cultivars. After the barley is harvested, cultivation is performed to break the barley residues and kill the weeds in the field. The cornmint plants adjacent to barley are topped and fertilized to promote rapid growth so that they can occupy the space left after the barley has been harvested.

5.18.2 Intercropping with Rapeseed

Intercropping of cornmint with rapeseed is widely followed in Jiangsu Province. The distance between the rows of rapeseed is about 130 cm and should not be less than 80 cm from where the cornmint is planted. The distance between the plants of rapeseed is about 25 cm. It is common for cornmint plants that are planted adjacent to rapeseed to die during their early stages of development. Therefore, the planting population of cornmint should be larger than that of a nonintercropped field by about 15%. Rapeseed seedlings are usually raised in mid-September and transplanted immediately after cornmint is planted. The rapeseed seedlings should reach about 20 cm in height with at least six leaves before being transplanted no later than November 20 in most areas in Jiangsu Province. The cultivars of rapeseed chosen to intercrop with cornmint should be resistant to lodging, compact in morphology, and should mature earlier

TABLE 5.8
The Effect of Intercropping Cornmint with Barley on the Yield and Quality of the Oil

Intercropping	Oil (kg/ha)	Alcohol Content (%)
33 cm mint intercropping with 33 cm barley	91.20	82.06
66 cm mint intercropping with 33 cm barley	92.25	82.52
100 cm mint intercropping with 33 cm barley	91.95	82.47
133 cm mint intercropping with 33 cm barley	91.95	83.84
Mint without intercropping (control)	92.25	84.89

than monocropped rapeseed cultivars. After rapeseed is harvested, cornmint plants are topped and fertilized to promote rapid growth. It has been reported that the yield of cornmint oil, rapeseed, and corn can reach up to 180 to 225 kg/ha and 4500 to 7500 kg/ha, respectively, when intercropped with rape and corn (Li and Liu, 2000; Xue, 1999).

5.18.3 Intercropping with Broad Bean

The hectarage of cornmint intercropped with broad bean is the second largest after its intercropping with rapeseed in China. Since broad bean plants are large and shade cornmint plants more than either barley or rapeseed does, the cultural practice is very critical to achieve a high oil yield and good quality of oil. First, the cultivars of broad bean chosen for intercropping with cornmint should be resistant to lodging and drought, compact in morphology, and mature earlier than monocropped broad-bean cultivars. Second, broad bean should be planted ahead of cornmint in mid-October in Jiangsu Province, and the distance between rows of broad bean should be 140 to 160 cm. Third, broad bean plants need to be physically supported and controlled to prevent overgrowing on the top of cornmint plants during their later developmental stages. Finally, the cornmint plants need to be topped and fertilized appropriately after broad bean is harvested.

5.18.4 Intercropping with Vegetables

Intercropping of cornmint with vegetables is a common practice, especially in areas near the cities in China. The vegetables commonly intercropped with cornmint or spearmint include vegetables with a short life cycle such as spinach and crown daisy. Cornmint is usually planted first under conventional cultural practices but the planting populations should be larger than that of a nonintercropped field by 20%. The seeds or seedlings of vegetables are planted later with a lower density than a nonintercropped vegetable field. In some areas, mints are also intercropped with vegetables that have a medium-length life cycle, such as leaf mustard, potherb mustard, and asparagus. These vegetables are usually harvested from the end of March to the middle of April in the Jiangsu Province. Therefore, they generally do not affect the growth of the mint plants. In addition, mints are also intercropped with vegetables that have a long life cycle such as peas. In this case, the distance between the rows of peas is about 150 cm. After peas are harvested around the end of May, the mint plants are topped and fertilized appropriately. Since most of the vegetable fields are covered and fertilized during winter, cornmint plants usually grow well when intercropped with vegetables.

5.18.5 Intercropping with Sesame

In some areas, the second cornmint crop is intercropped with sesame. Because sesame is very sensitive to soil pH, only fields with a sandy loam and/or silt loam should be used for intercropping with sesame. The cultivars of sesame chosen for intercropping with cornmint should have narrow leaves and few branches. Sesame seedlings are raised separately and transplanted to the field after the first crop is harvested. The distance between the rows of sesame is about 140 cm and that between plants is about 20 cm.

5.18.6 Intercropping with Other Crops

In some areas of China, cornmint is intercropped with corn. Since corn is a tall plant with a longer life cycle than other crops described earlier, cornmint plants grow poorly and lose more leaves due to reduced light intensity. Therefore, both the oil yield and the quality of the oil are low when intercropped with corn. In some areas, cornmint is also intercropped with

TABLE 5.9
The Effects of Crop Rotation on the Yield and Quality of Cornmint Oil

			Oil (kg/ha)		Alcohol Content (%)	
Year	Cultivar	Crop Rotation	First Harvest	Second Harvest	First Harvest	Second Harvest
1984		2 years of mint	110.25	47.25	81.7	85.1
	Haixuan	Corn, then mint rotation	118.50	51.75	83.6	86.4
		Cotton, then mint rotation	118.50	54.00	83.6	86.3
	Mint 73–8	2 years of mint	109.50	49.50	76.1	81.8
		Corn, then mint rotation	126.75	62.25	77.3	82.9
		Cotton, then mint rotation	126.75	63.00	77.8	83.1
1985		2 years of mint	89.25	27.75	80.8	84.3
	Hiaxuan	Corn, then mint rotation	95.25	45.75	82.8	86.4
		Cotton, then mint rotation	96.75	44.25	83.1	87.3
		2 years of mint	87.00	36.75	76.4	80.3
	Mint 73–8	Corn, then mint rotation	95.25	48.00	77.9	83.6
		Cotton, then mint rotation	93.00	50.25	78.1	83.8

onion, garlic, or cotton. However, the quality of mint oil is usually affected by either the mixing of onion and garlic plants with the cornmint during harvest or by the pesticides applied to the cotton.

5.19 CROP ROTATION

Irrespective of whether cornmint is planted as a monoculture or intercropped with other plants, rotation is required to control the pests, weeds, and disease and use the nutrients in the field efficiently. The data in Table 5.9 show the effects of crop rotation on the yield and quality of mint oil (Wang et al., 1992).

In the areas with two crops per year, the crops for the first year are mainly wheat, barley, broad bean, or rapeseed for the summer and cotton, rice, corn, jute, soybean, peanut, or sweet potato for the autumn.

The crops for the second year are cornmint for the summer and cornmint or rice for the autumn. In areas with five crops in 2 years, the crops for the first year are (a) barley, broad bean, or rapeseed, (b) corn or rice, and (c) carrot or sweet potato. The crops for the second, year are cornmint for the summer and cornmint or rice for the autumn.

5.20 MINT OIL PRODUCTION

Cornmint distillation is usually performed by the growers themselves using a steam and water system constructed of mild steel (Figure 5.1 and Figure 5.2). Oil produced by the growers is stored in 1-kg, 5-kg, and 10-kg containers (Watts, 1997). The growers have the option of storing or selling their oils depending on supply and demand and the current market process for crude oil. If they decide to sell their oil, they can either sell it to (a) small dealers who collect the oil in 100-kg containers or (b) large dealers who have collection stations. At one time the oil was sold to state-owned co-operatives who functioned like large dealers (Watts, 1997).

FIGURE 5.1 A battery of cornmint distillation units in Anhui Province.

The annual cornmint oil production over the last decade in China is shown in Table 5.10. It can be seen from these data that the amount of oil available for menthol isolation fluctuates, has decreased, and now stabilized. The reason for this decrease is the increased production of oil in India. That Indian oil is offered at a lower price than Chinese oil has directly affected production levels. Watts (1997) estimated that to satisfy the domestic or internal demand for menthol ca. 3000 metric tonnes of crude oil are required. The use of mints, mint oils, and menthol in China has expanded to a variety of products including toothpaste, chewing gum,

FIGURE 5.2 A single distillation unit in Anhui Province showing the condenser tank which houses a spiral tube condenser and receiver.

TABLE 5.10
Crude Cornmint Oil Production in China

Year	Oil Production
1994	4000
1995	4500
1996	3200
1997	4500
1998	4000
1999	3500
2000	4000
2001	3500
2002	3000
2003	3500

candy, synthetic drugs, and cigarettes. Recently mints have also been cultivated as a specialty vegetable crop for use in Chinese salads (Zhao, 2000).

REFERENCES

Dai, L. (1981). A preliminary study on *Mentha* species cultivated in China, *Acta. Pharm. Sinica*, 6, 849–859.

Fueng, W. (2000). Use of herbicide "Kewuzhong" to remove mint stem residues in the field after harvest, *J. Sci. Technol. Jiangsu Agric*, 1436.

Gu, S. (2000). The techniques for purification and rejuvenation of mint cultivar, *J. Sci. Technol. Jiangsu Agric*., 1430.

Guo, Y. and Chan, Y. (1993). Introduction and cultivation of new cultivar Mint 80-A-53, *Agric Sci. Technol. Anhui*, 21, 356–360.

Hu, M. and Shao, J. (1990). The test of herbicide "Tian Ben Da Song" application to control weeds in mint field, *Weed Sci.*, 3, 24–25.

Huang, T. (1992). Progresses in herbicide application in mint production, *Flav. Aroma Cosmet. Prods*., 4, 36–39.

Jian, G. and Zhou, R. (1993). The morphology of pollens from 7 species of *Mentha* genus, *J. Chinese Med. Sci. Univ*., 24, 150–156.

Jian, G., Zhou, Z., and Zhou, R. (1995). Chemical compositions of mint oil produced in Northern East of China, *Plant Resour. Environ*., 4, 60–62.

Lawrence, B.M. (1995). This isolation of aromatic materials from natural plant products. In: DeSilva, K.T., Ed., *A Manual on the essential oil industry*, UNIDO, Vienna, pp. 57–154.

Le, Z. (1993). Cultivation techniques of high efficient intercropping in mint production, *Commun. Agric. Sci. Technol*, 9, 11–12.

Le, C., Suen. X., and Lu, S. (1996). Analysis of the economical effects of potassium on mint production, *Shanghai Agric. Sci. Technol*., 2, 36–37.

Li, D. and Liu, L. (2000). Mint intercrops with rapeseed and corn, *J. Sci. Technol. Jiangsu Agric.*, 1463.

Liu, S. (1997). Study on the aroma compounds in the oil of peppermint and Asia mint, *J. Guailin Med. School*, 2, 300–302.

Liu, S. (2001). The effects of cultivation conditions on the quality of *Mentha piperita* L. oil, *Nat. Prod. Res. Dev*., 13, 55–57.

Manheimer, S.R. (1980). China: important raw material producer? *Perfum. Flav.*, 4, 19–22.

Ren, Y. (1995). Cultivation techniques for high yield in peppermint production, *Agric. Technol. Hinjiang*, 2, 21–22.

Sun, C. (1997). Technical aspects of fertilization for mints, *Anhuei Agric.*, 1, 23.
Wang, J. (1996). Cultivation techniques in mints, *Appl. Technol. Commun. Agric.*, 10, 26–27.
Wang, G. and Yang, C. (1997). The culture practices for high yield in mint production, *Nanyang Agric. Technol.*, 2, 8.
Wang, D., Zhao, G., and Zhang, Z. (1992). *Mint production and trade in China*, Scientific Technology Publisher, Jiangsu, China.
Watts, S. (1997). Menthol and cornmint from China, *Perfum. Flavor.*, 22 (1), 1–6.
Xu, et al. (1990). Cited in Wang et al. (1992).
Xue, Z. (1999). Mint intercropping with rapeseeds and corn, *J. Sci. Technol. Jiangsu Agric.,* 1349.
Yang, H. and Guo, S. (1993). A report on the application of potassium to mint in the field, *Agric. Sci. Technol. Jiangsu*, 3, 54–55.
Yang, R., Dai, K., and Shi, D. (1991). Morphology of 4 different species of *Mentha* genus, *J. Shanghai Med. Sci. Univ.*, 18, 223–227.
Ye, D. and Shi, J. (1993). Technical aspects in mint intercropping with rapeseeds and ration with rice, *Agric. Sci. Technol. Shanghai*, 5, 11.
Zhao, G. (1990). Variety mixture, productivity decline and their prevention in mint production, *Commun. Agric. Sci. Technol.*, 7, 13.
Zhao, D. (2000). Mint—a green vegetable crop, *Guiezhou Agric. Sci.*, 28, 5.
Zhao, Q.-H. (1991). Nantong—China's peppermint producing center, *Perfum. Flavor.*, 16, 37.
Zhang, R., Xie, Y., and Su, Z. (1995). A new species of peppermint and the composition of its oil, *Plant Res.*, 15, 213–214.
Zhou, Z. (1999). Field management at branching stages of mint development, *J. Sci. Technol. Jiangsu Agric.*, 1321.
Zhou, Z., Zhu, J., and Chen. Y. (1993). The characteristics of mint cultivar 80-A-53 and the techniques for its cultivation, *Agric. Sci. Technol. Shanghai*, 6, 37–39.
Zhu, Y. (1997). Cultivation techniques for high yield in mint production, *Anhui Agric.*, 1, 10.
Zhu, P., Wang, Z., and Huang, D. (2000). The effects of phosphate and potassium on the yield of mint biomass, *J. Jiangsu Agric. Sci.*, 1, 48–50.
Zuo, Q., Yi, J., and Hou, Z. (1995). A report on the performance of the United States peppermint during winter in the cold and semi-drought areas of China, *Agric. Technol. Inner-Mongolia*, 5, 14–16.

6 The Distillation of Mint Oils: History, Current Theory, and Practice

E.F.K. Denny and Brian M. Lawrence

CONTENTS

6.1 INTRODUCTION

Distillation with steam can recover volatile oils from several species in the *Mentha* genus. Because these oils are the products of distillation, they are by definition essences and are called "essential oils." The four species indicated here are those that yield the oils of most interest to commerce. Although the oils are different, the plants are rather similar:

Mentha piperita: peppermint: About 75% of the oil is a mixture of menthol and menthone in proportions of about 2:1 or 3:1, respectively.
Mentha canadensis: cornmint, or Japanese mint: This oil is the best source of natural menthol (ca. 70%).
Mentha spicata and *Mentha gracilis*: Native and Scotch spearmint: The oils comprise ca. 66% carvone.
Mentha pulegium: The European pennyroyal: The oil consists of up to 90% pulegone.

The characteristics of the principal components of these oils, which are important for distillation, fall within very narrow ranges. Under normal atmospheric pressure, they all boil with water at temperatures between 99.4°C and 99.6°C, and their calculated latent

heats of vaporization are all between 85.3 and 88.0 cal/g. They are classified as "superficial" oils because they are entirely secreted on the surface of the leaves. The tiny blister-like oil glands (trichomes) are easily seen with a low-power magnifier. The leaves themselves are also fairly similar. Cornmint and some strains of the pennyroyal possess hairs, but it is doubtful if this is enough to give them naturally absorptive surfaces. The leaves of each of the *Mentha* species should be treated as relatively glabrous when fresh, but they can be given slightly absorptive surfaces for distillation by prior wilting in the sun. Any significant amount of pubescence on the leaf surfaces would only reduce the amount of wilting required.

Mint distillation proceeds in the manner that is typical of plants that yield moderately volatile superficial oils from slightly absorptive herb surfaces. Within that generic group, differences among the herb surfaces or the oils themselves will, in theory, vary the course of distillation. However, as we have just seen, the differences these four mint herbs have are far too small to affect their recovery in any way that could be detected in the field. Therefore, it follows that if the factors that govern the distillation of any one of these oils can be mastered, then an adequate understanding for the other oils will be obtained.

The distillation process is very ancient and was in widespread use long before there was any knowledge of the natural laws that govern either the stills or the ancillary equipment. Over the years, especially in the United States, great practical improvements have been made on the relatively primitive methods inherited from Europe. These improvements may not have been the ultimate possible, but they worked much better than inherited primitive distillation processes.

Most of these advances were achieved by intelligent operators in the field during the time that their scientific advisors still believed any exposed mint oil must automatically vaporize to saturate the steam in the still. Some of the effects of this and other mistaken beliefs are still with us. However, it is now possible to point out the rules of physical chemistry to which these distillations must conform.

The basic distilling operation is deceptively simple. A charge of oil-bearing herb at ambient day temperature is loaded into a large vat, which we call the "still." Where appropriate, the plant material is tramped down firmly and evenly, with particular attention to ensuring that it is packed tightly against the still wall. Using steam to soften the plant material so that more can be pressed into each charge is not always advantageous. The yield of oil per metric tonne of herbage is usually reduced, and each charge spends so much longer in the still that the daily production rate declines. Where the mint is chopped to about 5 cm lengths and blown into a trailer (tub) still that is a little wider at the top than at the bottom, the herbage tends to settle and pack automatically during travel and when it shrinks under the influence of steam.

When the still is filled, it is closed with a steam-tight lid that often carries the outlet pipe. A flow of steam is introduced at the bottom of the still so that it percolates upward through the charge. Starting at the bottom, the steam condenses on the herb surfaces, and, surrendering its latent heat, raises successive layers to boiling point. When the boiling temperature reaches the top of the still, the moving steam will cause any oil that is exposed on the herb surface to start to vaporize. The oncoming steam will then drive a mixture of oil and water vapors off the top of the charge, and from there it is led through a condenser. When they are reduced back to the liquid state, the oil and water are immiscible and separate spontaneously according to their densities.

Our key phrase is "steam will cause oils to vaporize," because only oil that is actively vaporized inside the still will be recovered from the herb. Therefore, distillers need to understand the factors that promote this vaporization as well as those that hinder it.

The United States is the largest producer of peppermint and spearmint oil, while India and to a lesser extent China are the largest producers of cornmint oil. Most of the farms in the United States are large, with mint fields extensive enough for mechanization to exploit the economics of scale and offset the high cost of labor.

6.2 A HISTORICAL AND CURRENT PERSPECTIVE

Pennyroyal oil, which is produced in Spain and Morocco, is not a cultivated plant. It is collected from its natural habitat and is distilled in a direct-fired crude field still, as it has always been for the last 75 years. The type of field still that is used today can be seen in Figure 6.1. All other commercial mint oils are distilled from cultivated crops. Before discussing the theories and practices of modern-day field distillation of the other mints, it is of interest to review the progress of distillation technology that has taken place in the United States over the past 150 years. In 1850, Van Slyck described that mint distillation was performed using a fixed direct-fired copper kettle (still), in which the herbage was water-distilled (hydrodistilled) and condensed using a drip-type metal worm condenser (Van Slyck, 1850). Todd (1886, 1904) described the replacement of the copper kettle with round wooden vats made from pine or whitewood staves held together with hooped iron bands like a wooden barrel. In addition, the hydrodistillation process used previously was replaced with steam distillation using a fuel-fired satellite boiler. Landing (1969) noted that in Michigan in the 1890s, the round condenser tank was gradually replaced by a rectangular condenser tank within which the condenser pipe was housed. Over the next 30 years, numerous changes were made to the distillery equipment, such as the replacement of wooden vats with galvanized steel, the use of pulley systems to discharge the stills, multitubular condensers submerged in a tank of water either horizontally (mostly) or occasionally vertically, and the improvement of steam-generating boilers (Landing, 1969).

It is interesting to note that during the alcohol prohibition era of the United States (1920 to 1933), all mint stills required both state and federal permits and were regularly inspected by the Internal Revenue Service.

Trailer-mounted portable metal tanks that could be inserted into the distilling tubs, introduced in the 1920s and 1930s, were the forerunners of the fully portable trailer-mounted distilling tubs that came into use in the 1940s (Landing, 1969). This concept allowed the farmer to take the distilling tub into the field and fill it with the dried mint herbage. Children of the farmer were usually employed to climb into the distillation tub and pack down the

FIGURE 6.1 Typical direct-fired field still used for production of pennyroyal oil in Spain.

herbage by jumping up and down on it to tramp it down to prevent the formation of channels that would allow steam to escape during distillation.

The early distillation tubs were round. In fact, some of the round tubs were still in use in the early 1960s (Green, 1963). Most tubs, which were made of galvanized steel, varied in size from 6 to 9 ft (1.83 to 2.74 m) with a 6 to 7 ft (1.83 to 2.13 m) diameter (Sievers, 1952). The rims of some tubs were flattened so that a flat top could be clamped onto the tub by concentric clamps (C-clamps), under which a flat strip of composite material was fixed to the flattened rim to act as a gasket to prevent any steam escape when the top was clamped shut. Other tubs had a trough about 5 in. (12.7 cm) wide and 10 in. (25.4 cm) deep mounted on the rim, and the still top or cover had a turned-down edge that fitted the trough. Once the trough was filled with water, the top did not need to be clamped as the water seal was sufficient to prevent steam loss. The outlet for the steam and oil vapors was through a pipe that was mounted onto the side of the tub just below the top. The outlet diameter was several times greater than the steam inlet pipe to prevent pressure buildup in the tub. This outlet tube was connected to the condenser; however, most connections used the same water seal system as that used in the tub covers. Steam was introduced into the charged tub from a satellite boiler via a 1.5-in. (3-cm) pipe that was connected just above the base of the tub. The condenser systems used were varied including the old drip-type worm condensers in which the condenser was not inside a tank but was exposed, and water was dripped onto it from a trough above. Other condensers that were mounted inside a tank of water were horizontal tubes connected with hairpin bends to fit within the tank, coiled tube condensers, and even tubular condensers, although these latter condensers were not as popular because they were too expensive for most distillers. The mint oil receiving or separating cans were based on the design by Hughes (1952) who pioneered a lot of the practice of mint oil distillation. By the 1960s, the complete mint distillation as designed and tested by Hughes was manufactured for sale by the Cobb manufacturing company (Jefferson, Oregon), a company that is no longer in business.

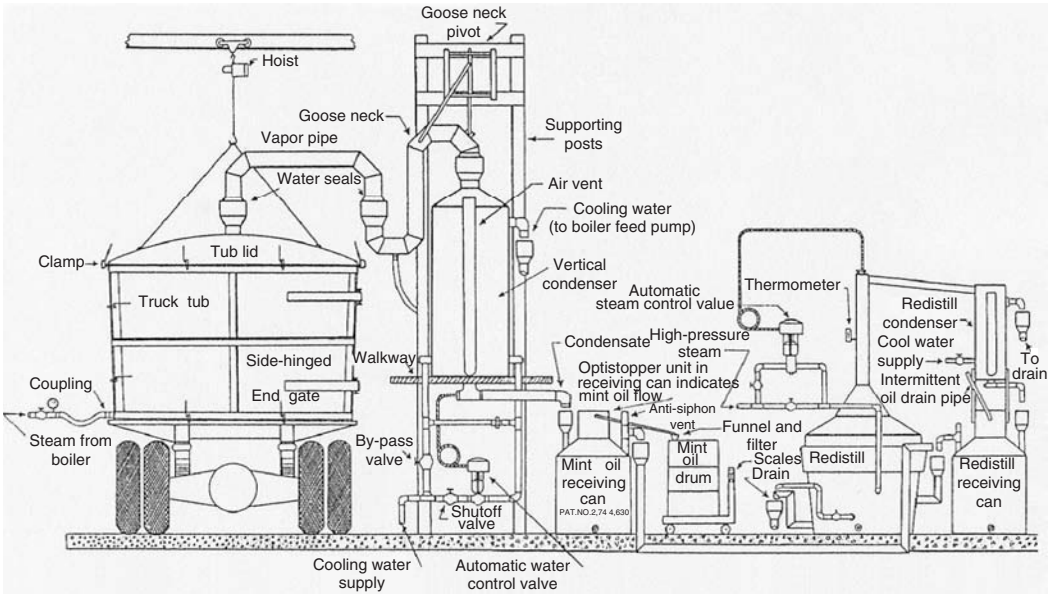


FIGURE 6.2 A schematic representation of the complete distillation system including a redistillery as manufactured by Cobb Manufacturing, Inc.

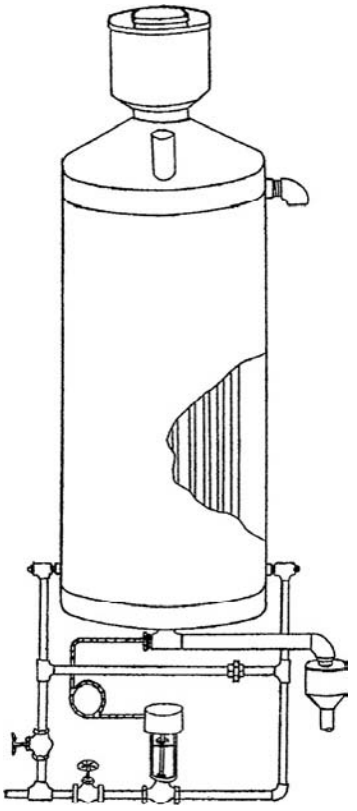


FIGURE 6.3 Vertical condenser of $5\frac{1}{2}$ ft (1.68 m) height and 28 in. (71 cm) diameter fitted with a large built-in vent to prevent pressure buildup, designed for use with an automatic water control valve and mint oil separator (receiving can) used in the 1960s.

A flow diagram of the equipment, including a redistillation system to isolate any oil that had dissolved in the condensate water, can be seen in Figure 6.2. Also, the vertical condenser and the receiving can that were used at that time can be seen in Figure 6.3. These tubs were generally permanent fixtures on a trailer (as shown in Figure 6.2) that are either pulled by tractor or other farm vehicles or they are mounted on a flatbed truck.

Over the past 50 years, the portable tubs (trailer stills) were fitted with an array of sparge tubes containing evenly distributed holes. These sparge tubes were arranged in parallel to the long dimension of the tub along the floor (bottom) of the tub connected at right angles to a common manifold header pipe across the front end of the floor. This header pipe was connected to a steam inlet fitted with a pressure gauge that juts out generally from the outside of the back of the tub. In the 1950s and 1960s, most tubs used were open at the top. The mint was and still is harvested using a reel or swather harvester, which lays down a windrow. After the windrow of mint herbage was left to wilt and dry in the field with a reduction of moisture by a minimum of 75%, it was and still is mechanically picked up by a silage chopper and transferred directly into the tub or trailer stills of 2 to 8 metric tonnes capacity. More recently, tubs have been designed with permanent tops so that the chopped mint herbage is blown from the silage chopper through a throat directly in it. The tubs with tops have been designed such that they are slightly wider at the top than the bottom so that the herbage will pack down when it is transported across the field as it receives more herbage. The tubs are transported either on a trailer or on a flatbed truck at the distillery. Once the

tub is taken to the distillery, the throat door is clamped shut and the outlet pipe is connected to the condenser by a flexible pipe, the steam inlet from the satellite boiler is connected and the steam is turned on. This allows distillation to proceed without any further handling of the herbage. The two popular condensers used are (a) a continuous series of pipes of decreasing diameter with hairpin bends in a horizontal tank or (b) a vertical tank containing a spiral condensing tube of fixed diameter (Lacy et al., 1981). Once the first drops of condensate appear in the receiver, the steam pressure is reduced depending upon the size of the distillation charge and the wetness or dryness of the chopped herbage. Lacy et al. (1981) recommended that for optimum oil production, the condenser water should be maintained between 33°C and 36°C for spearmint (both Native and Scotch) and between 42°C and 46°C for peppermint.

Examples of the style of tubs (trailer stills) that have been used in the United States over the years can be seen in Figure 6.4. One entrepreneur rents flatbed trucks on which open top rectangular tubs are welded to them for the distillation season. Once the season is over, he cuts off the weld and returns the trucks, thereby saving the costs of ownership of reliable flatbed trucks. Another entrepreneur has built his distillery very close to a large power plant so that he can purchase bleed steam at a rate lower than that for producing it from boilers. The largest distilleries in the United States can be found in the Columbia River basin (Oregon) and near Yakima (Washington). At these two distilleries, there are 28 bays for mint distillation. The most modern distillery can be found in Alberta (Canada). This distillery is under complete computer control with load cells and sensors on all equipment so that the economics of each distillation can be controlled. As a result, if the production of oil costs more than the value of the oil that is produced, the distillation of a specific tub automatically shuts down.

In many areas in the United States, the distillate water cannot be returned either to a river or as ground water unless the soluble volatiles have been removed from it. This is achieved by redistilling the distillate water prior to discarding it. Examples of two redistillation systems can be seen in Figure 6.5. Finally, the manufacturers of distillation equipment have developed a mobile distillery complete with boiler, condenser, receiving can, and connectors to the tub—all on a flatbed truck trailer as can be seen in Figure 6.6.

The harvest of cornmint in India and China is done by hand using a serrated edge sickle. Once harvested the herbage is left in the field to air-dry in the sun, after which it is collected and transported to the fixed field still in India by a variety of modes of transport including a tractor pulling a trailer, bullock cart, rickshaw, human-pulled trailer, or even balanced on a bicycle. The herbage is further air-dried with regular turning near the still before distillation. The stills used in India are basically of three types: (a) a field still, (b) a modified field still, or (c) a commercial still. Descriptions of these three stills can be seen in Chapter 4. The Central Institute of Medicinal and Aromatic Plants (CIMAP) has designed a new field still, a portable version of which they are taking to the rural areas to educate the distiller/growers on a more efficient process (Figure 6.7). As yet, no data has been reported on this distillation system although it is fitted with a vertical tube condenser. The receiver or collector (oil separator) generally used with the field still is a modified milk can as can be seen in Figure 6.8. This is highly inefficient as there is not enough cross-sectional area to slow down the distillate flow and give the small oil particles time to separate. In India, CIMAP has adopted an oil separator that is fitted with a funnel-shaped inlet pipe, which is J-shaped, inside the separator to direct the inlet water oil distillate upward, and a baffle plate welded to the base of the separator to isolate the water outlet from the inlet water/oil mixture (Figure 6.9) (Kahol et al., 1999). Although this upward pointing "J" at the end of the inlet pipe appears instinctively to be efficient, it is counterproductive because the effect of turbulence has not been taken into consideration.

In most areas, the capital cost for a satellite boiler is beyond the reach of most small growers or distillers. A steam generation system has been designed to overcome this problem

FIGURE 6.4 Examples of the tub (trailer still) designs that have been used in the United States over the years.

continued

(Nijjar, 1991, 1993). This system, which is known as ''Clandria'' or ''Calandria,'' is a rectangular steam generator specifically designed to fit under the cylindrical field stills. With this design, Nijjar has greatly increased the heat exchange area by substituting very deep corrugations for the earlier flat plate bottom of the cylinder and arranging for the flue gases to make a

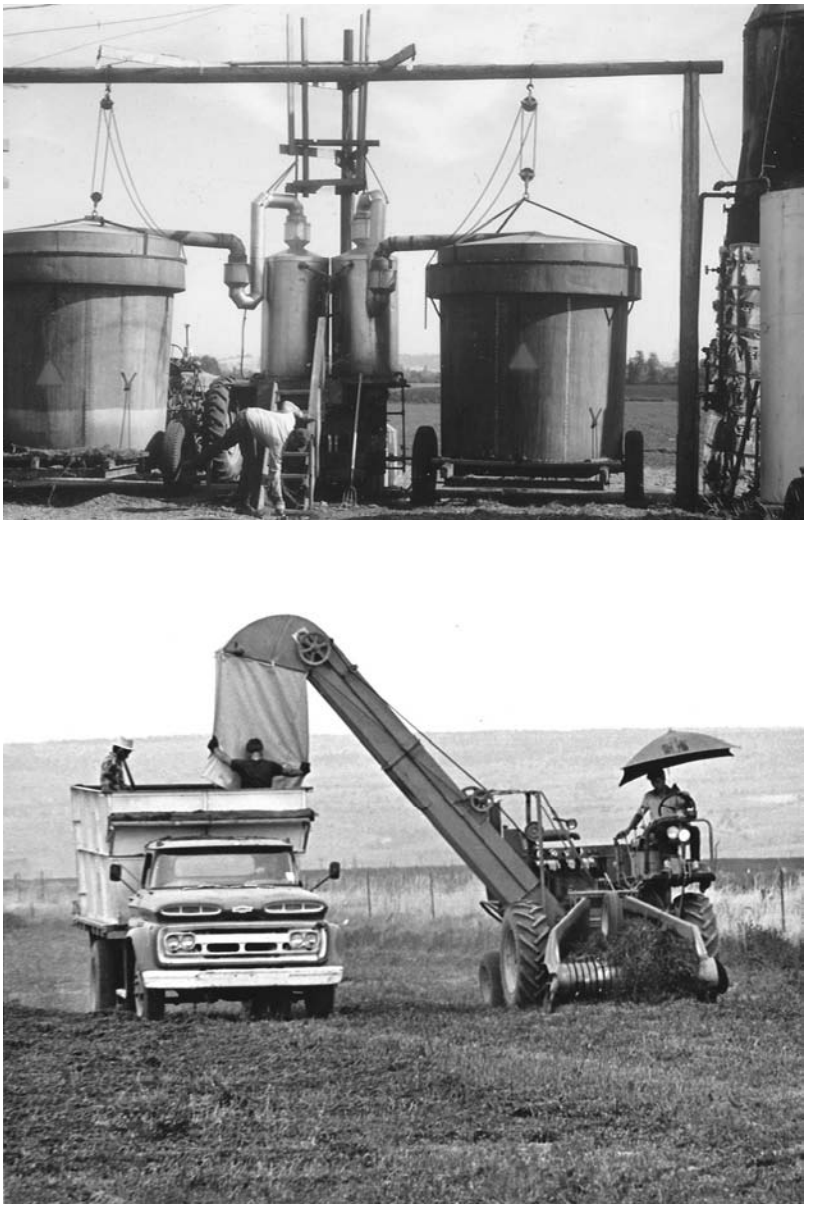


FIGURE 6.4 (continued)

triple pass in contact with the heat exchange area. This might be further developed if the corrugations were accompanied or even replaced by "Babcock" tubes. These are just pipes with a long arm and a short arm with respect to their lowest point and they induce rapid circulation of the water in the kettle. This would take advantage of the fact that the rate of heat exchange depends on a function of the speed with which the water and hot gases pass each other.

Field distillation in China is even more primitive than that used in India even though it is a fixed direct-fired cylindrical system. Because labor is cheap in China as it is in India, all mint

FIGURE 6.4 (continued)

harvesting is also done by hand and the cut herbage is transferred to the still on a cart. The dried herbage is added to the still by hand and tramped down before the goosenecked top is put on. The exit pipe from the gooseneck is connected to the unusual condenser, which is shown in Figure 6.8. The condenser is immersed in a tank of water with the exit pipe protruding through the side of the tank. The tank is sealed where the exit tube protrudes to prevent water loss from the tank. The condenser system is unique because the water or oil

FIGURE 6.4 (**continued**)

vapors run into the mouth of the condenser that is piped into the body of the condenser, which is itself surrounded by water (Figure 6.10). The water or oil condensate is then separated using the receiver collector as shown in Figure 6.10. Although the still is very primitive, the fact that the load of herbage in the still is less than 200 kg (stills are ca. one-third the size of Indian field stills) means that the distillation time is relatively short when compared with that in India. Nevertheless, distillation is very slow and oil recovery is incomplete in both China and India. In India, it takes about 8 h to distill 500 to 700 kg of herbage to get 14 to 18 kg of cornmint oil.

FIGURE 6.5 Two examples of distillate water redistillation systems. The one on the left designed by Cobb manufacturing and the one on the right by Newhouse Manufacturing, Inc.

FIGURE 6.6 Example of mobile distillery designed by Newhouse Manufacturing, Inc.

6.3 THE THEORY OF STEAM DISTILLATION

6.3.1 The Properties of Vapors

All vapors contain more energy than their parent liquids. To turn a liquid into a vapor, energy must be applied to it in the form of heat. The actual amount of heat required to vaporize a unit mass of liquid, without raising the temperature of the vapor above that of the liquid, is a precise characteristic of each individual compound and is called its "latent heat of vaporization." If a vapor is condensed back to liquid, the energy of the molecules is reduced and the characteristic quantity of latent heat is given out.

FIGURE 6.7 Newly designed field still by CIMAP.

Every liquid emits moving vapor molecules from its surface. If the vapor space were closed, the moving molecules would impinge on the enclosing walls and exert a pressure on them, which increases with rising temperature. At each temperature, the magnitude of this so-called vapor pressure, which is exerted by a dry "saturated" vapor that is still in contact with its liquid, is another precise characteristic of the particular compound. When continuous heat is applied to a liquid, its temperature will rise only until its vapor pressure becomes equal to the surrounding pressure, whatever that may be. Further heat will merely create more vapor. The liquid is then said to "boil" and the temperature at which this occurs is its "boiling point" under the prevailing pressure. If two immiscible liquids contribute molecules to the same vapor space, the mixture will boil when the temperature reaches the point at which the sum of the two individual vapor pressures becomes equal to the ambient pressure.

Mint stills work under virtual atmospheric pressure, the average of which at sea level is taken as equal to that at the foot of a column of mercury 760 mm tall. This is written as 760 mm Hg or 1-atm abs, in which "abs" stands for absolute. At about 99.6°C, water (steam) exerts 749 mm Hg of vapor pressure and menthol exerts 11 mm Hg. Therefore, this mixture exerts a vapor pressure of $11 + 749 = 760$ mm Hg at a temperature close to 99.6°C and will boil away at that temperature under normal atmospheric pressure. On its own, menthol boils at 212°C and tends to decompose. But in the presence of steam, menthol, and all other essential oil components, can be boiled away from the herb's surface at temperatures that are slightly below the boiling point of pure water.

Steam distillation for recovering herbaceous oils depends on bringing oil and water into contact at a temperature very close to the boiling point of water. Then the addition of a small amount of vapor pressure from the oil will make the total pressure of the mixture equal to the surrounding pressure. The oil and water mixture must then boil away from the surface of the herb at whatever rate the latent heat to support this vaporization can be applied to it. The way heat is applied to vaporize the oil needs special study. Understanding this factor makes the difference between an efficient operation and a bad one.

FIGURE 6.8 The converted milk can receiver (oil separator) used in India.

6.3.2 The Transference of Heat

At the start of the process, when steam first contacts the oil glands of the mint, they burst and form numerous patches of homogeneous oil on the herb surface. At the perimeter of each virtually circular oil patch, there is an oil–water *interface* in which oil is in contact with water from the steam that condensed on the herb to raise its temperature. At the point of oil evaporation, where it is in contact with water on the leaf, its vapor is saturated and exerting

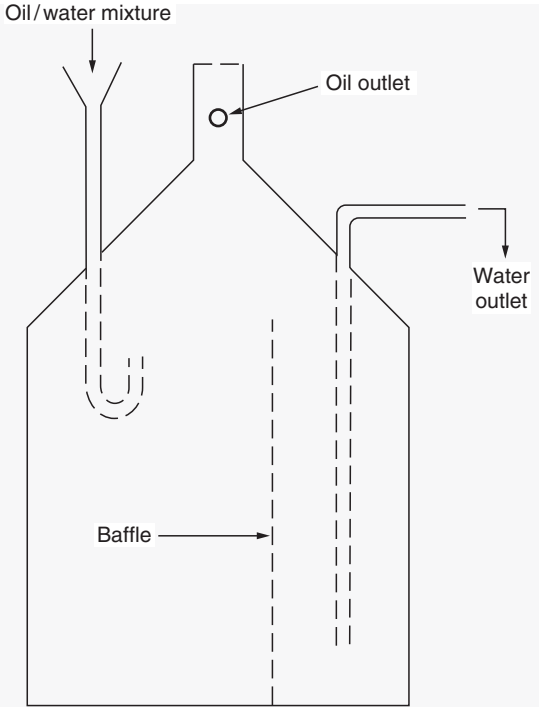


FIGURE 6.9 Modified oil separator designed by CIMAP.

its maximum vapor pressure. Then, the pressure and temperature required of the steam component are the lowest, which will give a boiling mixture of the two liquids under the prevailing pressure. When the oil vapor is subsequently dispersed through the steam in the general vapor space, it is diluted and exerts a smaller proportion of the pressure in the still. The steam must then remain at whatever higher temperature that enables it to exert the remainder of the ambient pressure.

This vapor pressure distribution in each microsystem ensures that the steam in the general vapor space will always be at a slightly higher temperature than that at which oil and water boil together on the surface of the herb. This creates a temperature gradient without which the process could not work. It is the only factor that leads steam to condense and surrender its latent heat into water surfaces that are in close contact with oil at the interface, and the temperature difference transfers this heat from the water to the oil. This is the only source of latent heat for the oil. Therefore, it can be vaporized only from these surface patch perimeters, because steam cannot condense into the homogeneous oil surfaces with which it is immiscible.

But water is a very poor conductor of heat and the distance across which the vaporizing oil can attract its latent heat is very short indeed. If the interface were just a thin circular line of contact round the oil patch, the area into which steam could usefully condense would be insignificant and very little oil would vaporize.

However, if the herb surface is even slightly absorptive, oil and water will intermingle at the interface by capillary action (Figure 6.11). This vastly enlarges the target area into which steam can usefully condense and ensures that the maximum amount of steam will be continually giving up heat to vaporize the oil. Then the surface patch shrinks rapidly as the oil is boiled away from its circumference, and the time taken by a given flow of steam to reduce the radii of the surface oil patches to nil is the "isolation time" for the oil.

FIGURE 6.10 Unusual condenser system and commonly used receiving can (oil separator) used in cornmint oil distillation in China.

FIGURE 6.10 (continued)

Of course, the water on the herb surface must be the right amount to promote the heterogeneous mixture at the interface. The original water that condensed to heat the charge does not follow up the receding perimeters of oil, and the water that condenses to vaporize the oil, replaces less than one-fifth of the fluid mass that it removes. Water from another source must be provided to bridge the widening gap between oil and water on the surface of the herb. Otherwise, the vital mixing of the liquids at the interface will be much reduced and the proportion of oil in the distillate will suffer a very serious and premature decline. On the other hand, since this intermingling of oil and water depends on capillary action, it is critical to ensure that there is never enough water present to flood the herb surfaces and overwhelm their absorptive capacity. This is even more injurious to the proportion of oil in the distillate. It also reduces the amount of oil that will be recovered.

Fresh unwilted mint cannot be steam distilled for its oil. All leaf surfaces become flooded by moisture from the plant cells that collapse in the presence of steam. There is no absorptive capacity left to make oil and water intermingle at the perimeters of the surface oil patches. There is relatively little transference of heat, and very little oil is vaporized. At the same time, excess moisture drips from one herb layer to the next carrying oil to the bottom of the still. This is why the cut herb is left wilting in the sun to lose cell moisture and create that slightly absorptive surface on which all transference of heat from the steam to the oil depends. This drying should be carried to the point at which the leaves are just short of turning brittle and liable to shatter during further handling. Ideally, the moisture content should be reduced to about 25% of the herb's remaining weight.

The happy medium between too much and too little water on the herb surfaces does not normally occur in nature, so the distiller must create it by a suitable preparation of the herb and adjusting the moisture content of the steam.

6.3.3 Matching the Steam to the Herb

Steam from ordinary commercial boilers consists of about 97% of dry saturated vapor and about 3% by weight of liquid water particles in the form of cloud. Some of these cloud particles can lodge on herb surfaces inside the still and help to bridge the widening gap between oil and water at the interface. But if the steam is generated by a kettle in the

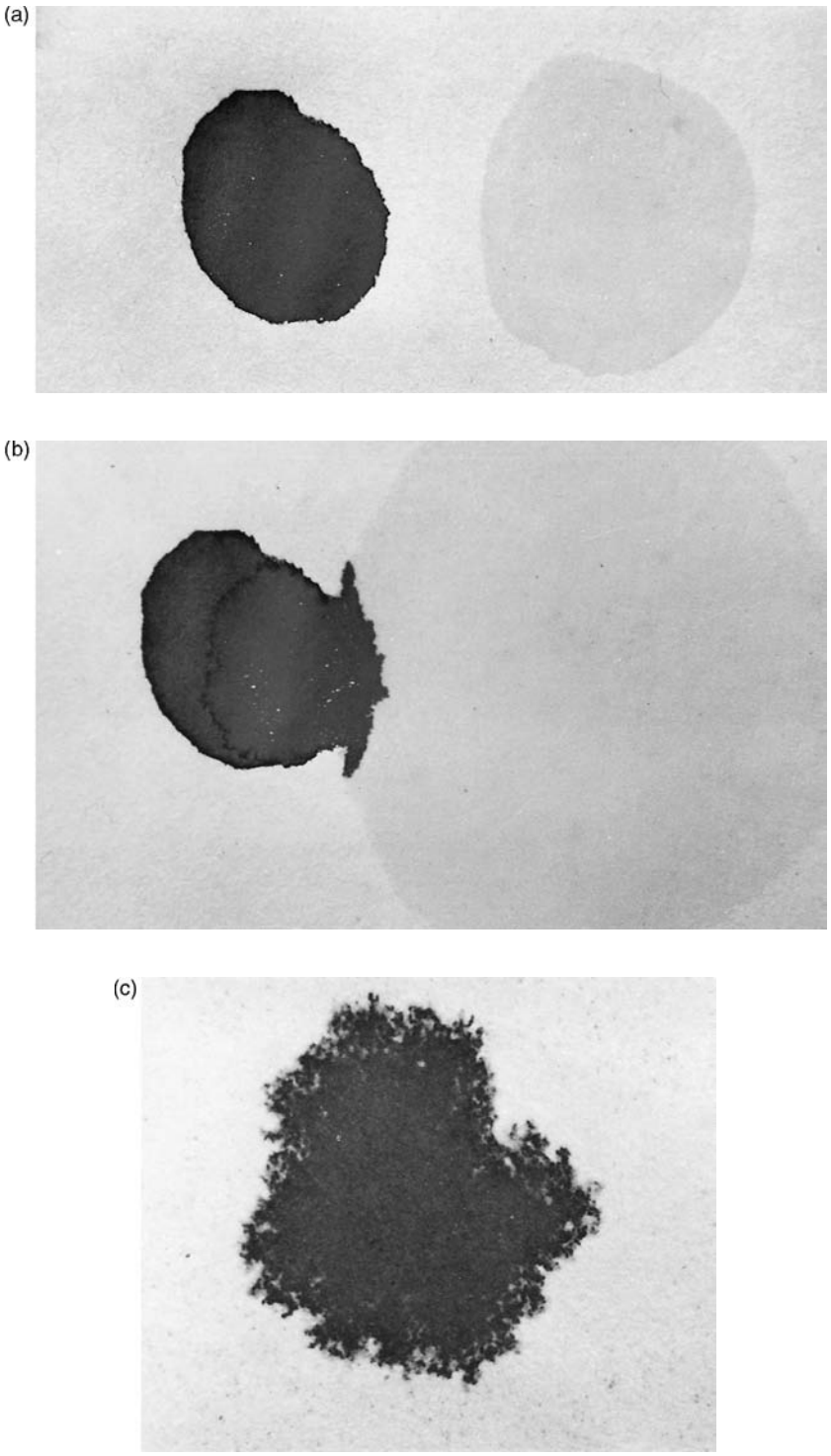


FIGURE 6.11 (See color plate following page 398.) Oil and water on an absorptive surface. (a) Drops of oil and water on absorptive paper (water that is not oil soluble is dyed red). (b) The spreading areas of oil and water meet and intermingle. (c) The intermingling of oil and water at the interface.

bottom of the still itself, this "Wetness fraction" is much larger and the wetter steam can deposit much more water on the herb; it may even be too much unless the mint herbage is very dry. Alternatively, steam from a satellite boiler can be injected into water in the bottom of any suitable fixed still to get the same effect, and in both these cases care will have to be taken to support the herb on a perforated grid above the reach of splashes from the boiling water.

If the steam is generated at a high temperature under significant gauge pressure in a satellite boiler, and expands to virtual atmospheric pressure at 100°C on entering a still, the surplus heat that is given out can vaporize some or all of the normal wetness fraction of the steam. Then the relatively dry steam will deposit little or no water on the herb. Further, if the equipment is well insulated against heat loss, the steam produced by a good boiler can even be made to take up a little surplus moisture from the herb surface. The actual amount will vary directly with the extent to which the generating pressure exceeds about 300 kPa. This is relevant if the mint carries more natural moisture than usual due, perhaps, to unfavorable weather hindering the wilting.

The satellite boiler offers the greatest variation in the moisture content of the steam and it is the only means of steaming trailer stills (tubs). For smaller charge weights processed in a fixed or stationary still, a well-designed undercharge kettle involves smaller capital outlay and will give excellent results with herbage that has been well wilted in the sun.

In practice, the amount of moisture remaining in the herb when it has to be picked up for distilling is dependent on the weather and this may cause difficulties in judging whether the steam should be generated under higher pressure and allowed to dry out more as it expands, or whether it should be passed through or over water to increase its wetness fraction. Unfortunately, experience derived from trial and error is the only guide to each individual distiller's best normal practice, as well as to the nature and extent of variations to suit herb abnormally affected by the weather. The distillate ratio of water passed per liter of oil in recovering a normal yield from the herb can show when the moisture content of the steam is matching the limited absorptive capacity of the leaf surfaces. If the herb has been suitably wilted and the wetness fraction of the steam is adjusted to the absorptive capacity of the herbage, efficient distillations should need to pass no more than 25 L of condensed water for each liter of oil recovered, a "distillate ratio" of 25:1 v/v. Failure to achieve this ratio is usually due to a corresponding failure to match the wetness fraction of the steam to the absorptive capacity of the herb surface.

In the early trailer stills (tubs), the steam was distributed from holes in an array of sparge pipes, parallel with the long dimension of the trailer and connected at right angles to a common header pipe across the front end of the floor. The herb was loaded directly onto the trailer floor along which the sparge pipes lay. In those circumstances, if the steam supply is not sufficient to maintain a gauge pressure of about 1 atm in the header pipe, the herb at the end of the trailer furthest from the header may receive a significantly greater flow of steam and finish distillation before the end nearest to it. Steam is then wasted at that end while the herb at the header end is finishing. Some amelioration might be made by graduating size of the holes in the sparge pipes with the largest diameters toward the header end (not the other way round). A better solution is to have the herb carried on a perforated false floor 150 mm above the trailer bottom. This creates a pressure-equalizing chamber. It ensures the even distribution of the steam. For a flow of at least 2 L of distillate per minute per square meter of charge top cross-sectional area, the pressure needed under the herb is equal to or less than 5 kPa $\approx$ 0.7 psi.

Where several trailers are steamed at the same time, it may be necessary to generate the steam under fairly high pressure to maintain the 1 atm of gauge pressure in the header pipes, which is necessary to achieve an adequate and reasonably even flow of steam up through the whole charge. When this steam expands, it is too dry to sustain the full potential heterogeneous mixture of oil and water at the interfaces. The oil perimeters soon retreat from the stationary surface water. Heat transference is then minimal and the rate of evaporation of the oil is greatly reduced. Overall distillate ratios in excess of 50:1 are quite common. In one example, in which the steam had to be generated under 7.8 atm of gauge pressure (115 psi), the ratio achieved was 70:1 v/v. Where the steam is as dry as this, the herb may be picked up a little sooner so that it retains just a little more moisture than when dry (about 25% moisture). When heat wave conditions make the mint hay very dry, the theoretical solution would be to expand the high-pressure steam through or over water on its way to the still or in the pressure-equalizing space beneath the charge.

The problem of steam that is generated under high pressure becoming too dry to support efficient transference of heat can be avoided by using low-pressure steam with the pressure-equalizing chamber under the false floor, provided both the outlet from the boiler and the diameter of the piping permit the transmission of enough low-pressure steam. If the steam generating pressure is only about 3-atm gauge, some condensation on uninsulated trailer walls may run to the bottom of the still and impart enough moisture to slightly dry steam. With well-wilted herb, the associated loss of oil is negligible.

With stationary cylinder stills, when the steam is generated in the bottom of the still itself, it will never be too dry. If the herb has been suitably wilted, this wet steam will usually return richer distillates than ''direct'' steam from medium- or high-pressure boilers. However, it can be too wet if bad weather has hindered the drying of the herb. A poor distillate ratio reveals wasteful use of steam when enough imported cloud particles are combining with moisture from collapsing aqueous plant cells to flood the leaf surfaces and cause an indifferent transference of heat. This condition also leads to the creation of a reflux flow, which reduces yield by carrying some oil to the bottom of the still. Unless suitably expanded, direct steam can be injected into a dry space beneath the charge of herb, instead of using the undercharge kettle; there is little that can be done to get better results from simple cylindrical stills when weather prevents adequate wilting of the mint. Boiling rates of 3 kg of steam or more per minute, per square meter of still top cross-sectional area, may help to reduce the reflux flow, but are unlikely to prevent it completely.

All mint distillations are particularly subject to the hydrophilic effect. Cloud particles that are carried by the steam strike the surfaces of the oil patches and roll up coatings of the oxygenated components of the oil. These coated particles are far too small to be caught by any normal separator. Any which fail to lodge on surfaces higher up the charge and which escape from the still in the liquid state will be lost in the discarded distillate water. With normal steam and ordinary stills, the loss of oil is proportional to the charge top cross-sectional area, and independent of charge height. This loss becomes, therefore, a decreasing proportion of the yield as stills get taller, and at about 200 mL/m^2 of charge top cross-sectional area, it is not an important proportion of the yield from stills of normal height. However, it combines with other effects to cause very misleading results from test stills only $\frac{1}{2}$ m tall or less. It is one of the reasons that such small steam stills are unsatisfactory for test distillations. An inexpensive test still for determining a plant material's characteristics for distillation is shown in Figure 6.12. For getting only a sample of oil and a guide to the potential yield of the herb, the Clevenger-type hydrodistillation apparatus is much to be preferred on ground of both cost and reliability.

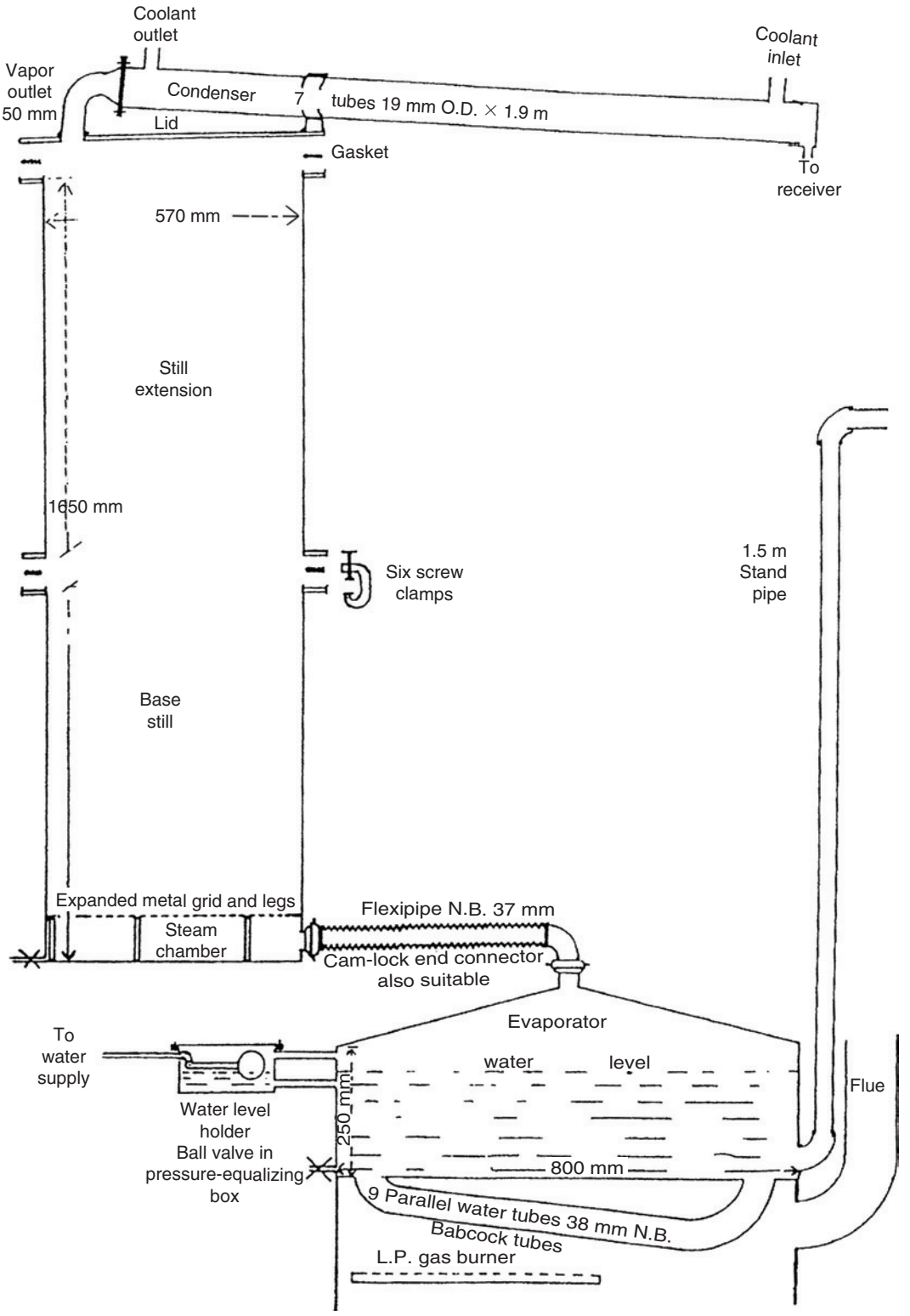


FIGURE 6.12 Schematic representation of test still designed by Denny.

6.3.4 The Steam Supply

The mixture of oil and water vapors that rises from the liquids' point of contact on the leaf is at the same temperature as the oil's liquid surface. Therefore, oil can vaporize only as fast as this saturated mixed vapor can be removed from over the oil–water interface. Similarly, the oil can vaporize only as fast as fresh steam can be delivered to replace that which has just condensed and surrendered its latent heat to the oil. Evidently, both these factors depend on the speed of the steam over the herb surfaces in terms of mass per unit time.

Provided other factors do not change, the time taken to isolate a surface-borne oil-like mint, by reducing the radii of the surface oil patches to nil, is exactly inversely proportional to the rate of displacement of the steam over the herb surfaces. The throughput of a mint distillery is proportional to its steam generating capacity. If we double the boiling rate, we halve the time each charge must occupy the still. If we double the diameter of a cylindrical still, without altering the boiler, the recovery of a normal oil yield will take four times as long. (Note that this does not apply to the distillation of oils that are not wholly borne on the surface of the herb.)

When the steam is supplied by an independent satellite boiler, there is no difficulty in having a suitable rate of flow through the still. In the United States, a boiler power of 100 hp commercial rating (1000 kW) is often allowed per trailer still (tub). If the boiler is in good order, this can achieve a steam displacement of 3 $kg/min/m^2$ for common trailers of about 8 m^2 in top area. But this is not always compatible with the desired wetness fraction when the steam has dried out on expanding as it enters the still.

In places where it is still believed that the herb has only to be immersed in steam for the oil to vaporize automatically, it is only thought necessary to fill the trailer with steam from any old boiler and the latter is often far too small. Until recently at least, this was frequently the case in Australia. The rate of steam flow through the charge of herb is far too slow. Even if the prolonged isolation time is acceptable, the loss of oil to reflux may not be. When satellite boilers service cylindrical stills that have much smaller top areas than the trailers, this is less likely to occur.

The problem is much more difficult in less developed situations in which a cylindrical still is heated by a fire directly underneath it like the stills found in India and China and the peasant stills that were common in Europe not so long ago. There is no way that a solid fuel fire under the plain flat bottom of a cylinder can possibly give a boiling rate approaching that required for efficient distillation. The very wet steam and the exceedingly slow rate of steam displacement induce a serious refluxed loss of oil. As stated earlier, any directly fired still must be equipped with Babcock tubes to have any chance of achieving an adequate flow of steam.

6.3.5 The Stills and Bulk Handling Systems

One aspect of the mint oil distilling industry that is inclined to be overlooked is the cost of getting the plant material, both to the distillery and away from it. The trailer stills (tubs) that are universally used in the United States have provided an answer to these potential problems. There will always be one or more extra processing places for trailers at the distillery so that arrivals and departures can be handled without interrupting continuous distillation in adjacent bays. This makes full use of the available steam supply. Then it is only necessary that the delivery of fresh trailers and the removal of spent ones should synchronize with the rate of processing at the distillery. Planners must consider not only how long it takes to load each trailer, but also how far and fast they can travel without the risk of damage. Since "down time" on maintenance will be greater with the harvesting machinery than at the distillery, it is advisable to allow for the capacity of the harvester to be about one-and-a-half times that of

the distillery. Then if harvesting gets too far ahead of processing, the field gang can be moved to areas of lighter crop, or more distant fields. The trailers solve the problem of removing the large bulk of spent material away from the distillery because they are usually fitted with hydraulic hoists to simplify the tipping and emptying.

Today, there are several different systems for dealing with the cylindrical stills. The one most likely to be used with mint is the simple traditional method in which loose herb is carted to the distillery and packed into the stills by hand. The grid that supports the bottom of the charge is equipped with wire ropes by which the spent charge can be hauled out of the still, using a geared hoist on an overhead rail leading to the tipping point. For ease of handling, it is advisable to have no more than 1-m depth of herb on a grid. Efficient usage of the steam and reasonable control of the hydrophilic effect require charge heights approaching 2 m tall. Therefore, a second coarser grid of crossed steel bars, with its own lifting ropes, should be put in place when the still is half full. The exhausted herb charge is then handled in two convenient halves. A small tractor with a front-end blade can push the spent herb from the dropping point to the compost heap or the drying area if it is to be used as boiler fuel.

Other systems involve having mesh-bottomed bins filled by the harvester in the field or at some filling station near the distillery. These bins are designed to be liners for the stills or even to be the stills themselves. Filled units are always waiting to replace exhausted charges, and they do it so quickly that the operation is virtually continuous. However, when the mint industry in any locality develops to the point where it could adopt this degree of refinement, the trailer stills are likely to be the best option. They provide an even more rapid changeover. The flexible steam pipe has only to be transferred from the exhausted unit to the fresh one standing by, and the steam valve reopened.

6.3.6 Ancillary Equipment—The Condenser

The essential oil distillery condensers have gone through almost as much development as have stills themselves. Three types are worth mentioning. The first of these is the traditional descending spiral of a single, tapering pipe immersed in a tank of static or running water. It can be made suitable for use with a small test still, but it is impractical to make it large enough to handle the rates of distillate flow that are now known to be desirable even for stills as small as 1 m^2 in the top cross-sectional area.

The horizontal zig-zag ''header'' condenser was developed in the United States to handle the distillate flows required for the trailer stills. They can always be built large enough to work with any practical still, but they must occupy a great deal of space. They certainly solved a problem, but the slow movement of the fluids in their very large volume gives them a few disadvantages, which a well-designed multitube condenser does not share.

Most modernized essential oil distilleries should now use multitube condensers, but they must be the single-pass and not the hairpin type (Figure 6.13). The vapor flow is distributed among a number of parallel stainless steel tubes, which have a relatively small volume of coolant water flowing rapidly past them. At the start of each distillation, they release air without inducing an unacceptable backpressure under the still lid. For each unit of heat exchange area, they are at least five times more economic and efficient in their use of cooling water than even the spiral coil type. Large, or very small adjustments of the rate of coolant flow will give immediate corrections to the condensate delivery temperature and this can be thermostatically controlled. They are very compact and easy to install. For example, a unit to serve a trailer still of 8 m^2 top area, passing 2 L of distillate per square meter per minute, is only 2.5 m (8 ft 3 in.) long and 380 mm (15 in.) in diameter. For changing from one oil to another, the tubes can be cleaned in the same way as the barrels of a shotgun. Other condensers do not have these advantages.

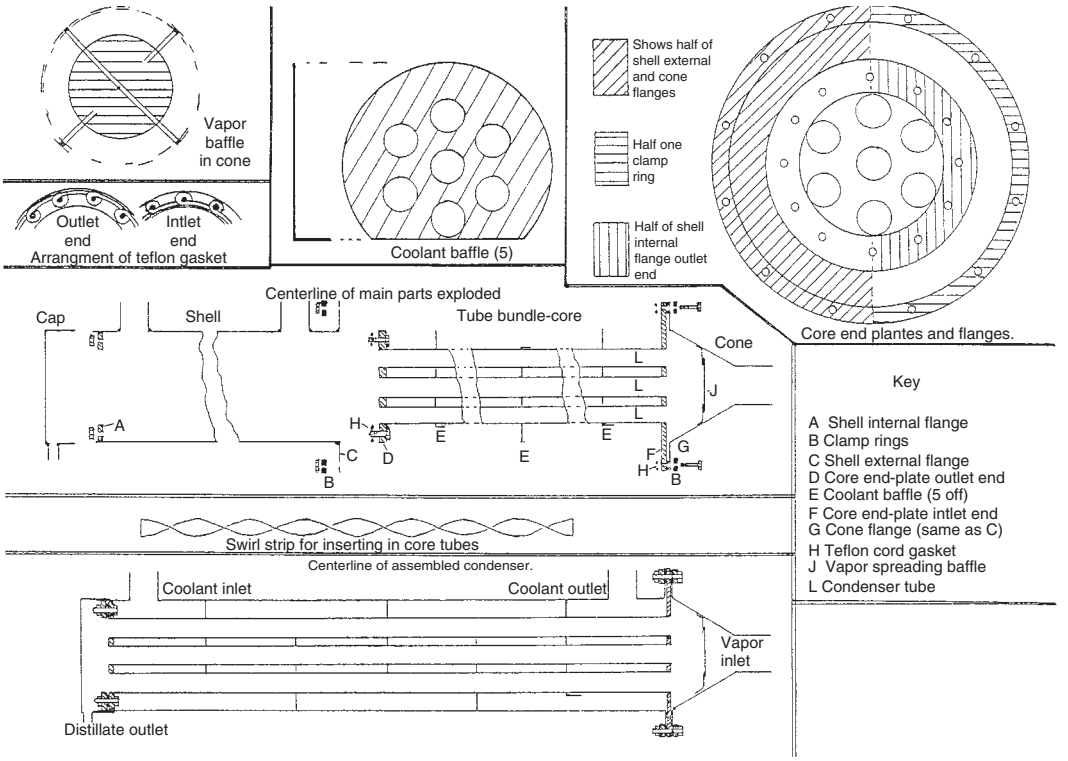


FIGURE 6.13 Vapor-in-tube condenser, a high efficiency single-pass seven-tube condenser for a test still; however, this mandatory arrangement applies to all condenser sizes.

There has been some reluctance to adopt these single-pass multitube condensers because it was originally believed that they had to be installed in the vertical position, which was hard to arrange. In fact, the difference in their performance between upright and near horizontal positions is almost negligible. Many conservative operators, having known the difficulties in using the coil condensers with trailer stills, doubted if such a compact unit could do as much work as the huge horizontal zig-zag condensers that were nearly as big as the trailer stills they served. The well-known difficulties of designing vapor-in-tube condensers also deterred those engineers who lacked the empirical data required to confirm theoretical assumptions.

The sketch in Figure 6.13 shows the mandatory arrangement for industrial, single-pass, multitube condensers. They can be taken apart to clean the tubes if calcareous salts in the cooling water cause encrustation of their outer surfaces. To save space, the diagram shows only a small condenser with just seven tubes, suitable for working with the test still drawn in Figure 6.12. All larger units follow the same arrangement. Where the quality of the cooling water is not in doubt, a simpler unit, using the same arrangement, can have the shell and inlet cone permanently welded to the end plates.

The number and length of the parallel tubes required in both simplified and industrial types to condense a range of distillate flows in temperate climate areas can be seen in Table 6.1. It allows the connecting pipe from the still to be 3 m (10 ft) long and to have one 90° bend. It is calculated for stainless steel tubes up to 3 m long, having 20 or 25 mm outside diameter and wall thickness not exceeding 1 mm. They are arranged on a hexagonal or triangular diamond

TABLE 6.1
Tube Condenser Parameters for Standard Temperature Climates

Distillate Flow Rate Metric		Distillate Flow Rate Pounds WT		Diameter Connecting Pipe		Outside Diameter of Condenser Tubes		Number of Required Tubes and Length of Pattern Tubes		
kg/h	L/min	lb/h	lb/min	in.	mm	in.	mm	Hex or D	ft	m
30	0.50	66	1.10	1.5	37	0.75	20	7 H	2.70	0.83
45	0.75	99	1.66	1.5	37	0.75	20	7 H	4.05	1.23
60	1.00	132	2.20	2	50	0.75	20	7 H	5.40	1.65
100	1.67	220	3.66	3	75	0.75	20	15 D	4.21	1.30
150	2.50	330	5.50	3	75	0.75	20	19 H	4.98	1.52
200	3.33	440	7.33	3	75	0.75	20	27 D	4.68	1.42
250	4.17	550	9.17	4	100	0.75	20	31 H	5.09	1.55
300	5.00	660	11.00	4	100	0.75	20	31 H	6.10	1.86
350	5.83	770	12.83	4	100	0.75	20	37 H	5.97	1.82
400	6.66	880	14.67	4	100	0.75	20	42 D	6.01	1.83
500	8.33	1100	18.33	5	125	0.75	20	55 H	5.74	1.75
600	10.00	1320	22.00	5	125	1.00	25	37 H	8.20	2.50
800	13.33	1760	29.33	6	150	1.00	25	42 D	9.20	2.80
1000	16.66	2200	36.66	6	150	1.00	25	61 H	8.20	2.50
1250	20.83	2750	45.83	8	200	1.00	25	69 H	9.10	2.75
1500	25.00	3300	55.00	8	200	1.00	25	85 H	9.00	2.74

Note: Hexagon: 7-tube bundle diameter = 4 × tube O.D.; 19-tube bundle diameter = 7 × tube O.D.; 31-tube bundle diameter = 9 × tube O.D.; 37-tube bundle diameter = 10 × tube O.D.; 55-tube bundle diameter = 12 × tube O.D.; 61-tube bundle diameter = 13 × tube O.D.; 85-tube bundle diameter = 15 × tube O.D.; 91-tube bundle diameter = 16 × tube O.D. Diamond: 27-tube bundle diameter = 9 × tube O.D.; 42-tube bundle diameter = 10.5 × tube O.D.; 69-tube bundle diameter = 13.5 × tube O.D.; 102-tube bundle diameter = 16.5 × tube O.D. The shell diameter must not be more than 50 mm greater than that of the tube bundle (Figure 6.13).

pattern with the tube centers $1\frac{1}{2}$ tube diameters apart. The shell diameters are only 50 mm greater than that of the tube bundle and the coolant baffles are not more than $1\frac{1}{2}$ shell diameters apart. The coolant flow is 15 times that of the distillate and the condensate emerges at a temperature nearly 22°C above that of the entering coolant water. If the coolant fails or is not turned on, the backpressure that can develop under the still lid because of the pipe and condenser is less than 1.4 kPa (= 0.20 psi).

6.3.7 Ancillary Equipment—The Receiver–Separator

The principle involved in oil separation from the distillate water is very simple. When the distillate emerges from the condenser and is caught by the receiver, the oil rises to the top of the water. The large droplets of oil separate out quite quickly whereas the smaller ones rise through water much more slowly. If the receiver is suitably designed, oil can be made to pour from an outlet at the top of the vessel whereas water is continuously discharged from the bottom.

If the rate of distillate (both oil and water) flow and the dimensions of the receiving vessel are such that the small oil particles rise through the water more slowly than the water flowing downward toward the discharge outlet, it is easy to see that quite a lot of oil can be discharged with the water. This oil can only be partly recovered, and only in a degraded state, by

redistilling the partially separated water. Present thinking insists that the speed at which small oil particles rise through water at different temperatures shall be measured, and that the separator is then designed to ensure that the water travels downward at no faster rate.

It was once believed that the oil and water should be separated at cool temperatures to avoid a loss of oil due to its presumed solubility in warm water. But the main loss of oil susceptible to this solubility occurs at 100°C in the condenser and it is difficult to demonstrate any further significant loss of condensed oil in water even at 55°C.

The separation of oil and water is promoted by the difference in their densities and resisted by the viscosity of the water. In this case, the former increases and the latter decreases with rising temperature (Porter and Lammerink, 1994). So the conditions for the separation of the mint oils from water improve dramatically at higher temperatures. Tests have shown the distances per minute, by which small particles of peppermint oil rise through water to be 4.5 mm at 35°C, 6.2 mm at 45°C, and 9 mm at 55°C. Since the separator must have water traveling downward more slowly than the droplets of mint oil that are rising, the cross-sectional area of the travel path at 55°C of the water is only half of what it needs to be at 35°C.

Although modern separators that allow for the speeds of distillate travel are much larger in cross section than the less effective types of former times, the higher temperatures do restrain their size. For a trailer still of 7.5-m^2 area passing 15 L ($\approx$ 4 U.S. gal) of distillate per minute, the diameter of the separator would be 208 mm (82 in.) at 35°C, 170 mm (67 in.) at 47.5°C, and 147 mm (58 in.) at 55°C. The last of these options is perfectly convenient and manageable, but it is advisable to cap any open vessels or take other reasonable precautions against loss of oil by evaporation. In addition, the separator should be insulated against heat loss.

The equivalent diameters for separators to work with a cylindrical still passing 8 L of distillate per minute would be 152 mm (60 in.) at 35°C, 124 mm (49 in.) at 47.5°C, and 108 mm (41 in.) at 55°C.

An efficient distillate receiver–separator designed to work with the small-scale still can be seen in Figure 6.14. The inlet funnel and pipe lead distillate to the bottom of an open topped, inner cylinder, which can hold the first 3 min of distillate from a new charge. A number of years ago, Hughes (1952) pointed out that the early oil-rich distillate may be cooler than that remaining in the separator from the previous run. As a result, it will go straight to the bottom and out the water discharge pipe if it is not caught and held until the temperatures have stabilized. However, compared with those days, the multitube condenser has greatly reduced the amount of distillate at risk to only about the first 3 min of full flow.

The core of the separator is a column pipe on an inverted funnel, which is inserted down the inner cylinder to rest on the inlet pipe. The outlet of the latter is angled to create a swirl in the funnel. This reduces turbulence and gives a primary stage separation for which the outlet at the top of the column discharges oil just above the fluid level held in the neck of the main vessel. Hughes suggested that the completion of the distillation could be indicated by the oil flow from the column outlet coming to an end. If the flow of distillate is very constant it can be helpful, but not if the flow rate is at all unsteady.

The main cylinder encloses the outer annulus of which the cross-sectional area is such that the water will travel downward more slowly than the oil particles will rise through it. The water outlet pipe connects at the bottom of this annulus and is taken to a height that causes a depth of 6 to 8 cm of oil to be retained in the neck of the separator. This allows any suspended water to sink away from the level of the main oil outlet pipe so that only oil that is relatively free of water will be delivered for collection. Even so, the oil should be dried over anhydrous sodium sulfate to ensure its keeping qualities. The oil can be tested for dryness before it is finally stored.

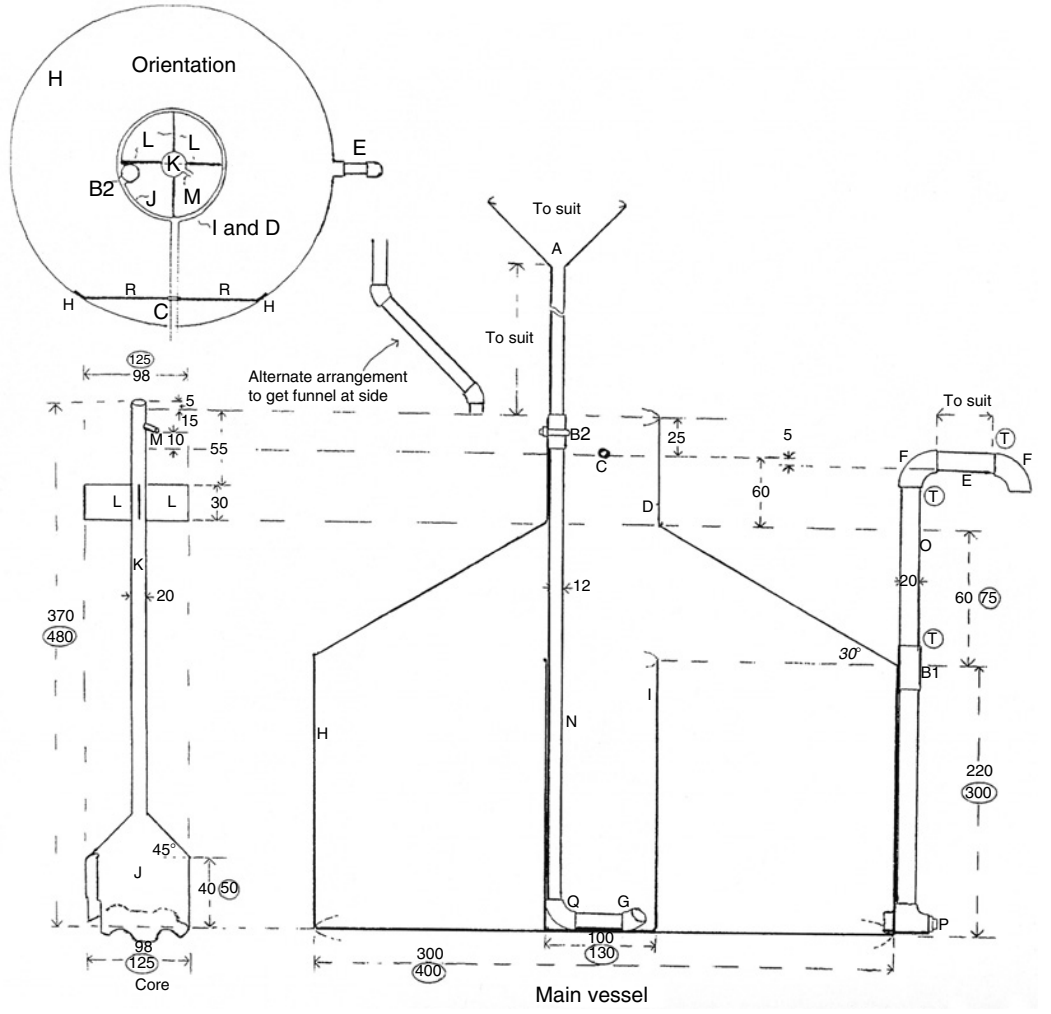


FIGURE 6.14 Schematic representation of a distillate receiver–separator for mint oil. This is a compact design to be used with pilot stills (Figure 6.12) with a distillate flow of 40 L/h. Core is inserted down the neck of the main cylinder to rest on the base (floor) of the inner cylinder.

Key A. distillate inlet funnel; B1. $\frac{3}{4}$ in. BSP socket soldered to cylinder; B2. $\frac{1}{2}$ in. BSP socket clamped to neck wall (see also N); C. oil outlet pipe 10 × 160 mm (see R for support rods from main vessel); D. wall of cylinder neck (ID 100 mm); E. water outlet final pipe (20 mm). Low side of pipe is at least 5 mm below low side of oil pipe C and adjusted by threaded joints (T); F. $\frac{3}{4}$ in. (20 mm) BSP threaded elbows; G. $\frac{1}{2}$ in. 45° elbow angled to make swirl; H. wall of main outer cylinder; I. wall of open topped inner cylinder (if given a sealed bottom it can be free standing if suitable); J. core inverted funnel with cutout at side to pass and locate pipe N; K. core tube (20 mm); L. vanes to hold core central in neck; M. oil telltale outlet at top of core; N. distillate pipe assembly (12 mm). To have funnel A to one side a 45° elbow may connect to socket B2; O. galvanized water outlet pipe (20 mm); P. stainless steel "T" and plug (20 mm); Q. 12 mm 90° elbow; R. Strut rods for **C** silver soldered to H. Joints marked with (T) must remain threaded to allow adjustment of water outlet level for holding 80 mm depth of oil in the separator neck.

In Figure 6.13, the height of both the internal and main cylinders of the separator are shown as 200 mm. In larger models made to the same pattern, these cylinders also have equal heights. However, a height of 400 mm is ample to inhibit turbulence and adequate for all industrial scale purposes and sizes. The distance traveled by the condensate in receivers designed for nonstop operation does not affect the separation of the two liquids.

6.3.8 Distillery Throughput

6.3.8.1 Change Time and Heating Phase

The important question of how much herbage a distillery can handle in a given period can be answered only if the mass of each charge and the time it must occupy the still are known. This time falls into three separate periods of which the first is the "change time." With modern stills, it may be taken as the period from which the steam is turned off from an exhausted charge until it is turned on again to the replacement. With trailers and prefilled cylindrical stills, it need not exceed 3 min. For practical purposes in modern distilleries, the change time is negligible.

With hand-filled stationary cylinders, the steam may be turned on, or the undercharge kettle left boiling, while the still is loaded, provided no extra effort is made to compress the herb with the help of the steam. The change time is then inseparable from the "heating time" and is again virtually negligible.

The "heating time" lasts from the first admission of steam to a fresh charge until all the air has been expelled from the still and the distillate is flowing normally. The point is indicated when no more fog is seen issuing from the condenser. (At the start of all distillations, warm air from the still is cooled below its dew point as it passes through the condenser, and issues carrying visible quantities of cloud.) The duration of this time depends on the amount of herb and steel to be heated, their specific heats, and the rise in temperature to boiling point. It is inversely dependent on the rate that steam is applied and surrenders its latent heat at the rate of 540 kcal/kg to the herb and its container. (The specific heat of steel is about 0.117 and that of mint herbage in distilling condition ranges from about 0.6 if very dry to about 0.8 if a little moist.)

The activity on the herb surfaces during this heating phase largely determines the course of the rest of the distillation.

Consider the diagram in Figure 6.15. Picture the plant charge as a number of hypothetical layers, each 1 cm thick. Let steam be admitted to the still at a rate sufficient both to raise one layer to boiling point in 7 sec and to vaporize oil for a period of 7 sec. When the lowest layer attains boiling temperature, a mixture of steam and oil vapor will rise off it and condense onto the second layer for a period of 7 sec. When the second layer boils, the vapors that rise from it and condense onto the third layer will comprise the usual 7-sec worth of steam and the oil distilled from the second layer in 7 sec, as well as the oil lifted from the first layer to the second layer while the latter was heating up. When the third layer boils, the vapors that condense onto the fourth layer will contain the first 7 sec of oil that were distilled from the three layers below, as well as the usual quantity of steam.

Under favorable conditions, this will continue up the still with each successive heating layer receiving an amount of condensing oil equal, in our model, to the first 7 sec of oil distilled from every layer below it in the charge. But when the herb is only slightly absorptive like wilted mint, a practical limit to the quantity of rising oil is reached when the increasing amount becomes sufficient to fill the absorptive capacity of the herb surface. Then, no more oil–water interface can be created on each single layer of herb. When this occurs, the amount of oil that can be vaporized from a recently heated layer, and carried upward in the uniform time that each higher layer takes to boil, is necessarily at its maximum. Then for practical purposes, the heating vapors passing to all the higher layers will have a constant proportion of

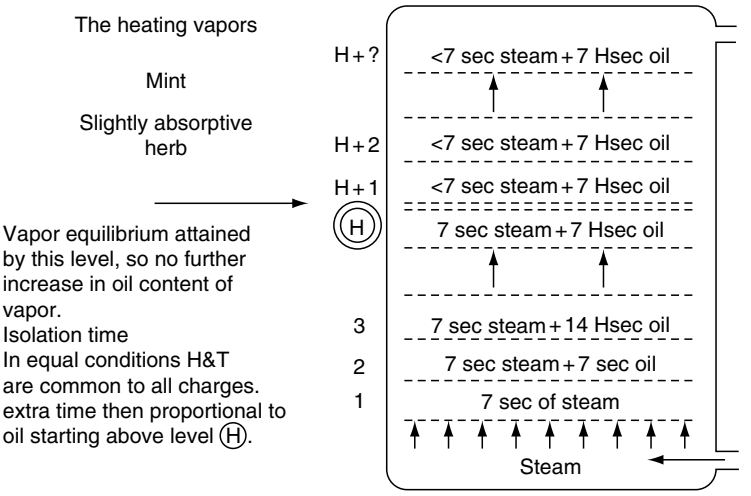


FIGURE 6.15 Hypothetical schematic representation of steam or oil movement throughout the charge during steam distillation (H = charge height; T = time).

oil to water. For the sake of discussion, it can be called the "equilibrium composition" of the heating vapors. Figure 6.15 illustrates how this occurs and gives an impression of the way the oil is distributed on the herb surfaces when the steam breaks through the top of the charge and the isolation of the oil begins.

6.3.8.2 Oil Isolation Phase

The "oil isolation time" starts when the full design rate of distillate flow is reckoned as starting and lasts until the rate of oil production has declined to the point where there is no reason to continue the flow of steam. This period divides into two distinct phases.

The proportions of oil and water in the first vapors to rise off the top layer of the charge will be the same as in the equilibrium composition of the heating vapors. The oil removed from the top layer will be replaced by oil from the same length of oil–water interface on the layer below and will therefore have the same proportions of oil and water. This state must prevail so long as the oil leaving the top layer, or any other, is replaced by an equal quantity of oil from the same length of oil–water interface on the layer below. It follows that, irrespective of the size of the charge, all the oil from above the level at which the heating vapors attained equilibrium composition, will be isolated at a constant distillate ratio of oil to water and the graph of oil produced against water condensed will be a straight line.

When the layer in which the heating vapors first attained equilibrium composition reaches the top of the still and its oil passes off to the condenser, it will be replaced by oil from a slightly smaller quantity and shorter oil–water interface on the layer below. The next layer below will have its oil replaced from an even shorter interface and so on. With the oil becoming an ever-smaller proportion of the total distillate, the graph of oil against water must curve away toward becoming parallel with the water axis.

If the quality of both the mint herbage and the steam do not change, the level at which heating vapors' equilibrium occurs will be the same distance up the charge from the bottom. Therefore, the amount of steam that must pass to isolate the oil from below the equilibrium level is proportional to the cross-sectional area of the charge. With stills that are wider at the top than the bottom, accurately self-compensating factors allow calculations to be based on the cross-sectional area of the top of the charge.

The graphs of two charges of different heights and cross-sectional areas show the virtually equal fixed distillate ratios applying to the two different amounts of oil from above the vapor equilibrium levels and the slightly smaller amount of oil recovered from below the equilibrium level in the still with the slightly smaller area. The two lines for the fixed distillate ratios are not absolutely perfectly parallel because of the fact that the smaller charge was slightly less well wilted than the larger one (Figure 6.16).

It is not possible to set out all the detailed calculations here, but they are available for those who wish to study the subject in greater depth (Denny, 2001). Suffice to say that the factors governing the amount of water that must pass to exhaust the oil from a charge of peppermint, for example, distilled under given conditions, can be combined and simplified to give uncomplicated relations for the isolation times on which to base reliable estimates of the handling capacity of a distillery.

While the heating time is proportional to the mass of plant material in the charge, the oil isolation time depends on the amount of oil to be recovered and is independent of the amount of herbage in the still. For calculating the oil isolation phase of the time the charge must occupy the still, we do not need to know the weight of the charge, only the amount of oil it is expected to produce.

Let any still have top cross-sectional area A square meters and let the charge of herb return W liters of oil. If the distillate flow rate is F liters of water per minute, the oil isolation time, T minutes, for moderately wilted peppermint processed with low-pressure steam as mentioned earlier is given by

$$T = (23.84A + 15.38W) \div F$$

This is intended as a general formula for "middle-of-the-road" conditions as regards herb and steam moisture, in which all the charges of average herb, not less than 1.3 m tall, would return

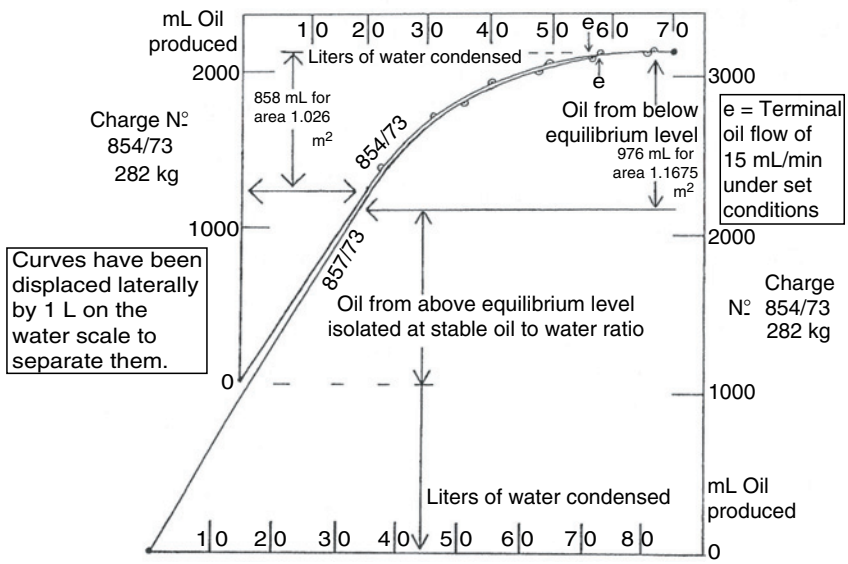


FIGURE 6.16 Graphical representation of the distillation of two mint charges with different heights and top areas. The temperature gradient and vapor composition equilibrium occur during initial heating at a level below the top of the charge.

overall distillate ratios of 1 part oil to 25 parts water v/v or better. If the steam is too dry, the oil isolation times calculated by this formula will be too short; if the herbage is drier than the test material, they will be too long. In the series of five test runs, which included the charges graphed in Figure 6.16, the greatest difference between the observed oil isolation times, which ranged from 19.5 to 29.5 min, and those calculated by the formula is only 20 sec. The method is sound but the constants would need to be varied directly with any expected factor of change in the distillate ratio, if that could be predicted.

6.4 CONCLUSION

The perfect mint distillery suitable for all situations has not yet been designed and it is most unlikely that it ever will be. But, as stated at the beginning of this chapter, in the 20th century huge improvements have been made to that which has been inherited. But the inspired concept of the trailer stills, for example, was developed 30 years or more before their inventors or their scientific advisers understood what made this distillation work. These advances were mainly based on reducing labor and other costs by increasing the size of the stills and exploiting the development of farm mechanization. As the orthodox explanation of the process at that time actually defied the second law of thermodynamics, it is not surprising that some of these practical changes do not make the best use of the rules of physical chemistry, and others, like the redistillation of the distillate waters, have been made to look like virtues by necessity.

Now that our understanding of this distilling process has improved, we can produce "wisdom after the event" and state two principles on which further advances could be based, or present ones adjusted. The first of these is that, because the oil must be vaporized to be recovered, the moisture content of the steam must be correctly adjusted to match the absorptive capacity of the mint leaf surfaces so that it promotes the capillary action, which alone makes possible the transference of latent heat to vaporize the oil.

The second vital principle is one for which due allowance is very seldom made. The four units, still, condenser, separator, and steam generating system are all mutually dependent. The scale of one cannot be altered without requiring corresponding changes in the scale and size of all others. Although any one item, even the separator, could be made the starting point from which to calculate the matching capacities required of the other three, probably the most practical fundamental factor is the cross-sectional area of the still. This is because the performance of the distillery depends directly on the rate of displacement of the steam in relation to the area of the top of the still.

If a trailer still that has three times the cross-sectional area of the cylindrical still that it replaces is adopted, the steam generation must be trebled without resorting to an unduly high pressure in the boiler. The effective heat exchange area and free air passage through the condenser must be recalculated both for condensing capacity and for backpressure. It is equally essential that the cross-sectional area of the separator must also be increased by the same factor of three in accordance with the area of the new trailer. In regions where the trailers are in normal use, it may well be that there are a few places where some of these things could be improved, if they were not known, or had been overlooked when the early trailer stills were introduced.

In places where the vertical cylinders are the standard stills, the same matching up of the various pieces of equipment must apply. In particular, the boiler must be able to supply the steam for a distillate flow equal to at least 2 L/min for each square meter of the top cross-sectional area of the still. Normally that will not be a problem. But where the steam is generated by an undercharge kettle in the bottom of the still itself, a faster boiling rate is

desirable to counter the greater risk of reflux from the very wet steam depositing excessive water on the herb surfaces. It is virtually certain that Babcock tubes need to be employed to achieve an adequate boiling rate and flow of distillate.

If essential oil production is to hold its own in competition with artificial flavors, the producers cannot afford to ignore any possibility of improving the value of what they are offering to the buyer. The proper management of the distillation process is vital in two aspects that greatly affect this value, the economy and the quality of the product.

REFERENCES

Denny, E.F.K. (2001). *Field Distillation for Herbaceous Oils*. 3rd ed., Denny, McKenzie Associates, Lilydale, Tasmania, Australia.

Green, R.J. (1963). Mint Farming. Agric. Inform. Bull. No. 212. A.R.S. USDA & Purdue Univ. Agric. Exp. Stn. U.S. Government Printing Office, Washington, D.C.

Hughes, A.D. (1952). Improvements in the Field Distillation of Peppermint Oil. Bull. No. 31, Engineer. Exp. Stn., Oregon State College, Corvallis, OR, pp. 1–64.

Kahol, A.P., Tandon, S. and Ahmed, J. (1999). Effect of the design parameters on the efficiency of *Mentha arvensis* oil/water separator. *Fafai J*., 1, 21–30.

Lacy, M.L., Stephens, C.T., Green, R.J. and York, A.C. (1981). Mint Production in the Midwestern United States. North Central Regional Extension Publication Number 155, Co-operative-Extension Service, Michigan State University, East Lansing, Michigan, 1–18p.

Landing, J.E. (1969). *American Essence, A History of the Peppermint and Spearmint Industry in the United States*. Copyright A.M. Todd Foundation, Kalamazoo Public Museum, Kalamazoo, MI.

Nijjar, G.S. (1991). How to obtain best results from energy balanced (calandria type) distillation stills. *Ind. Perfum*., 35(4), 197–203.

Nijjar, G.S. (1993). Optimising efficiency of energy balance (calandria type) distillation stills. In: *Newer Trends in Essential Oils and Flavours*, Dhar, K.L., Thappa, R.K. and Agarwal, S.G., Eds., Tata McGraw-Hill Publ. Co., New Delhi, pp. 352–363.

Porter, N.G. and Lammerink, J.P. (1994). Effect of temperature on the relative densities of essential oils and water. *J. Essent. Oil Res*., 6, 269–277.

Sievers, A.F. (1952). Methods of Extracting Volatile Oils from Plant Material and the Production of Such Oils in the United States. USDA Tech. Bull., No. 16 Revised, U.S. Government Printing Office, Washington, D.C., pp. 1–28.

Todd, A.M. (1886). The essential oil industry of Michigan. *Proc. Am. Pharm. Assoc*., 34, 121.

Todd, A.M. (1904). The American Peppermint Industry. 5th International Congress. *Angew. Chem*., 807–808.

Van Slyck, D.C. (1850). Cultivation of Peppermint. Report Commissioner of Patents 1849. U.S. House of Representatives Exec. Doc. No. 20 Part II, p. 389.

7 The Composition of Commercially Important Mints

Brian M. Lawrence

CONTENTS

The main oils of the *Mentha* genus that have achieved high economic importance are cornmint, peppermint, Native spearmint, and Scotch spearmint; however, two other oils *Mentha citrata* and pennyroyal are of commercial importance but to a much lesser extent. The oil compositions of each of these six mint oils are examined separately in the order *Mentha citrata*, cornmint, peppermint, pennyroyal Native spearmint, and Scotch spearmint. A brief discussion on the cultivation and oil production precedes the review of the oil compositions of these commercially important mints.

7.1 *Mentha citrata* OIL ex *Mentha aquatica* L. var. *citrata* (EHRH.) FRESEN

7.1.1 Cultivation and Oil Production

Mentha citrata, Lemon mint, bergamot mint, or lavender mint are some collective names under which this linalool/linalyl acetate-rich mint oil of commerce is known. It is a tall (30 to 60 cm) perennial herb with decumbent branches with erect ends. The leaves are smooth, ovate-elliptical in shape. The flowers, which appear on short dense terminal spikes, are pale

purple in color. As it is a natural hybrid between *M. aquatica* L. and *M. spicata* L., it is found in its natural habitat in Europe where these two species are quite prevalent.

A few years ago, Todd and Murray (1968) and Murray (1969) developed two synthetic hybrids between *M. aquatica* var. *citrata* and a crisp leaved *M. spicata* that had oil yield that was superior to that of the natural hybrids. These became known as EO661 and EO664. The hybrid EO661 was developed to yield an oil that possessed a fresh, green, lavender-like odor, whereas EO664 was developed to yield an oil that possessed a sweet, lavender-bergamot-like odor. Both of these synthetic hybrids were grown commercially in the western U.S.; however, as the oil yield of EO664 was greater than EO661, it was preferred by the growers. A few years ago, more than 10 tonnes of EO664 were produced annually. More recently, the area under cultivation of this synthetic hybrid has decreased to the point at which EO664 oil (now known as TODD-664) is currently only produced in limited amounts (3 to 4 tonnes) on one farm in the western U.S.

Selections of *M. aquatica* var. *citrata* (the taxonomic origin of *Mentha citrata* oil) are also grown on a limited scale in both India and China. It was first introduced into India in 1959, in the Jammu and Kashmir region. As a result of agronomical studies, the technology associated with growing it as an oil producing crop was developed so that by 1988 a new cultivar "Kirin" was introduced into cultivation (Anonymous, 1988) from which ca. 10 tonnes of oil was produced (Hussain et al., 1988). This high yielding plant was reported to possess an oil with an average composition of linalool (48%) and linalyl acetate (37%).

Varshney (1986) reported that the production of oil in India in 1985 was ca. 7 tonnes. Since then, oil production increased to 15 tonnes by 1989 and 50 tonnes by 1993 (Krishnamoorthy, 1989, 1993). The current annual oil production is estimated to fluctuate between 15 tonnes and 50 tonnes depending on supply and demand, ease of sale, and the economic return that can be generated by growing cornmint, peppermint, or spearmint for oil production rather than bergamot mint. Prasad (1997) reported that cultivation of the Kirin cultivar had recently increased to ca. 500 ha with an annual oil production of 50 tonnes.

A survey of the literature reveals that the oil of *M. aquatica* var. *citrata* has been the subject of considerable study, although most of these studies have been directed toward the establishment of cultivation in India (Choudhury et al., 1979; Singh and Nand, 1979; Singh et al., 1979a, 1979b; Arumugam and Kumar, 1980; Kumar et al., 1980; Bhardwaj et al., 1983, 1984; Rajeswara Rao et al., 1984).

In 1924, *M. aquatica* var. *citrata* was promoted as a new crop by the U.S. Department of Agriculture. In a brief report (Anonymous, 1924), it was noted that under suitable conditions experienced in Virginia, 35.6 kg/ha of oil, which had a linalyl acetate content of 50%, could be produced.

7.1.2 Oil Composition

A survey of the literature reveals that it has been known for some time that an oil known as lemon mint, lavender mint, or bergamot mint contained linalool and linalyl acetate as major components (Gildemeister and Hoffman, 1961). Between 1960 and 2004, the commercial oil of *Mentha citrata* has been the subject of moderate study by Handa et al. (1964); Nigam and Levi (1964); Murray and Lincoln (1970); Virmani et al. (1972); Kapil et al. (1974); Bhagat et al. (1974); Singh et al. (1980); Lincoln et al. (1986); and Bahl et al. (2000). The components characterized in this oil can be seen categorized according to the class of compounds such as hydrocarbons, alcohols, esters, aldehydes, ketones, and miscellaneous compounds as can be seen in Table 7.1a, Table 7.1b, Table 7.1c, and Table 7.1d.

Mutation studies on *M. aquatica* var. *citrata* by Kak and Kaul (1978) using gamma rays and x-rays revealed that the linalool and linalyl acetate contents could be affected. They found that on irradiation of the parent clone [the main components of which were linalool

TABLE 7.1a
Hydrocarbons Found in *Mentha citrata* Oil

Compound	References
α-Pinene (0.6%–1.7%)	Handa et al. (1964); Virmani et al. (1972)
β-Pinene (0.6%–2.0%)	Handa et al. (1964); Virmani et al. (1972); Lincoln et al. (1986)
Limonene (0.2%–1.9%)	Handa et al. (1964); Virmani et al. (1972); Lincoln et al. (1986); Bahl et al. (2000)
β-Phellandrene (3.3%)	Virmani et al. (1972)
1-Vinyl-*p*-menth-4(8)-ene (<0.1%)	Singh et al. (1980)

(56.0%) and linalyl acetate (16.3%)], two mutant strains were selected because they possessed commercial potential. The main components of the oils of these two mutant strains (C-408 and C-326) were 72.1% and 7.6%, and 40.1% and 40.0% for linalool and linalyl acetate, respectively.

Lawrence (1978) analyzed the oils of the two synthetic hybrids (EO661 and EO664). The results of these analyses are shown in Table 7.1e. As can be seen, the hybrid EO661 was more lavender-like in composition whereas EO664 was more bergamot-like.

7.1.2.1 Chirality

The enantiomeric distribution of some of the components found in *Mentha citrata* oil can be seen in Table 7.1f.

7.2 CORNMINT OIL ex *Mentha canadensis* L.

7.2.1 Cultivation and Oil Production

The oil of *M. canadensis* that was originally selected for commercialization because of its richness in menthol has been referred to commercially as cornmint, Japanese peppermint,

TABLE 7.1b
Alcohols and Esters Found in *Mentha citrata* Oil

Compound	References
Linalool (19.0%–51.5%)	Handa et al. (1964); Murray and Lincoln (1970); Virmani et al. (1972); Bhagat et al. (1974); Lincoln et al. (1986); Singh et al. (1980); Bahl et al. (2000)
Methanol (<0.1%)	Kapil et al. (1974)
3-Octanol (0.2%–0.3%)	Bahl et al. (2000)
α-Terpineol (0.4%–5.9%)	—
Geraniol (0.5%–8.4%)	—
Citronellol (1.4%–2.9%)	—
Linalyl acetate (14.8%–60.0%)	Handa et al. (1964); Murray and Lincoln (1970); Virmani et al. (1972); Bhagat et al. (1974); Lincoln et al. (1986); Singh et al. (1980); Bahl et al. (2000)
Geranyl acetate (0.2%–6.2%)	Bahl et al. (2000)

TABLE 7.1c
Aldehydes and Ketones Found in *Mentha citrata* Oil

Compound	References
Furfural, acetone	Kapil et al. (1974)
Pulegone (8.1%)	Handa et al. (1964)
Piperitone (4.2%)	—
Isopinocamphone (0%–1.3%)	Lincoln et al. (1986)

Chinese peppermint, Manchurian peppermint, or Brazilian mint, although more recently cornmint has become the accepted name. A summary of the production of this oil in India and China can be seen in Chapters 4 and 5 of this book.

7.2.2 Oil Composition

Because of its commercial importance, cornmint has been the subject of considerable study. A survey of the pre-1960 literature (Gildemeister and Hoffman, 1961) revealed that the chemical composition of cornmint oil was fairly well known. The components listed in the review are as follows:

α-pinene
camphene
limonene
p-menth-4,(8)-ene
β-caryophyllene
methanol
ethanol
butanol
hexanol
isoamyl alcohol

TABLE 7.1d
Miscellaneous Compounds Found in *Mentha citrata* Oil

Compound	References
1,8-Cineole (0.2%–1.3%)	Handa et al. (1964); Lincoln et al. (1986) Bahl et al. (2000)
Menthofuran (0%–0.1%)	Handa et al. (1964); Nigam and Levi (1964) Lincoln et al. (1986)
Piperitone oxide* (3.0%)	Handa et al. (1964)
Formic acid, acetic acid (<0.1%)	Kapil et al. (1974)
cis-Linalool oxide† (0.1%–2.6%)	Bahl et al. (2000)
trans-Linalool oxide† (0.1%–2.5%)	—

*Correct isomer not identified.
†Furanoid form.

TABLE 7.1e
Percentage Composition of the Oils of Synthetic Hybrids of *Mentha aquatica* var. *citrata*

Compound	EEO661	EEO664
β-Pinene	0.1	—
Sabinene	*t*	—
Myrcene	0.1	0.2
Limonene	0.1	0.3
1,8-Cineole	2.3	0.5
p-Cymene	*t*	0.2
3-Octyl acetate	1.2	0.7
1-Octen-3-yl acetate	0.5	0.3
cis-Linalool oxide[†]	1.2	1.6
trans-Linalool oxide[†]	1.3	1.7
Linalool	55.2	24.9
Linalyl acetate	34.0	57.3
β-Caryophyllene	0.1	0.1
α-Humulene	*t*	*t*
α-Terpineol	1.0	2.8
Germacrene D	0.2	0.8
Geranyl acetate	0.7	1.8
Citronellol	*t*	0.7
Caryophyllene oxide	0.1	0.2

[†]Furanoid form.
t = trace (<0.1%).

TABLE 7.1f
The Enantiomeric Distribution of Constituents of *Mentha citrata* Oil

Enantiomeric Ratio	References
(1S,5S)-(−)-α-pinene (15%–21%):(1R,5R)-(+)-α-pinene (79%–85%)	—
(1S,5S)-(−)-β-pinene (92%–95%):(1R,5R)-(+)-β-pinene (5%–8%)	—
(4S)-(−)-limonene (92%–99%):(4R)-(+)-limonene (1%–8%)	Mosandl et al. (1991); Lawrence (1998)
(3S)-(−)-citronellol (47%):(3R)-(+)-citronellol (53%)	Ravid et al. (1992a)
(3R)-(−)-linalool (>99%):(3S)-(+)-linalool (<0.1%)	Shimizu (1988); Lawrence (1998)
(3R)-(−)-linalyl acetate (>99%):(3S)-(+)-linalyl acetate (<0.1%)	Ravid et al. (1994d); Lawrence (1998)

3-methylpentanol
3-octanol
3-hexenol*
menthol[†]
neomenthol
carvacrol
thymol
acetaldehyde

isovaleraldehyde
furfural
3-hexenyl phenylacetate*
menthyl formate
menthyl acetate
menthyl isovalerate
menthyl hexanoate
menthyl phenylacetate
acetone
3-methylcyclohexanone
6-methyl-5-hepten-2-one
menthone
isomenthone
pulegone
piperitone
formic acid
acetic acid
isovaleric acid
hexanoic acid
heptanoic acid
3-hexenoic acid*

[†]Correct isomer not identified.
*Major component.

As can be seen, the data presented earlier was not specific to a particular geographic source of cornmint. Over the years, commercially important cornmint oil has been available from Japan, Taiwan, Brazil, Paraguay, China, and India with limited quantities from Argentina, Korea, Thailand, and the United States In addition, because of the obscurity of the early literature, a few pertinent studies were not included in the Gildemeister and Hoffmann review, consequently they will be included in this review. For example, Benezet et al. (1947) reported that Brazilian mint oil contained α-pinene, camphene, limonene, furfural, 3-octanol, menthone, neomenthol, β-caryophyllene, menthol (major constituent), formic acid, valeric acid, hexanoic acid, and nonanoic acid. Garnero (1951) further identified borneol in the same oil.

Nakamura et al. (1952) characterized the presence of isocaryophyllene alcohol in a sample of Japanese peppermint oil.

Hiraizume (1959) reported that cornmint oil of Japanese origin contained minor quantities of menthyl formate, 3-hexenyl formate, 3-hexenyl acetate, 3-hexenyl isovalerate, 3-hexenyl hexanoate, and 3-hexenyl phenylacetate as trace constituents in Japanese cornmint oil.

Nagasawa (1959) examined the aliphatic carbonyl content of a Japanese oil and identified acetaldehyde, acetone, 2-butanone, 2-heptanone, 3-methylcyclohexanone, and 6-methyl-5-hepten-2-one as minor constituents.

Kobayashi et al. (1960) reported that trace amounts of δ-3-carene, a piperitone oxide isomer, and octanoic acid could also be found in a sample of Japanese peppermint oil.

Komatsu (1960) determined that the three major alcohols found in dementholized cornmint oil of Japanese origin were 3-octanol, neomenthol, and menthol. It was also determined that for dementholized cornmint oil produced in two regions in Japan, the compounds given here were found in the approximate ratio of 2:3:21, respectively.

Nikolaeva (1964) characterized menthol (73% to 88%), neomenthol, menthone, isomenthone, α-pinene, and 1,8-cineole in the oil of *M. canadensis* (as *M. sachalinensis*) originally

collected from its natural habitat. She also characterized menthol (69% to 91%), menthone, α-pinene, limonene, pulegone, neomenthol, and furfural in *M. canadensis* (as *M. sachalinensis*) originally obtained from Japan where it was grown commercially for oil production and menthol isolation. In addition, the oil of clone MC-41 that was produced by the artificial hybridization of Japanese *M. canadensis* with *M. longifolia* ssp. *longifolia* was found to contain menthol (82.4% to 88.1%), menthone, neomenthol, isomenthone (3.0%), α-pinene, and a piperitone oxide isomer. Nikolaeva recommended that this new hybrid be used as a source of cornmint oil from which menthol could be isolated.

Using colchicine treatment of seeds or seedlings of *M. canadensis* (as *M. arvensis* var. *piperascens*), Ikeda et al. (1966) examined the main components of oils produced from 23 artificial polyploids (tetraploids) once they reached the flowering stage. They found that the oils could be divided into six groups according to their main components. Group 1. Eight clones with menthol (<75%), Group 2. Two clones with menthone (<80%), Group 3. Five clones with menthol (60% to 75%) and menthone (13% to 20%), Group 4. Five clones with menthone (60% to 75%) and menthol (6% to 25%), Group 5. Two clones with pulegone (30% to 40%) and Group 6. One clone with carvone (44%). Oils rich in menthol were used to develop cornmint clones that were grown commercially because of their richness in menthol.

Between 1961 and 1975, commercially available dementholized or partially dementholized cornmint oils of various origins were analyzed by Smith and Levi (1961), Porsch and Farnow (1962), Hawkes and Wheaton (1967), and Hefendehl and Zeigler (1975). A summary of these analyses can be seen in Table 7.2a.

Nakayama et al. (1970) examined the main components of the oils of the "Sanbi," "Hakubi," and "Ryokubi," cultivars of cornmint (Japanese peppermint) grown in Japan. The menthone, menthyl acetate, and menthol contents of "Sanbi," "Hakubi," and "Ryokubi" oils were 7.5%, 1.5%, and 85.7%; 9.1%, 5.6%, and 81.2%; and 7.3%, 1.4%, and 86.4%, respectively. They further reported the development of a new Japanese peppermint (commonly known as "Shubi") by crossing a tetraploid *M. piperita* with *M. canadensis*. Analysis of the oil of this hybrid revealed the presence of α-pinene, β-pinene, myrcene, limonene, 1,8-cineole, isoamyl isovalerate, 3-octanol, *trans*-sabinene hydrate, menthone, menthofuran, isomenthone, (Z)-3-hexenyl isovalerate, neomenthyl acetate, terpinen-4-ol, neomenthol, menthyl acetate, menthol, pulegone, β-caryophyllene, piperitone oxide (no isomer given), piperitone, caryophyllene oxide, α-bourbonene, and menthofurolactone. Unfortunately, the authors did not present much quantitative information; however, from the chromatographic data obtained, they did report that "Shubi" oil contained menthone (21.0%), menthyl acetate (2.8%), and menthol (68.2%).

Ikeda et al. (1971) compared the major components found in the oils of the Ryokubi and Sanbi clones of cornmint grown in Japan. These oils were found to possess the following components:

α-pinene (0%–0.5%)
β-pinene (0.2%–1.1%)
limonene (0.7%–2.3%)
3-octanol (0.5%–0.8%)
menthone (2.8%–6.1%)
isomenthone (0.8%–2.1%)
menthyl acetate (2.7%–9.3%)
neomenthol (0.9%–2.2%)
menthol (76.8%–86.3%)
pulegone (0%–0.8%)
piperitone (1.4%–2.7%)

TABLE 7.2a
Comparative Percentage Composition of Dementholized Cornmint Oils of Various Commercial Origins

Compound	Brazil	China	Japan	Korea	Taiwan
α-Pinene	0.2–1.5	*t*–4.0	0.7–4.0	0.1–4.0	0.1–1.0
Camphene	—	0–0.6	0–0.1	—	—
β-Pinene	*t*–2.5	*t*–4.0	1.3–4.0	0.3–4.0	0.1–1.7
Sabinene	0–1.0	0–1.0	0–1.0	0–1.0	0–1.0
Myrcene	0–1.0	0–1.0	0–1.0	0–1.0	0–1.0
Limonene	4.9–10.0	1.0–8.1	1.0–12.4	0.7–4.0	0.7–6.8
1,8-Cineole	*t*–1.2	*t*–0.2	0.1–1.2	0.1–0.2	0.1–0.6
3-Octanol	0.1–0.7	0.5–4.0	0.2–3.8	0.1–1.0	0.1–1.0
Menthone	21.5–35.0	25.0–29.4	22.0–25.0	19.6	4.0–30.9
Menthofuran	0–0.2	0–0.2	0–0.2	0–0.2	0–0.2
Isomenthone	6.6–10.0	6.8–8.8	2.9–4.0	1.8	2.8–7.9
Linalool	0–0.1	0–0.2	0–0.8	—	0–0.2
Menthyl acetate	1.0–7.4	0.9–7.0	0.9–4.0	1.0–8.3	1.0–9.6
Isopulegol	0–2.0	0–1.0	0–1.0	0–1.0	0–1.0
Neomenthol	1.0–4.6	2.0–5.0	1.0–5.0	1.3–4.0	2.0–10.0
β-Caryophyllene	0–4.0	0–4.0	0–4.0	0–1.0	0–4.0
(E)-β-Farnesene	0–0.2	0–0.2	0–0.2	—	0–1.0
Neoiso(iso)pulegol	0–1.0	0–0.5	—	—	—
Neoisomenthol	0–1.0	0–1.0	—	—	—
Menthol	33.2–53.1	36.0–52.0	36.3–38.9	56.6	33.9–60.8
Isomenthol	0–1.5	0–2.0	—	—	—
Pulegone	0.1–1.5	0–4.9	0.1–1.8	0.2–1.0	0.2–2.1
Germacrene D	0–4.0	0–1.0	0–4.0	0–1.0	0–1.0
Piperitone	1.5–4.4	2.4–6.0	6.4–11.6	1.0–10.4	1.0–8.5

t = trace (<0.1%).

As a potential replacement for growing the opium poppy, the Government of Thailand explored the possibility of growing cornmint for oil production in the northern regions around MaeTho, Chang Khian, Buak Chan, Nong Hoi, and Doi Ang Khang villages in the hills and vicinity of Long Pong village (Chomchalow et al., 1976). Two cultivars "Ryokubi" and "Akamaru" were obtained from Japan and "Shivalik" was obtained from India. The results of the oil analyses of these cultivars produced over two seasons, which were reported by Pichitakul and Sthapitanonda (1977) can be seen in Table 7.2b.

Kak and Kaul (1980) developed a number of mutants of *M. canadensis* (as *M. arvensis* var. *piperascens*) using either x-rays or gamma-rays to develop mutants that could be characterized by either profuse branching as in MA-14 or by increasing the yield/ha from 57 kg/ha (mother clone) to 73 kg/ha and 77 kg/ha for MA-37 and MA-14, respectively. Two other clones that were developed (S-12/1 and S-118/3) possessed oils in which menthol content was 78% to 80%. Other mutants developed were RL-85/4 and RL-78, oils of which were rich in pulegone (55% and 45%, respectively), and one mutant, the oil of which contained menthone (45%).

Sakata and Koshimizu (1980) sampled Japanese peppermint (cornmint) plants over a range of development stages for their menthyl β-D-glucopyranoside, menthone, and menthol contents. They determined that to obtain the highest oil yield, the plant should be harvested at the preflowering stage.

TABLE 7.2b
Percentage Composition of the Oils of Three Cultivars Grown in Thailand

Compound	Ryokubi Oil	Akamaru Oil	Shivalik Oil
α-Pinene	0.4–0.6	0.6–0.9	0.6
β-Pinene	0.9–1.4	1.5–1.8	1.5
Limonene	1.6–1.8	1.7–2.0	2.4–2.7
3-Octanol	0.6–0.9	0.2	0.8
Linalool	0.2–0.5	0.2–0.3	0.1
Menthone	5.1–5.3	10.5–19.3	6.1–7.4
Menthyl acetate	2.7	11.3–21.9	9.1–11.9
Neomenthol	2.0–2.6	3.1–4.1	2.6–4.0
Menthol	81.9–82.8	53.2–55.4	67.7–73.5
Isomenthol	0.7–0.8	0.3–0.5	0.6–0.7
Pulegone	1.0–1.5	0.4–0.5	1.0–1.1
Piperitone	0.9	3.7–4.3	1.1–1.3

Sakata et al. (1982) reported the analysis of four new hybrid clones that were rich in menthol. The clones in question were "Hokkai" 19 ($2n = 120$), "Hokkai" 20 ($2n = 120$), "Wasenami" ($2n = 72$), and "Sayakaze" ($2n = 120$). Sakata et al. noted that "Hokkai" 19 and "Hokkai" 20 were complex hybrids between a menthone-rich crisp-leafed clone of *M. spicata* and *M. canadensis* (as *M. arvensis* var. *piperascens*). They noted that the main components of "Hokkai" 20 oil were as follows:

α-pinene (0.1%)
β-pinene (0.3%)
myrcene (0.2%)
limonene (0.3%)
β-ocimene* (0.1%)
γ-terpinene (0.3%)
3-octanol (0.5%)
menthone (12.7%)
isomenthone (4.0%)
β-bourbonene (0.4%)
menthyl acetate (4.2%)
neomenthol (1.7%)
β-caryophyllene (1.2%)
menthol (69.1%)
piperitone (4.0%)
1-acetoxymenthone (0.4%)

*Correct isomer not identified.

They also found that "Hokkai" 19 oil contained 1-acetoxymenthone.

These new sources of "cornmint oil" are of interest because of their pedigree, because at one time it was fairly safe to say that the detection of dementholized cornmint oil adulteration of peppermint oil was almost routine. The reason for this statement is that cornmint oil does not contain any more than trace amounts of *trans*-sabinene hydrate, menthofuran, and viridiflorol. Therefore, attention is directed toward those particular compounds when

examining the authenticity of peppermint oil. With the new pedigree of "cornmint" like "Hokkai" 20, the amount of *trans*-sabinene hydrate, menthofuran, and viridiflorol present in "Hokkai" 20 has now changed in comparison to the original *M. canadensis* (as *M. arvensis* var. *piperascens*). In fact, it is quite possible that these compounds cited earlier are not valid as indicators of adulteration of dementholized "Hokkai" 20 in true peppermint oil. Fortunately, in this instance one can use the occurrence of 1-acetoxymenthone as an indicator of the addition of either "Hokkai" 19 or "Hokkai" 20 to peppermint oil.

Peiris et al. (1982) compared the composition of cornmint oils produced from plants harvested at three distinct development stages in Sri Lanka. Their results are summarized in Table 7.2c.

As can be seen, there was a dramatic change in the menthone to menthol in the oil as the plant reached its full flowering stage.

Lawrence (1983) compared the composition of a number of samples of crude oils of cornmint and samples of dementholized oils, all of Indian origin. The results of these analyses are shown in Table 7.2d.

Bicchi et al. (1989) compared the headspace composition of healthy and diseased cornmint clone 701 (as *M. arvensis* var. *piperascens*) plants. The comparative compositions are shown in Table 7.2e. As can be seen, there were major quantitative differences found between the headspace obtained from healthy and diseased plants. In particular, the healthy plants were richer in menthol whereas the diseased plants were richer in menthyl acetate.

TABLE 7.2c
Percentage Composition of Cornmint Oils Produced in Sri Lanka from Plants Harvested at Different Development Times

Compound	Vegetative Stage (2 Months)	Full Blooming Stage (3 Months)	Postflowering Stage (4 Months)
α-Pinene	0.1	0.1	0.9
Camphene	*t*	*t*	*t*
β-Pinene	0.1	0.2	1.2
α-Phellandrene	0.2	0.2	*t*
Limonene	1.4	1.1	1.9
γ-Terpinene	*t*	*t*	*t*
p-Cymene	*t*	—	*t*
3-Hexenol*	*t*	—	0.1
3-Octanol	1.0	0.8	0.7
Menthone	81.4	4.8	6.8
Isomenthone	8.3	2.7	2.5
Linalool	0.4	0.3	0.2
Menthyl acetate	0.3	—	3.3
Piperitone oxide*	1.9	3.3	9.4
β-Caryophyllene	*t*	*t*	*t*
Menthol	0.6	77.7	70.0
Pulegone	0.3	0.5	1.1
Carvone	0.8	0.6	0.4
Piperitone	4.1	2.1	1.7

*Correct isomer not identified.
t = trace (<0.01%).

TABLE 7.2d
Chemical Composition (%) of Crude and Dementholized Cornmint Oils

Compound	Crude Oil	Dementholized Oil
α-Pinene	0.6–0.9	0.5–1.8
Camphene	*t*	*t*
β-Pinene	0.6–0.9	0.5–2.0
Sabinene	0.2–0.4	0.2–0.7
Myrcene	0.4–0.7	0.1–0.8
Limonene	0.7–6.2	2.7–4.7
1,8-Cineole + (Z)-β-ocimene	0.1–0.3	0.2–0.8
γ-Terpinene	*t*–0.1	*t*–0.1
p-Cymene	*t*–0.1	*t*–0.1
3-Octanol	0.2–0.8	0.3–1.8
1-Octen-3-yl acetate	*t*–0.1	*t*–0.1
1-Octen-3-ol	*t*–0.1	*t*–0.1
Menthone	3.4–14.9	16.5–30.9
Menthofuran	*t*–0.3	0.4–0.8
Isomenthone	1.9–4.8	7.0–12.5
β-Bourbonene	0.1–0.2	0.3–0.5
Neomenthyl acetate	*t*	*t*–0.1
Linalool	*t*–0.1	0.1–0.3
Menthyl acetate	1.0–2.6	1.9–5.5
Isopulegol	0.1–0.2	0.3–0.4
Neoisomenthyl acetate	0.6–0.8	0.9–2.0
Neomenthol	0.9–1.6	2.5–2.9
β-Caryophyllene + terpinen-4-ol	0.6–1.6	2.1–5.1
Neoiso(iso)pulegol	0.3–0.8	0.5–2.1
Neoisomenthol	*t*–0.3	0.2–0.9
Menthol	65.4–80.3	34.8–40.1
Pulegone	0.2–0.5	0.2–3.4
(E)-β-Farnesene	*t*–0.1	*t*–0.3
α-Terpineol	0.1–0.2	0.1–0.5
Germacrene D	0.3–1.0	0.1–1.3
Piperitone	0.2–1.7	0.5–3.8
p-Cymen-8-ol	*t*–0.1	*t*–0.1
Piperitenone	*t*	*t*–0.1
Caryophyllene oxide	0.1–0.2	0.1–1.2

t = trace (<0.1%).

The headspace composition of *M. canadensis* (as *M. arvensis* f. *piperascens*) was determined by Osaki (1992) and the following constituents were identified:

dimethylsulphide (0.2%)
(E)-2-hexenal (0.4%)
(Z)-3-hexenol (1.2%)
(Z)-3-hexenyl acetate (0.1%)
methanol (0.8%)
3-octanol (3.3%)
α-pinene (3.8%)

TABLE 7.2e
Comparative Percentage Headspace Composition of Living Healthy and Diseased Cornmint Plants

Compound	Healthy Plant Headspace	Diseased Plant Headspace
3-Hexenal*	—	0.3
(Z)-3-Hexenol	0.8	3.2
α-Pinene	—	*t*
Sabinene	*t*	*t*
β-Pinene	—	0.3
Myrcene	—	0.6
(Z)-3-Hexenyl acetate	*t*	1.9
(E)-3-Hexenyl acetate	—	*t*
Limonene	—	0.4
β-Ocimene*	*t*	*t*
γ-Terpinene	—	*t*
Menthone	2.1	3.7
Isomenthone	2.1	1.4
Isomenthol	1.9	1.6
Menthol	66.7	33.7
Neomenthol	*t*	*t*
(Z)-3-Hexenyl propionate	*t*	*t*
Piperitone	1.5	4.7
Isomenthyl acetate	*t*	0.6
Menthyl acetate	9.5	31.3
Neomenthyl acetate	*t*	0.5
β-Bourbonene	*t*	0.5
β-Caryophyllene	0.4	1.4
(E)-β-Farnesene	1.5	3.4
Germacrene D	2.3	0.4

*Correct isomer not identified.
t = trace (<0.1%).

β-pinene (1.8%)
myrcene (3.2%)
sabinene (1.8%)
limonene (14.1%)
(Z)-β-ocimene (0.1%)
(E)-β-ocimene (0.2%)
β-caryophyllene (0.6%)
1,8-cineole (0.3%)
menthone (8.7%)
linalool (0.2%)
menthol (35.1%)
pulegone (2.1%)
carvone (0.2%)
piperitone (6.5%)
piperitenone oxide (0.1%)

Trace amounts of methyl 2-methylbutyrate, ethyl 2-methylbutyrate, and γ-terpinene were also found as components of the headspace volatiles.

The results of an analysis of cornmint oil produced in Russia from three cornmint cultivars were reported by Voronina et al. (1990). A summary of their data can be seen in Table 7.2f. There was no explanation for the lack of monoterpene hydrocarbons in Vniiemk 20. The authors recommended that this latter cultivar be grown for menthol production.

Topalov and Zheljazkov (1991) compared the composition of cornmint oils produced from plants grown in Bulgaria that were harvested at 50% flowering, 100% flowering, and post-flowering plants. The results of this study are shown in Table 7.2g. As can be seen, the oils contained moderate levels of menthofuran. This would indicate that the taxonomic origin of this oil was from a hybrid plant rather than *M. canadensis* in which menthofuran content rarely exceeds 0.2%.

Gasic et al. (1992) examined the differences in chemical composition of cornmint oil produced from seven different cultivars grown in Yugoslavia. The range of composition of selected compounds over two harvesting seasons can be seen in Table 7.2h.

Over a 6-year period, the menthol content of cornmint oil produced from plants grown in Serbia was reported by Adamovic (1993) to range from 71% to 82%. He also reported that the menthol content of oils produced from six genotypes ranged from 74% to 81%. In 1978, Central Institute for Medicinal and Aromatic Plants, Lucknow (CIMAP), embarked on a genetic improvement of *M. canadensis* (as *M. arvensis* var. *piperascens*) grown in India. In addition to making a worldwide collection of germplasm, a budsport of a strain of Thai origin was subjected to multiple selectional studies; as a result, a strain (cultivar) known as MAS-1

TABLE 7.2f
Comparative Chemical Composition (%) of Russian Cornmint Oil Produced from Three Different Cultivars

	Cultivars		
Compound	**Simferopolskaya 200**	**Vniiemk 20**	**Standard**
α-Thujene	0.1	—	0.1
α-Pinene	0.1	0.1	0.2
Camphene	0.3	—	0.2
Sabinene	0.2	—	0.5
Myrcene	*t*	—	0.1
1,8-Cineole	1.9	0.1	0.8
(Z)-β-Ocimene	0.1	—	0.1
Linalool	0.1	0.1	0.1
Menthone	9.3	5.8	11.2
Isomenthone	4.1	0.9	2.5
Menthofuran	0.1	—	—
Neomenthol	1.1	1.2	2.3
Menthol	76.0	83.2	80.0
Isomenthol	0.2	0.2	0.2
Carvotanacetone*	1.8	0.1	0.2
Piperitone	0.9	2.0	0.3
Menthyl acetate	1.7	3.9	0.7
β-Caryophyllene	0.1	1.4	0.2
α-Humulene	0.1	1.1	0.8

*Questionable identity.

TABLE 7.2g
Comparative Percentage Composition of Cornmint Oil Produced from Plants Harvested with Different Amounts of Flowers Formed

Compound	Oil from 50% Flowering Plants	Oil from 100% Flowering Plants	Oil from Postflowering Plants
α-Pinene	0.4	0.2	0.4
Sabinene	0.1	0.4	0.3
β-Pinene	0.3	0.1	0.4
Myrcene	0.4	0.3	0.4
Limonene	0.7	0.5	0.1
1,8-Cineole	0.1	0.5	0.1
γ-Terpinene	0.1	0.1	0.1
p-Cymene	<0.1	0.1	<0.1
Hexanol	0.2	0.2	0.2
Menthone	12.8	11.7	6.1
Menthofuran	11.8	12.2	10.3
Isomenthone	0.3	0.3	0.4
Menthyl acetate	5.4	6.4	15.4
β-Caryophyllene	1.6	1.2	1.3
Neomenthol	2.2	2.6	2.7
Neoisomenthol	0.6	0.6	0.7
Menthol	59.3	59.7	57.8
Pulegone	1.1	1.3	1.1
Carvone	0.3	0.2	0.4

was released to farmers in 1983. Two years later, a second cultivar known as "HY-77" was released to farmers in 1985 (Hussain, 1993). It was found that the oil yield and menthol content between the standard cultivar (MA-2) that was grown at that time, "MAS-1" and "HY-77" was 183 kg/ha and 66%, 316 kg/ha and 83%, and 428 kg/ha and 81%, respectively.

TABLE 7.2h
Comparative Range (%) of Selected Components over Two Consecutive Harvesting Seasons*

Compound	First Season	Second Season
α-Pinene	0.1–1.5	*t*–1.4
β-Pinene	0.2–2.9	0.1–1.8
Limonene	0.6–8.4	0.5–17.7
1,8-Cineole	*t*–2.6	*t*–1.8
trans-Sabinene hydrate	0–0.8	0–0.6
Isomenthone	0.1–6.0	0.5–4.1
Neomenthol + neoisomenthol	2.4–7.1	3.0–12.3
Menthol	56.7–80.2	63.4–75.3
Pulegone	0.3–4.0	1.2–2.2
Piperitone	0.1–5.9	0.2–4.7

t = trace (<0.1%).
*Eight clones.

TABLE 7.2i
Average Composition (90%) of Seven Selected Constituents of Indian and Chinese Dementholized Cornmint Oils (DMO)

Compound	Indian DMO	Chinese DMO
Limonene	2.200	2.875
1,8-Cineole	0.500	0.440
trans-Sabinene hydrate	0.005	0.022
Isomenthone	10.270	9.200
Isopulegol	2.020	1.218
Neoiso(iso)pulegol	1.800	1.030
Piperitone	1.080	1.406

Spencer et al. (1997) reported the average amounts of seven selected constituents in Indian and Chinese dementholized cornmint oil. These results can be seen in Table 7.2i.

Machale et al. (1997) determined that the distillation water obtained during the steam distillation of cornmint was found to contain the following:

limonene (*t*)
1,8-cineole (15 ppm)
(Z)-3-hexenol (8.3 ppm)
3-octanol (*t*)
menthone (284 ppm)
menthofuran (*t*)
menthyl acetate (94 ppm)
neomenthol (203 ppm)
menthol (689 ppm)
piperitone (*t*)

t = trace (<10 ppm)

Rajeswara Rao et al. (1999) studied the composition of cornmint oil produced from different plant parts (shoot stem, shoot leaf, stolon stem, and stolon leaf) of *M. canadensis* (as *M. arvensis* f. *piperascens*) grown under the semiarid tropical climatic conditions experienced in Hyderabad. The results of this study are found in Table 7.2j. As can be seen, there were some variations in the menthol, menthone, pulegone, and menthyl acetate contents depending on the part of the plant from which the oil was obtained.

The composition of Indian cornmint oils produced from cultivars grown in the Hyderabad region of Andhra Pradesh with oils produced from the same cultivars grown in the Sambhal region of Uttar Pradesh was the subject of analysis by Rajeswara Rao et al. (1999). The results of this study are summarized in Table 7.2k. Examination of this data revealed that the menthol content of the oil of the Shivalik cultivar grown in Uttar Pradesh was higher, whereas the other cultivars appeared to be well suited to the semiarid tropical climate because their oils were richer in menthol than the Shivalik cultivar.

In India, a number of cultivars of cornmint are grown commercially. Before 1990, "Shivalik" was the main cultivar grown; however, throughout the 1990s cultivars such as MAS-1, Kalka, Gomti, Himalaya (introduced in 1996), and Kosi (introduced in 1997) have all been grown commercially for oil production (Gupta, 2000; Kumar et al., 2000). A summary of the oil contents of these various cultivars harvested at different development stages can be seen in

TABLE 7.2j
Percentage Comparison of the Oils of Different Parts of Cornmint

Compound	Shoot Leaf	Shoot Stem	Stolon Stem	Stolon Leaf
(Z)-3-Hexenol	0.2	*t*	*t*	0.1
α-Thujene	—	—	—	*t*
α-Pinene	0.5	0.1	0.4	0.6
Camphene	—	—	—	*t*
Sabinene	0.2	0.1	0.2	0.3
β-Pinene	0.6	0.1	0.4	0.7
Myrcene	1.3	0.3	0.5	1.1
3-Octanol	0.3	0.1	0.1	0.2
α-Phellandrene	—	—	*t*	*t*
p-Cymene	*t*	*t*	*t*	*t*
Limonene	1.5	0.3	3.1	3.7
1,8-Cineole	0.2	0.1	0.2	0.1
(Z)-β-Ocimene	0.1	*t*	*t*	0.1
(E)-β-Ocimene	0.1	*t*	*t*	0.1
trans-Sabinene hydrate	0.1	0.1	0.1	0.1
Terpinolene	—	—	*t*	*t*
Linalool	*t*	0.1	0.1	0.1
Menthone	12.6	4.6	13.0	17.6
Isomenthone	3.1	2.1	2.0	3.2
Neomenthol	2.0	1.8	2.0	1.6
Menthol	69.8	78.2	43.7	60.5
α-Terpineol	*t*	0.1	0.1	0.1
Pulegone	0.3	0.6	22.1	3.1
Piperitone	0.7	0.5	0.3	0.7
Geraniol	0.1	0.1	5	0.1
Menthyl acetate	3.9	7.2	8.3	3.8
β-Elemene	0.1	*t*	0.2	0.1
β-Caryophyllene	0.2	0.2	0.2	0.2
Germacrene D	0.1	0.1	0.1	0.2
γ-Cadinene	0.2	0.1	0.1	0.1
(E)-Nerolidol	0.1	0.2	0.3	*t*
Caryophyllene oxide	0.1	*t*	—	—
Oil yield (%)	0.62	0.02	0.02	0.35

t = trace (<0.1%).

Table 7.2l. In addition, a comparison of the major components found in oils of each of the Indian cultivars harvested at normal harvest time (first) and again at the second cutting of the ratoon crop (second) is shown in Table 7.2m. Rajeswara Rao et al. (2000) compared the composition of an oil produced from the whole aboveground herbage of the Shivalik cultivar of cornmint with the oils produced from the flowers, leaves, and stems of the same cultivar. Their results can be seen in Table 7.2n.

Lawrence (2001) compared the main component composition of dementholized cornmint oil of Indian and Chinese origins. An example of oil analyses from bulked commercial samples can be seen in Table 7.2o. As can be seen, the differences were found to be minor although oils could be differentiated based on the isopulegol and neoiso(iso)pulegol contents

TABLE 7.2k
Percentage Composition of Oils Produced from Different Cultivars in Different Regions

	Cultivar					
Compound	**Gomti (S)**	**MAS-1 (S)**	**HY-77 (S)**	**Shivalik (S)**	**Fresh Shivalik Oil (N)**	**Commercial Shivalik Oil (N)**
(E)-2-Hexenal	—	0.1	—	—	—	—
(Z)-3-Hexenol	0.1	0.3	—	—	—	<0.1
α-Pinene	0.7	0.6	0.6	0.6	0.6	0.6
Camphene	—	—	—	—	<0.1	0.1
Sabinene + 1-octen-3-ol	0.2	0.2	0.2	0.2	0.3	0.3
β-Pinene	0.7	0.6	0.6	0.5	0.7	—
Myrcene + 3-octanol	1.4	0.9	1.0	1.1	1.1	1.1
Limonene + 1,8-cineole	0.9	0.7	2.0	2.0	1.0	1.8
(E)-β-Ocimene	—	—	—	—	<0.1	—
trans-Sabinene hydrate	—	—	<0.1	—	0.1	—
Terpinolene	—	0.1	—	—	<0.1	—
Linalool	0.2	0.3	0.2	0.3	0.1	—
Menthone	4.7	1.7	7.6	10.2	7.6	9.6
Isopulegol	—	—	—	—	0.1	—
Isomenthone	2.2	1.5	1.8	2.0	4.9	4.3
Neomenthol	1.6	1.7	1.6	1.6	2.1	1.7
Menthol	78.8	87.2	73.6	70.5	75.5	70.4
Isomenthol + α-terpineol	0.2	0.2	0.1	0.1	1.2	0.2
Pulegone	0.5	1.3	5.9	6.6	0.4	0.5
Piperitone	0.8	0.8	0.5	0.5	0.3	0.3
Menthyl acetate	5.3	0.2	0.2	—	1.7	1.2
β-Caryophyllene	0.4	0.3	0.3	0.4	0.5	0.6
Germacrene D	—	—	0.2	0.5	0.6	0.6
(E)-Nerolidol	—	—	0.1	—	—	—

N, Production in Sambhal (Uttar Pradesh); S, production in Hyderabad (Andhra Pradesh).

and their component ratios. In addition, trace amounts (<0.1%) of α-terpinene, (Z)-β-ocimene, (E)-β-ocimene, γ-terpinene, 3-octanone, 3-heptanol, β-bourbonene, borneol, dihydrocarveol, isopiperitenol, citronellol, methyl salicylate, *p*-cymen-8-ol, geranyl acetate, (Z)-jasmone, caryophyllene oxide, eugenol, thymol, and (Z)-3-hexenyl phenylacetate were also found in the same oil.

Singh et al. (2001) reported that the oil congealing (crystallizing) point was the most important criterion used for determining the price that a buyer would give to the farmer in India for his oil. Because the congealing point is affected, not only by menthol, but also by the other constituents of the oil, the authors obtained some partially dementholized oils of seven cultivars (Shivalik, Gomti, Kalka, SS-1-4, Himalaya, Kosi, and MAS-1) and determined their major constituents (menthol, neomenthol, menthone, isomenthone, menthyl acetate, and other components). A summary of the analyses can be seen in Table 7.2p. A congealing point was determined for each of these oils after which incremental amounts of menthol were added to each of the oils and their analyses and congealing points were obtained. From the data obtained, linear correlation coefficients and regression equations were calculated for

TABLE 7.2l
Oil Content (%) of Whole Plants of Six Cornmint Cultivars Harvested at Different Developmental Stages

Growth Stage at Harvest for Oil Production	Cultivars					
	MAS-1	Kalka	Shivalik	Gomti	Himalaya	Kosi
Plants from previous season with early sucker production (Stage 1)	0.2	0.5	0.3	0.2	0.2	0.2
Plants from previous season with late sucker production (Stage 2)	0.4	0.5	0.5	0.4	0.3	0.4
Main crop early harvest time (Stage 3)	0.3	0.4	0.4	0.2	0.6	0.5
Main crop normal harvest time (Stage 4)	0.5	0.6	0.6	0.4	0.7	0.7
Main crop late harvest time (Stage 5)	0.5	0.5	0.8	0.6	0.7	0.7
Second cutting (ratoon crop) early harvest time (Stage 6)	0.6	0.8	1.2	0.9	1.2	1.3
Second cutting (ratoon crop) late harvest time (Stage 7)	0.5	0.7	1.0	0.9	1.1	1.2

the congealing points and the three most important constituents, namely menthol, menthone, and menthyl acetate. To make this computation more user friendly and not cultivar specific, the authors pooled the data and combined these with a larger set of data obtained from increased sample size. They found that there was a significant positive correlation between the congealing point and the menthol content and a negative correlation between the congealing point with menthone and menthyl acetate. As a result, they were able to develop a universal ready reference chart irrespective of cultivar. This can be seen in Table 7.2q.

A review of these data reveals that the average difference between the menthol, menthone, and menthyl acetate contents for each degree (°C) change in the congealing

TABLE 7.2m
Comparative Percentage Composition of the Main Components of Various Indian Cornmint Cultivar Oils

	MAS-1		Kalka		Shivalik		Gomti		Himalaya		Kosi (%)	
Compound	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Limonene	1.4	2.6	2.1	3.8	1.1	1.6	0.9	1.5	1.8	2.1	1.2	3.6
3-Octanol	0.7	0.6	0.6	0.5	0.6	0.7	1.1	1.2	0.9	1.0	0.4	0.2
Menthone	3.6	4.1	3.2	4.4	7.4	8.5	12.8	17.7	0.5	8.2	3.4	7.0
Isomenthone	1.4	1.8	2.0	2.3	4.3	5.5	3.4	3.8	3.1	3.4	3.1	3.6
Menthyl acetate	0.5	0.6	0.8	0.6	1.7	1.6	3.0	1.0	1.0	1.9	4.5	3.2
Neomenthol	2.6	2.2	2.4	1.9	1.9	2.0	1.8	2.1	1.9	2.1	1.9	2.3
Menthol	85.6	80.5	80.7	76.3	75.3	71.0	71.3	63.3	73.2	73.2	78.7	69.6
Isomenthol	0.4	0.4	0.3	0.3	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.4

1st, normal harvest time; 2nd, second cutting harvest time.

TABLE 7.2n
Percentage Composition of the Oils of the Flowering Whole Plant, Flowers, Leaves, and Stems of the "Shivalik" Cultivar of Cornmint

Compound	Flowering Whole Plant Oil	Flower Oil	Leaf Oil	Stem Oil
α-Pinene	0.6	0.6	0.5	*t*
Sabinene	0.2	0.3	0.2	*t*
β-Pinene	0.6	0.6	0.5	*t*
Myrcene + 3-octanol	1.6	1.7	1.6	0.5
Limonene + 1,8-cineole	1.2	1.9	1.1	0.2
(Z)-β-Ocimene	0.1	0.1	0.1	*t*
(E)-β-Ocimene	—	0.1	0.1	*t*
trans-Sabinene hydrate	0.1	0.1	0.1	*t*
Linalool	0.1	0.2	*t*	0.1
Menthone	13.9	30.2	14.9	5.2
Isomenthone	3.0	3.5	3.0	2.1
Neomenthol	1.7	2.0	1.8	0.9
Menthol	68.4	53.2	71.1	82.3
Isomenthol + α-terpineol	*t*	0.1	0.1	0.1
Pulegone	0.4	0.3	0.4	0.2
Piperitone	0.7	0.7	0.7	0.5
Menthyl acetate	0.7	0.1	0.3	3.4
β-Cubebene	0.1	0.1	0.1	0.1
β-Elemene	0.1	0.1	0.2	0.1
β-Caryophyllene	0.5	0.8	0.6	0.7
Germacrene D	0.5	0.6	0.4	0.5
γ-Cadinene	0.1	—	*t*	—
δ-Cadinene	—	0.1	*t*	0.1
(E)-Nerolidol	0.3	0.1	0.1	0.1
Caryophyllene oxide	—	—	*t*	*t*

t = trace (<0.1%).

point is 1.19%, 0.54%, and 0.12%, respectively. Consequently, if a congealing point of 13.3°C was obtained for a cornmint oil, then the menthol content would be (69.73 + 0.36) 69.09%, the menthone content would be (13.62–0.16) 13.45%, and the menthyl acetate content would be (2.83–0.04) 2.87%.

The Kalka cornmint cultivar was grown in the open (unshaded) and under the partial shade of poplar trees by Singh et al. (2002a). They noted that as cornmint is a long day plant that flowers profusely during the summer, cultivation under partial shade decreases the proportion of flowers produced, which, in turn, results in a lower menthone content (Table 7.2r) and a higher menthol content. Unfortunately, the authors did not comment on the effect (if any) of partial shade on oil content.

Rajeswara Rao (2002) compared the composition of cornmint oil produced from plants that were grown as a monoculture or as an intercrop with rose-scented geranium in the vicinity of Hyderabad (India). The results of this study can be seen in Table 7.2s. The authors found that the differences in oil yield of cornmint were not significant between different methods of culture. It should be noted, however, that the intercropping study was not done to support cornmint oil production but to support rose-scented geranium oil production.

TABLE 7.2o
Comparative Percentage Composition of Indian and Chinese Dementholized Cornmint Oils (DMO)

Compound	Indian DMO	Chinese DMO
α-Pinene	1.8	5.0
Camphene	0.2	0.1
β-Pinene	2.1	2.6
Sabinene	0.8	0.6
δ-3-Carene	0.4	*t*
Myrcene	1.1	0.9
Limonene	3.1	4.2
1,8-Cineole	0.9	0.7
p-Cymene	0.1	*t*
Terpinolene	0.1	*t*
(Z)-3-Hexenol	0.1	*t*
3-Octanol	2.5	2.7
Menthone	19.4	20.2
Octyl acetate	0.3	0.3
Isomenthone	12.2	9.3
Linalool	0.2	*t*
Menthyl acetate	4.6	4.0
Isopulegol	1.2	2.0
cis-Isopulegone	0.9	*t*
Neomenthol	5.6	5.4
Neoiso(iso)pulegol	2.7	1.2
Neoisomenthol	1.2	1.2
Menthol	32.6	31.0
Isomenthol	0.4	0.2
Pulegone	0.7	1.2
Lavandulol	1.0	1.1
Decyl acetate	0.2	0.2
α-Terpineol	0.6	0.7
Germacrene D	1.1	0.6
Piperitone	1.0	1.7
Carvone	0.2	0.9
δ-Cadinene	0.1	*t*
Decanol	0.1	*t*
Piperitenone oxide	*t*	0.2

t = trace (<0.1%).

The minor constituents that have been found in some of the studies performed in cornmint oil over the past 44 years can be seen categorized according to the class of compound such as hydrocarbons, alcohols, esters, aldehydes, ketones, acids, phenols, nitrogen heterocyclic compounds, and miscellaneous compounds.

7.2.2.1 Hydrocarbons

The monoterpene hydrocarbons routinely found in cornmint oil are α-pinene, camphene, β-pinene, sabinene, myrcene, limonene, (Z)-β-ocimene, (E)-β-ocimene, γ-terpinene, *p*-cymene, and terpinolene in various cornmint oils. In addition, the sesquiterpene hydrocarbons such as

TABLE 7.2p
Comparative Percentage Composition of Selected Constituents of the Partially Dementholized Oils of Seven Indian Cornmint Cultivars

Compound	Shivalik	Gomti	Kalka	SS-1-4	Himalaya	Kosi	MAS-1
Menthone	20.6	34.2	12.7	21.9	17.7	11.8	28.0
Isomenthone	10.2	8.3	7.7	12.0	8.9	6.7	4.6
Menthyl acetate	1.6	1.6	0.9	6.8	2.8	3.8	6.7
Neomenthol	7.0	5.2	8.8	<0.1	8.1	3.8	3.2
Menthol	56.4	45.0	63.8	53.4	45.9	65.8	41.6

β-caryophyllene, (E)-β-farnesene, β-elemene, β-bourbonene, germacrene D, and δ-cadinene have also been routinely characterized in cornmint oil.

Since 1960, 25 other aliphatic, monoterpene, and sesquiterpene hydrocarbons have been occasionally characterized in this commercially important oil (see Table 7.2t). A summary of the data reveals that 41 hydrocarbons have been identified in cornmint oil.

TABLE 7.2q
The Relationship between the Congealing Point of Cornmint Oil and the Menthol, Menthone, and Menthyl Acetate Contents (%)

Congealing Point (°C)	Menthol	Menthone	Menthyl Acetate
3	56.84	18.94	4.05
4	58.02	18.42	3.93
5	59.22	17.88	3.81
6	60.40	17.35	3.69
7	61.59	16.82	3.56
8	62.78	16.28	3.44
9	63.97	15.75	3.32
10	65.16	15.21	3.20
11	66.35	14.68	3.08
12	67.54	14.14	2.95
13	68.73	13.61	2.83
14	69.92	13.07	2.71
15	71.11	12.54	2.59
16	72.30	12.00	2.47
17	73.49	11.47	2.34
18	74.68	10.93	2.22
19	75.87	10.40	2.10
20	77.06	9.87	1.98
21	78.25	9.33	1.86
22	79.44	8.79	1.73
23	80.63	8.26	1.61
24	81.82	7.72	1.49
25	83.01	7.19	1.37
26	84.19	6.65	1.25
27	85.38	6.12	1.12

TABLE 7.2r
Comparative Percentage Composition of Cornmint Oil Produced from the Kalka Cultivar Grown in the Open and in Partial Shade

Compound	Oil from Unshaded Plants	Oil from Partially Shaded Plants
(Z)-3-Hexenol	0.1	0.2
α-Pinene	0.7	0.8
Sabinene	0.3	0.3
β-Pinene	0.7	0.9
3-Octanone	*t*	*t*
Myrcene	0.5	0.7
3-Octanol	0.6	0.3
α-Phellandrene	*t*	*t*
p-Cymene	*t*	—
Limonene + 1,8-cineole	2.0	2.7
(Z)-β-Ocimene	0.3	0.2
(E)-β-Ocimene	0.1	*t*
trans-Sabinene hydrate	0.1	0.1
Linalool	0.1	0.1
Isopulegol	0.5	0.8
Menthone	11.7	4.8
Isomenthone	5.0	5.7
Menthol	70.9	78.2
Isomenthol	0.2	0.2
α-Terpineol	0.2	0.1
Citronellol	0.2	0.3
Pulegone	0.2	0.1
Piperitone	0.5	0.3
Neomenthyl acetate	*t*	*t*
Menthyl acetate	1.9	1.8
Isomenthyl acetate	0.1	0.1
β-Caryophyllene	0.4	0.2
Germacrene D	0.4	0.3
δ-Cadinene	0.1	*t*
Caryophyllene oxide	0.1	—

t = trace (<0.1%).

The reports that *p*-menth-4(8)-ene was a component of cornmint oil (Gildemeister and Hoffmann, 1961) could not be confirmed from the recent (1960–2004) literature.

7.2.2.2 Alcohols

The alcohols routinely found as constituents of cornmint oil are (Z)-3-hexenol, 3-octanol, linalool, terpinen-4-ol, α-terpineol, isopulegol, neoiso(iso)pulegol, neomenthol, neoisomenthol, menthol, and isomenthol. Since 1960, 47 other alcohols have been occasionally characterized in this commercially important oil (see Table 7.2u). In summary, it would appear that 58 alcohols have been identified in cornmint oil. It should be noted that the iso(iso)pulegol characterized by Lawrence et al. (1989) and Kubeczka and Formacek (2002) was a misidentification of neoiso(iso)pulegol. The report noting that butanol (Gildemeister and

TABLE 7.2s
Comparative Percentage Composition of Cornmint Oil Produced from a Monoculture or Intercropped Plants

Compound	Monoculture Oil	Intercrop Oil
α-Pinene	0.7	0.6
β-Pinene	0.6	0.3
Myrcene + 3-octanol	1.6	1.4
Limonene + 1,8-cineole	1.5	1.2
(Z)-β-Ocimene	0.1	0.1
(E)-β-Ocimene	0.1	0.1
trans-Sabinene hydrate	0.1	0.1
Linalool	0.2	0.3
Menthone	9.0	9.5
Isomenthone	2.1	2.8
Neomenthol	1.7	1.2
Menthol	75.2	74.5
Isomenthol	0.1	0.2
Pulegone	1.3	1.9
Piperitone	0.5	0.8
Menthyl acetate	3.8	3.0
β-Caryophyllene	0.3	0.4
Germacrene D	0.1	0.2
(E)-Nerolidol	0.1	0.1

Hoffmann, 1961) and isocaryophyllene alcohol (Nakamura, 1952) as cornmint components could not be confirmed from the recent (1960–2004) literature.

7.2.2.3 Esters

The esters that have been routinely characterized as constituents of cornmint oil are 3-octyl acetate, menthyl acetate, neomenthyl acetate, and neoisomenthyl acetate. Since 1960, 26 other esters have been occasionally characterized in this commercially important oil as shown in Table 7.2v. Based on the known data, it would appear that a total of 30 esters have been identified in cornmint oil.

The identification of menthyl formate, 3-hexenyl formate, and 3-hexenyl hexanoate (Hiraizume, 1959) and menthyl isovalerate, menthyl hexanoate, and menthyl phenylacetate (Gildemeister and Hoffmann, 1961) could not be confirmed from the recent (1960–2004) literature.

7.2.2.4 Aldehydes

No aldehydes have been routinely characterized in cornmint oil above the trace (<0.1%) level. Since 1960, a total of 13 aliphatic aldehydes have occasionally been characterized in this commercially important oil (see Table 7.2w).

7.2.2.5 Ketones

The ketones that have been found as constituents of cornmint oil are menthone, isomenthone, pulegone, and piperitone. Since 1960, 18 aliphatic and monoterpene ketones have been

TABLE 7.2t
Additional Aliphatic Monoterpene and Sesquiterpene Hydrocarbons Found in Cornmint Oil

Compound	References
Santene†	Malla et al. (1962)
γ-Elemene†	Sacco et al. (1969)
α-Copaene, β-copaene, γ-muurolene, α-amorphene bicycloelemene	Sakata et al. (1982)
β-Cadinene†	Zhu et al. (1993)
Bicyclogermacrene	Benn (1998)
γ-Cadinene	Rajeswara Rao et al. (1999);
α-Cubebene†, β-Bisabolene†, α-Elemene†	Pandey and Chowdhury (2002)
Undecane	Li et al. (2001)
α-Farnesene*	Rohloff (2002)
α-Bourbonene	Sakata and Hashizume (1971); Sakata et al. (1982)
α-Terpinene	Hefendehl and Ziegler (1975); Benn (1998); Lawrence et al. (1999)
Aromadendrene	Sakata and Hashizume (1971); Sakata et al. (1982); Li et al. (2001)
α-Humulene	Sakata et al. (1982); Veronina et al. (1990); Zhu et al. (1993)
β-Cubebene	Sakata et al. (1982); Qiu et al. (1990); Rajeswara Rao et al. (2000)
δ-3-Carene	Kobayashi et al. (1960); Mathur et al. (2001); Lawrence (2001); Rohloff (2002)
β-Phellandrene	Baslas and Baslas (1968); Sakata et al. (1982); Kubeczka and Formacek (2002)
α-Thujene	Malla et al. (1962); Baslas and Baslas (1968); Sacco and Nano (1970); Veronina et al. (1990); Benn (1998); Rajeswara Rao et al. (1999)

*Correct isomer not identified.
†Questionable identity.

occasionally characterized in this commercially important oil as shown in Table 7.2x. A summary of the data reveals that a total of 22 ketones have been identified in cornmint oil.

The characterization of acetone, 2-butanone, 2-heptanone, and 6-methyl-5-hepten-2-one by Nagasawa (1959) could not be confirmed from the recent (1960–2004) literature.

7.2.2.6 Acids

No acids have been routinely characterized in cornmint oil above the trace (<0.1%) level. Since 1960, a total of 13 aliphatic and aromatic acids have been occasionally characterized in this commercially important oil (see Table 7.2y). The reported occurrence of formic acid and 3-hexenoic acid as trace components of cornmint oil (Gildemeister and Hoffman, 1961) could not be confirmed from the recent (1960–2004) literature.

7.2.2.7 Phenols

Phenols have not been routinely characterized as trace (<0.1%) constituents in cornmint oil. Since 1960, a total of four phenols (Table 7.2z) have been occasionally found as constituents of this commercially important oil.

TABLE 7.2u
Additional Alcohols Found in Cornmint Oil

Compound	References
2,6-Nonadienol*	Talwar et al. (1963)
trans-p-Menthane-2-5-diol	Hashizume and Sakata (1967)
2,6-Dimethyl-2-octanol†	Belafi-Rethy et al. (1973a)
Viridiflorol	Karasawa and Shimizu (1976)
Carveol*, 1,2-epoxyneomenthol, α-muurolol, T-muurolol, α-cadinol, caryophylladien-I-ol	Sakata et al. (1982)
3-(5′,5′-dimethyltetrahydrofuran-2′-yl)-(Z)-2-butenol	—
	Sakurai et al. (1983a)
Methanol	Osaki (1992)
6-Methyl-3-heptanol	Zhu et al. (1993)
3-Methylcyclohexanol, octanol, 3-Nonanol, myrtenol, terpin hydrate†, *p*-menth-8-ene-diol*	Boelens (1993)
2-Methylbutanol, spathulenol, 2-phenethyl alcohol, *p*-menthanol*	Benn (1998)
Geraniol	Rajeswara Rao (1999)
o-Mentha-1(7),8-dien-3-ol*†	Pandey and Chowdhury (2000)
Dihydrocarveol, isopiperitenol, 3-heptanol	Lawrence (2000)
(E)-2-Nonenol, *cis*-sabinene hydrate, *trans*-verbenol, pulegol*, (Z)-nerolidol	Rohloff (2002)
Iso(iso)pulegol	Kubeczka and Formacek (2002)
Ethanol	Talwar et al. (1963); Qiu et al. (1990)
p-Cymen-8-ol	Lawrence (1983); Boelens (1993)
Borneol	Sakata et al. (1982); Lawrence (2001)
Hexanol	Topalov and Zheljazkov (1991); Zheljazkov et al. (1996)
β-Terpineol*	Zhu et al. (1993); Boelens (1993)
Isoamyl alcohol	Boelens (1993); Benn (1998)
(E)-Nerolidol	Boelens (1993); Rajeswara et al. (1999)
(E)-2-Hexenol	Lawrence (2001)
1-Octen-3-ol	Lawrence (1981); Benn (1998); Rajeswara Rao et al. (1999)
Menth-2(7)-dien-3-ol*†	Pandey and Chowdhury (2000)
Decanol	Zhu et al. (1993); Boelens (1993); Lawrence (2000)
Citronellol	Boelens (1993); Lawrence (2000); Singh et al. (2002a)
Lavandulol	Benn (1998); Lawrence (2001); Rohloff (2002)
trans-Sabinene hydrate	Nakayama et al. (1970); Sakata et al. (1982); Gasic et al. (1992); Spencer et al. (1997); Benn (1998); Rajeswara Rao et al. (2000); Singh et al. (2002b); Rohloff (2002)

*Correct isomer not identified.
†Questionable identity.

7.2.2.8 Nitrogen Heterocyclic Compounds

Nitrogen heterocyclic compounds have not been found in amounts exceeding 100 ppm in cornmint oil. Since 1960, a total of five nitrogen heterocyclic compounds have been occasionally characterized in this commercially important oil as shown in Table 7.2aa.

TABLE 7.2v
Additional Esters Found in Cornmint Oil

Compound	References
2-Hexenyl acetate*	Malla et al. (1962)
Menthyl valerate	Hefendehl and Ziegler (1975)
Bornyl acetate, carvyl acetate*, isopulegyl acetate, piperitenyl acetate, 1,2-epoxyneomenthyl acetate	Sakata et al. (1982)
(E)-3-Hexenyl acetate, (Z)-3-hexenyl propionate	Bicchi et al. (1989)
Methyl 2-methylbutyrate	Osaki (1992)
Nonyl acetate	Zhu et al. (1993)
α-Terpinyl acetate	Boelens (1993)
Ethyl isovalerate amyl 2-methylbutyrate, amyl isovalerate, octyl isovalerate	Benn (1998)
Geranyl acetate	Rajeswara Rao (1999)
Methyl salicylate	Lawrence (2001)
Hexyl isovalerate	Sakata et al. (1982); Benn (1998)
Ethyl 2-methylbutyrate	Osaki (1992); Benn (1998)
Decyl acetate	Zhu et al. (1993); Lawrence (2001)
(Z)-3-Hexenyl phenylacetate	Pandey and Chowdhury (2000)
Isoamyl isovalerate	Sakata and Hashizume (1971); Sakata et al. (1982); Boelens (1993)
(Z)-3-Hexenyl isovalerate	Nakayama et al. (1970); Sakata et al. (1982); Boelens (1993); Benn (1998)
Octyl acetate	Zhu et al. (1993); Boelens (1998); Benn (1998); Lawrence (2001)
Isomenthyl acetate	Emberger and Hopp (1985); Bicchi et al.(1989); Boelens (1993); Singh et al. (2000b)

*Correct isomer not identified.

TABLE 7.2w
Aldehydes Found in Cornmint Oil

Compound	References
Furfural	Talwar et al. (1963)
3-Hexenal*	Bicchi et al. (1989)
(E)-2-Hexenal	Osaki (1992)
Isovaleraldehyde, (Z)-4-heptenal, octanal, 2,4-nonadienal*	Benn (1998)
Acetaldehyde, valeraldehyde	Lawrence (2001)
(E,Z)-2,6-Nonadienal	Talwar et al. (1963); Benn (1998)
Isobutanal	Hefendehl and Ziegler (1975); Lawrence (2001)
Nonanal	Boelens (1993); Benn (1998)
2-Methylbutanal	Benn (1998); Lawrence (2001)

*Correct isomer not identified.

TABLE 7.2x
Additional Ketones Found in Cornmint Oil

Compound	References
2-Isopropylcyclopentanone	Onogaki (1962)
α-Thujone†	Baslas and Baslas (1969)
Camphor, isopiperitenone, 1-acetoxymenthone, 3-acetoxycarvomenthone	Sakata et al. (1982)
Carvotanacetone†	Veronina et al. (1990)
β-Ionone*, (E)-β-damascenone	Benn (1998)
cis-Isopulegone	Lawrence (2001)
Geranyl acetone	Benn (1998); Lawrence (2001)
Carvomenthone†	Malla et al. (1962); Talwar et al. (1964); Baslas and Baslas (1969)
3-Octanone	Sakata et al. (1982); Lawrence (1983); Boelens (1993); Benn (1998)
(Z)-Jasmone	Sakata et al. (1982); Benn (1998); Pandey and Chowdhury (2000); Lawrence (2001)
Piperitenone	Lawrence (1981); Sakata et al. (1982); Lawrence (1983); Kirichenko (1989); Benn (1998)
3-Methylcyclohexanone	Talwar et al. (1963); Sakata and Hashizume (1972); Sakata et al. (1982); Boelens (1993); Benn (1998); Pandey and Chowdhury (2000)

*Correct isomer not identified.
†Questionable identity.

7.2.2.9 Miscellaneous Compounds

To date only 1,8-cineole has been routinely identified as a constituent of cornmint oil. Since 1960, the 14 miscellaneous compounds that have been occasionally characterized in this commercially important oil can be seen in Table 7.2ab. As a result, a total of 15 miscellaneous compounds have been found in cornmint oil.

TABLE 7.2y
Acids Found in Cornmint Oil

Compound	References
Heptanoic, nonanoic	Kobayashi et al. (1960)
Anisic, 3-methyladipic	Sakata and Hashizume (1972)
Acetic	Boelens (1993)
Isovaleric, hexanoic, benzoic, salicylic, 3-isopropenylpentane-1,5-dioic	Tsuneya et al. (1998)
Octanoic	Kobayashi et al. (1960); Tsuneya et al. (1998)
(E)-2-Hexenoic	Sakata et al. (1982); Tsuneya et al. (1998)
2-Methylbutyric	Tsuneya et al. (1998); Benn (1998)

TABLE 7.2z
Phenols Found in Cornmint Oil

Compound	References
Guaiacol, 4-vinylguaiacol	Benn (1998)
Thymol	Hefendehl and Ziegler (1975); Benn (1998); Lawrence (2001)
Eugenol	Kobayashi et al. (1960); Tsuneya et al. (1998); Benn (1998); Lawrence (2001)

TABLE 7.2aa
Nitrogen Heterocyclic Compounds Found in Cornmint Oil

Compound	References
3-Phenylpyridine,5-phenyl-2-propylpyridine, 4-phenyl-4-propylpyridine	Sakurai et al. (1983b)
4-Methylquinoline	Benn (1998)
6-Methylpyrimidone*	Pandey and Chowdhury (2000)

*Questionable identity.

TABLE 7.2ab
Miscellaneous Compounds Found in Cornmint Oil

Compound	References
trans-2,5-Diethyltetrahydrofuran	Itahara et al. (1970)
Dehydromenthofurolactone 2,3-dimethyl-4-hydroxy-2-nonenoic acid lactone	Sakata and Hashizume (1973)
Mint sulphide	Takahashi et al. (1981)
Pulegone oxide*	Sakata et al. (1982)
Methyl eugenol	Boelens (1993)
2-Ethylfuran	Lawrence (2001)
Dimethyl sulfide	Peyron (1961); Lawrence (2001)
Piperitone oxide*	Nigam et al. (1963); Sakata and Hashizume (1971)
Menthofurolactone	Ito et al. (1969); Sakata and Hashizume (1971)
Methyl octyl ether	Boelens (1993); Benn (1998)
Caryophyllene oxide	Hashizume and Sakata (1970); Lawrence (1981); Boelens (1993)
Piperitenone oxide	Sakata et al. (1982); Osaki (1992); Benn (1998)
Menthofuran	Nigam and Levi (1964); Hefendehl and Ziegler (1975); Lawrence (1978); Sacco et al. (1989); Veronina et al. (1990); Topalov and Zheljazkov (1991); Shimizu (1985); Kubeczka and Formacek (2002)

*Correct isomer not identified.

TABLE 7.2ac
Enantiomeric Distribution of Cornmint Oil Constituents

Enantiomeric Ratio	References
(1R,5R)-(+)-α-pinene (26.5%–28.0%):(1S,5S)-(−)-α-pinene (72.0%–73.5%)	Kries, et al. (1990); Coleman and Lawrence (2001)
(1R,5R)-(+)-β-pinene (44.6%–50.9%):(1S,5S)-(−)-β-pinene (49.1%–55.6%)	Kreis et al. (1990); Coleman and Lawrence (2001)
(4R)-(+)-limonene (0.1%–1.9%):(4S)-(−)-limonene (98.1%–99.9%)	Kreis et al. (1990a); Coleman and Lawrence (2001)
(1S,4R)-(+)-menthone (<0.5%):(1R,4S)-(−)-menthone (>99.5%)	Emberger and Hopp (1985)
(1R,4R)-(+)-isomenthone (>99%):(1S,4S)-(−)-isomenthone (<1%)	Emberger and Hopp (1985)
(1S,3S,4R)-(+)-menthol (0-<0.5%):(1R,3R,4S)-(−)-menthol (>99.5%–100%)	Emberger and Hopp (1985)
(1S,3S,4R)-(+)-menthyl acetate (0%):(1R,3R,4S)-(−)-menthyl acetate (100%)	Emberger and Hopp (1985); Kries et al. (1990)
(1R,3S,4R)-(+)-isomenthol (>99%):(1S,3R,4S)-(−)-isomenthol (<1%)	Emberger and Hopp (1985)
(1R,3S,4S)-(+)-neomenthol (>99%):(1S,3R,4R)-(−)-neomenthol (<1%)	Emberger and Hopp (1985)
(1R,3R,4R)-(+)-neoisomenthol (>99%):(1S,3S,4S)-(−)-neoisomenthol (<1%)	Emberger and Hopp (1985)
(4S)-(+)-piperitone (79%):(4R)-(−)-piperitone (21%)	Ravid et al. (1994a)

7.2.2.10 Chirality

The enantiomeric distribution of some constituents of cornmint oil can be seen in Table 7.2ac. In addition, Nakayama et al. (1970) determined that (1R,4S,5S)-(+)-*trans*-sabinene hydrate, (4R)-(−)-terpinen-4-ol, and (−)-caryophyllene oxide were the major enantiomers found in cornmint oil. König et al. (1997) confirmed that the major enantiomer of *trans*-sabinene hydrate was as reported by Nakayama and colleagues. They also determined that the major enantiomers of isopulegol and menthofuran were (1R,3R,4S)-(−)-isopulegol and (6R)-(+)-menthofuran. Galle-Hoffman and König (1998) also determined that (4R)-(−) -piperitone was found in cornmint oil. This is the opposite to the enantiomer (4S)-(+) piperitone that has been found in peppermint oil and oils of other *Mentha* species.

7.3 PEPPERMINT OIL ex *Mentha piperita* L.

7.3.1 Introduction

The first botanical record of peppermint appeared in 1696 when the plant was collected in Hertfordshire (England) by Dr. Eales (Landing, 1969). A preserved specimen of the plant can be found in the herbarium of the British Museum. Sometime later the plant was given the name peppermint because it resembled spearmint and it possessed a pepper-type taste. The botanical origin of peppermint is *Mentha* ×*piperita* L, a natural hybrid of *M. aquatica* L. × *M. spicata* L.

7.3.2 Cultivation and Oil Production

Peppermint cultivation began in England around 1750 in the vicinity of Mitcham (Surrey) and by the end of the century, oil production had commenced. Between 1812 and 1830, peppermint had been introduced in Ashfield (Western Massachusetts, United States) and oil production had commenced (Landing, 1969). Over the next 50 years, cultivation and oil

TABLE 7.3a
Peppermint Oil Productions (Tonnes) 1990–2000 in the United States by Region

Year	MID	WIL	MAD	LAG	IDA	MON	YAK
1990	442.7	931.2	487.2	N/A	641.8	N/A	838.7
1991	634.6	958.4	430.0	N/A	671.8	N/A	611.0
1992	621.0	952.5	480.8	N/A	792.4	N/A	802.0
1993	500.0	680.4	400.5	N/A	762.0	N/A	747.5
1994	475.8	909.5	371.5	N/A	1006.5	N/A	964.3
1995	684.0	919.4	420.0	N/A	1281.4	N/A	1354.4
1996	435.0	802.9	358.8	N/A	1427.0	N/A	1454.2
1997	526.2	635.2	317.5	N/A	1510.5	N/A	1555.8
1998	677.2	505.3	296.7	273.1	985.7	216.8	1620.2
1999	497.6	427.3	255.4	273.5	702.2	135.6	1210.2
2000	388.7	355.2	205.5	282.6	543.4	68.6	1327.2

MID, Midwest; WIL, Willamette; MAD, Madras; LAG, LaGrande; IDA, Idaho; MON, Montana; YAK, Yakima; N/A, data not available.

production had moved westward to Michigan, Indiana, and Wisconsin. By the beginning of the 20th century, cultivation and oil production had moved further west to Oregon, Washington, and Idaho. This change in venue for cultivation was a direct result of severe winterkill and, more important, the devastating Verticillium root disease that decimated many of the eastern and Midwest plantings. Today, the western states produce a lion's share of peppermint oil in the United States. A summary of production levels between 1990 and 2000 can be seen in Table 7.3a (Lawrence, 2000). More recent data can be found in Chapter 9.

Over the last 100 years, numerous other countries such as Argentina, Australia, Brazil, Bulgaria, Canada, England, France, Hungary, India, Italy, Japan, Morocco, New Zealand, Poland, Romania, Russia and the former Soviet Union, Serbia and Montenegro and the former Yugoslavia, South Africa, and Spain have been producing peppermint oil. Currently, only Argentina, Australia, India, and Italy produce quantities greater than 10 tonnes. India is by far the largest producer. The current production levels for the four countries are 25 tonnes, 15 tonnes, 350 tonnes, and 30 tonnes, respectively.

7.3.3 Oil Composition

A survey of the pre-1960 literature (Gildemeister and Hoffman, 1961) reveals that the constituents that have been characterized in peppermint in various origins were as follows:

α-pinene
α-terpinene
α-phellandrene
limonene
β-caryophyllene
cadinene*
amyl alcohol
isoamyl alcohol
hexenol*
3-octanol
menthol

neomenthol
trans-sabinene hydrate
thymol
carvacrol
caryophyllene alcohol
clovanediol*
β-betulenol
acetaldehyde
isovaleraldehyde
acetone
3-methylcyclohexanone
jasmone*
menthone
isomenthone
pulegone
piperitone
menthyl acetate
menthyl isovalerate
menthyl octenoate*
acetic acid
isovaleric acid
octanoic acid
octenoic acid*
1,8-cineole
menthofuran
dimethyl sulfide

*Correct isomer not identified.

In other literature, Kremers (1926) identified 2-methylbutanol as a low boiling component of Midwest U.S. peppermint oil. Reitsema (1958) determined that the main components of a similar oil were as follows:

menthone (25%)
menthofuran (2%–15%)
pulegone (2%)
menthol (50%)
piperitone (1%)
piperitenone (1%)

Smith and Levi (1961) compared the major constituents of commercial samples of peppermint oil produced in the Midwest, Oregon, and the Yakima Valley of Washington. A summary of the results can be seen in Table 7.3b. These same authors also compared the analyses of peppermint oil produced in Italy, England, Bulgaria, South Africa, Argentina, Canada, Netherlands, Poland, and Spain characterizing the same components.

Porsch and Farnow (1962) compared the composition of North American peppermint oil with peppermint oils produced in Bulgaria, France, and Hungary. the results of the study are shown in Table 7.3c.

Hefendehl (1962) studied the differences in oil composition of old and young leaves, leaf position effect, and effect of withering on peppermint grown experimentally in Germany.

TABLE 7.3b
Comparative Composition of North American Peppermint Oils

	Midwest (3)*	Oregon (3)	Yakima (4)
α-Pinene	0.8	0.7–0.8	0.5–0.8
Camphene	0–0.2	0–0.2	0–0.2
β-Pinene	0.9–1.0	0.9–1.6	1.1–1.7
Limonene	3.0–3.6	3.2–4.3	3.5–3.7
1,8-Cineole	7.1–8.7	7.5–8.3	6.4–13.5
Menthone	29.9–31.6	19.5–23.8	8.9–17.9
Menthofuran	0.7–1.9	2.6–3.3	6.2–9.4
Isomenthone	4.8–5.9	3.2–4.5	2.2–3.4
Menthyl acetate	4.2–5.1	4.4–6.1	5.3–11.6
Neomenthol	3.4–3.6	3.6–4.1	2.8–3.9
Menthol	37.0–39.3	41.8–46.2	40.2–48.7
Pulegone	0.4–0.8	0.9–2.3	0.9–2.6
Piperitone	0.5–1.3	1.7–3.1	0.7–1.6

*Number of samples.

He found that the age of the leaves, their state of maturity, and freshness were the main causes in oil composition variance. He found that as the leaves increased in age, the reduction of menthone to menthol increased proportionally. Young leaves were found to have higher levels of pulegone. Also, when the plant was in flower, the menthofuran content was at its maximum level.

Ognyanov and Vlahov (1962, 1965) and Vlahov and Ognyanov (1964) characterized a number of constituents in Bulgarian peppermint oil such as amyl alcohol, (Z)-3-hexenol, acetaldehyde, isovaleraldehyde, phenylacetaldehyde, 3-octanone, 3-octyl formate, 3-octyl

TABLE 7.3c
Comparative Chemical Analysis of Peppermint Oils of Various Origins

Compound	N. American	Bulgarian	French	Hungarian
α-Pinene	1.5	2.0	1.5	1.0
β-Pinene	1.0	1.0	1.0	0.5
Limonene	*t*	*t*	*t*	*t*
1,8-Cineole	6.5	6.0	7.5	4.5
3-Octanol	0.5	0.5	0.5	0.5
trans-Sabinene hydrate	1.0	0.5	2.0	1.0
Menthol + menthofuran	26.0	28.0	25.0	24.0
Isomenthone	*t*	5.0	*t*	7.0
Neomenthol	3.0	4.0	4.0	3.5
Menthol + Neoisomenthol	48.0	41.0	48.0	49.0
Menthyl acetate	9.0	7.0	9.0	7.5
Isomenthol	3.0	2.0	3.0	2.0
Piperitone	1.0	1.0	0.5	0.5

t = trace (<0.5%).

acetate, *trans*-sabinene hydrate, menthone, menthofuran, menthyl acetate, menthol, amyl formate, amyl acetate, 3-octanol, (E)-2-hexenal, α-pinene, camphene, β-pinene, limonene, 1,8-cineole, γ-terpinene, terpinolene, and a β-ocimene isomer.

Katsuhara et al. (1964, 1966, 1967) identified a number of new constituents in peppermint oil of Japanese origin. The components that were characterized were, β-bourbonene, β-caryophyllene, an elemene isomer, ε-cadinene, aromadendrene, α-calacorene, guaiazulene, a calamenene isomer, *trans-p*-menth-2-ene-1,4-diol, *p*-menthane-1,3-diol, and *p*-menthane-2,3-diol.

Vlahov et al. (1967a) isolated the sesquiterpene hydrocarbons in pure form from Bulgarian peppermint oil and determined their optical activities.

Over the next 30 years, peppermint oil has been the subject of analysis by numerous groups and individuals around the world. This has led to the identification of more than 300 constituents of the oil. Rather than documenting the plethora of studies, the more important ones are discussed in this review.

Von Schantz and Norri (1968) compared the composition of the leaf oil obtained from Ukranian peppermint plants that were harvested at different development times. The results of this study are shown in Table 7.3d.

Malingré (1969) obtained oil from the flowers, leaf tips, and leaves of peppermint grown experimentally in Groningen (Netherlands). A composition of the major constituents identified in these oils can be seen in Table 7.3e. He also determined that oil produced from the

TABLE 7.3d
Comparative Chemical Composition of Peppermint Leaf Oil

Compound	Before Flowering	Initial Flowering	End of Flowering
α-Pinene	0.4	0.7	0.8
Camphene	*t*	*t*	*t*
β-Pinene + sabinene	0.9	1.4	1.8
+Myrcene	0.2	0.4	0.4
α-Terpinene	*t*	0.1	*t*
Limonene	5.0	9.8	8.3
1,8-Cineole	4.6	5.1	7.8
β-Ocimene*	*t*	*t*	*t*
γ-Terpinene	0.1	0.6	0.4
p-Cymene + unknown	0.2	0.4	0.2
Terpinolene + unknown	0.1	0.2	0.2
3-Octanol	0.4	0.4	0.4
Menthone	26.2	3.1	5.5
Menthofuran	*t*	*t*	*t*
Isomenthone	2.6	0.9	1.3
Menthyl acetate	6.3	22.1	18.0
Neomenthol + β-caryophyllene	5.4	7.4	6.0
Menthol	42.8	40.5	42.4
Isomenthol + δ-cadinene	0.4	0.9	1.0
Piperitol + unknown	0.7	1.6	1.4
Piperitone + unknown	2.2	2.2	1.9

*Correct isomer not identified.

TABLE 7.3e
Chemical Composition of Peppermint Oil Obtained from Various Parts of the Plant

Compound	Flower Oil	Leaf Tips Oil	Leaf Oil
α-Pinene	0.3	0.3	0.3
β-Pinene	0.5	1.2	1.0
Limonene	1.9	5.3	0.9
1,8-Cineole	1.3	1.0	3.6
3-Octyl acetate	0.2	0.1	0.8
3-Octanole	0.2	0.1	0.3
Menthofuran	20.1	4.2	1.0
trans-Sabinene hydrate	1.4	1.2	2.5
Menthone	25.4	59.0	2.2
Isomenthone	4.1	6.3	0.9
Menthyl acetate	2.4	0.6	10.8
Neomenthol	1.7	1.7	7.9
Menthol	20.1	6.8	58.6
Pulegone	16.2	2.5	0.9
Piperitone	1.2	2.1	1.2

same peppermint plants that were harvested at their optimum yield time possessed the following composition:

α-pinene (0.1%–0.3%)
β-pinene (1.0%–2.2%)
limonene (0.7%–1.2%)
1,8-cineole (1.5%–2.2%)
3-octyl acetate (0.1%–0.2%)
3-octanol (0.3%–0.9%)
menthofuran (1.5%–2.3%)
trans-sabinene hydrate (0.7%–1.2%)
menthone (2.6%–3.0%)
isomenthone (1.4%–1.8%)
menthyl acetate (12.0%–13.9%)
neomenthol (4.4%–5.0%)
menthol (51.1%–56.6%)
pulegone (2.1%–2.5%)
piperitone (2.4%–3.3%)

An oil produced commercially in the Willamette Valley (Oregon, United States) was the subject of analysis by Lawrence et al. (1972). It was found to contain the following components:

2-methylbutanal + isovaleraldehyde (0.1%)
α-thujene + α-pinene (0.7%)
β-pinene (0.8%)
sabinene (0.4%)
myrcene (0.1%)

isoamyl alcohol (0.2%)
limonene (1.2%)
1,8-cineole (5.6%)
(Z)-β-ocimene (0.1%)
γ-terpinene (0.5%)
p-cymene (0.1%)
terpinolene (0.1%)
3-octanol (0.2%)
trans-sabinene hydrate (0.8%)
menthone (24.2%)
menthofuran (1.2%)
isomenthone (3.5%)
β-bourbonene (0.6%)
menthyl acetate + neomenthol (3.7%)
neoisomenthyl acetate (4.9%)
terpinen-4-ol (1.2%)
β-caryophyllene (0.8%)
menthol (45.8%)
(E)-β-farnesene (0.1%)
pulegone (1.0%)
α-terpinene (0.1%)
α-amorphene (0.1%)
germacrene D (0.9%)
α-muurolene (0.5%)
piperitone (0.1%)
ledol (0.1%)

Trace amounts (<0.1%) of ethanol, butanal, 2-methylfuran, 2-ethylfuran, camphene, α-terpinene, β-phellandrene, (E)-2-hexenal, hexanol, (Z)-3-hexenol, (E)-2-hexenol, tetradecane, 1-octen-3-ol, pentadecane, α-copaene, *cis*-sabinene hydrate, *trans*-*p*-menth-2-en-1-ol, α-gurjunene, β-copaene, hexadecane, β-ylangene, γ-muurolene, ledene, γ-cadinene, δ-cadinene, octadecane, α-cadinene, *p*-menth-1-en-9-yl acetate, nonadecane, a calamenene isomer, *p*-menthan-9-ol, eicosane, 2-phenethyl isovalerate, caryophyllene oxide, heneicosane, thymol, eugenol, 2-methylbutyric acid, isovaleric acid, hexanoic acid, heptanoic acid, octanoic acid, and decanoic acid were also found in the same oil. Subsequent to this analysis, Lawrence (1978) determined that ledene was identified in error. It was, in fact, viridiflorene, the epimer of ledene. In addition, Karasawa and Shimizu (1976) and Rojan et al. (1977) showed that the ledol identified by Lawrence et al. (1972) was a misidentification of the epimeric viridiflorol.

Sheldon et al. (1972) reported that they characterized 100 components in North American peppermint oil. The components were quantitatively distinguished as >10% (2 components), 1% to 10% (10 components), 0.1% to 1.0% (19 components), 0.01% to 0.1% (14 components), 0.001% to 0.01% (29 components) and <0.001% (26 components). Unfortunately, the authors only reported the identities of the newly characterized components and not the full 100; they did not present any quantitative data either.

It is well known that aged peppermint oil has a more desirable aroma than freshly distilled oil. As a result, Wong (1972) compared the chemical composition of both fresh and aged peppermint oil (see Table 7.3f). In addition, trace amounts (<0.1%) of acetaldehyde, isobutanal, and camphene, were also characterized, although the fresh oil was richer in the trace aliphatic aldehydes than the aged oil. As can be seen from the results presented in Table 7.3g, the fresh oil was found to be slightly richer in 1,8-cineole, menthone, menthofuran, menthyl

TABLE 7.3f
Comparative Percentage Composition of Fresh and Aged Peppermint Oil

Compound	Fresh Oil	Aged Oil
Isovaleraldehyde	0.2	0.3
α-Pinene	0.8	0.8
β-Pinene	1.6	1.6
Myrcene	0.2	0.2
α-Terpinene	0.4	0.3
Limonene	1.6	1.4
1,8-Cineole	5.4	5.2
γ-Terpinene	0.7	0.6
p-Cymene	0.1	0.3
trans-Sabinene hydrate	1.2	1.2
Menthone	24.1	22.0
Menthofuran	2.3	1.2
Isomenthone	2.5	3.2
Linalool	0.5	0.5
Menthyl acetate	4.7	4.3
Neomenthol	3.8	3.8
α-Caryophyllene[†]	1.5	1.3
Terpinen-4-ol	3.1	2.5
Menthol	38.2	42.3
β-Caryophyllene	0.5	0.5
Isomenthol	0.5	0.5
Pulegone	1.1	0.8
(E)-β-Farnesene	0.4	0.3
α-Terpineol	0.3	0.4
Piperitone	0.2	0.6
α-Elemene[‡]	0.1	0.1

[†]Probably a misidentification of β-elemene.
[‡]Probably a misidentification of germacrene D.

acetate, terpinen-4-ol, and pulegone, whereas the aged oil was richer in isovaleraldehyde, *p*-cymene, isomenthone, piperitone, and menthol. Wong inferred that storage of peppermint under temperature-controlled conditions after both water and oxygen had been removed from the oil created an optimum situation for the aging of peppermint oil. A comparison between some selected component ratios demonstrates the differences between oils stored under optimum or nonoptimum conditions as can be seen in Table 7.3g.

Kartnig and Still (1975) examined the oils of a number of commercial cultivars of peppermint used for oil production outside of the United States and compared the oils to an oil commercially available in Germany. The results of this study are summarized in Table 7.3h.

An oil produced solely from the flowering heads of peppermint grown in Oregon was found (Lawrence, 1978) to contain the following constituents:

α-thujene + α-pinene (0.8%)
β-pinene (0.9%)
sabinene (0.4%)

TABLE 7.3g
Ratios of Components Found in Oils Aged (Stored) under Optimum and Nonoptimum Conditions

Component Ratio	Oil Stored Under Optimum Conditions	Oil Stored Under Nonoptimum Conditions
Menthol/menthone	1.9	2.0
Menthol/piperitone	52.6	72.3
Menthone/piperitone	28.0	36.1
Neomenthol/piperitone	4.5	5.9

myrcene (0.1%)
limonene (2.6%)
(Z)-β-ocimene (0.1%)
(E)-β-ocimene (0.2%)
p-cymene (0.1%)
terpinolene (0.1%)
trans-sabinene hydrate (0.5%)
menthone (18.2%)
menthofuran (21.5%)
isomenthone (6.8%)
β-bourbonene (0.1%)
neomenthol (0.2%)
trans-isopulegone (0.1%)
β-caryophyllene (1.1%)
menthol (24.1%)
pulegone (13.9%)
(E)-β-farnesene (0.1%)
germacrene D (1.0%)
piperitone (0.2%)

In addition, trace amounts (<0.1%) of camphene, α-terpinene, γ-terpinene, 2-methylbutyl 2-methylbutyrate, linalool, *cis*-sabinene hydrate, neoisomenthyl acetate, β-copaene, *trans*-*p*-mentha-2-en-1-ol, α-terpineol, and carvone were also characterized in this same oil.

TABLE 7.3h
Comparative Percentage Composition of Various Peppermint Oils

Compound	"Erdinger"	"Neckaperle"	Bulgarian	Ukranian	"Commander"	Commercial
α-Pinene	0.6–0.8	0.7–0.9	0.1–0.5	0.6–0.8	0.6	1.2
β-Pinene	1.4–1.7	1.5–1.9	0.5–1.1	1.4–1.7	1.9	1.6
Limonene	1.0–1.2	1.3–1.8	0.3–0.6	5.4–6.4	1.8	6.4
1,8-Cineole	7.8–7.9	7.7–8.2	2.5–3.8	5.4–6.1	2.8	0.4
p-Cymene	0.1–0.2	0.1–0.2	*t*–0.1	—	—	0.2
Menthone	24.1–36.2	26.3–39.5	51.6–51.8	41.2–45.8	67.2	31.8
Menthofuran	0.6–2.6	0.5–2.5	0.3–1.2	0.1–0.3	—	0.1
Isomenthone	3.7–4.6	3.9–4.9	9.8–10.0	4.6–5.0	7.6	7.2
Menthyl acetate	6.4–9.1	6.2–8.6	4.5–5.6	6.0–6.3	0.4	9.5
Menthol	31.4–41.9	29.1–39.2	22.3–23.2	23.2–28.0	0.9	35.4
Piperitone	0.9–1.1	1.0–1.1	1.9–2.8	3.2	—	3.3

The oils produced from Auvergne peppermint (*M. piperita* var. *officinalis* f. *rubescens*) that was harvested both before and during flowering were analyzed by Carnat and Lamaison (1987). The composition of the oils was compared with that of a commercial peppermint oil that was also produced in France (Table 7.3i). Murray et al. (1988) produced an oil from the stolons of *M. piperita* that was grown commercially in Michigan (United States) and found that it contained the following major components:

α-pinene (1.1%)
β-pinene (1.6%)
limonene (4.5%)
1,8-cineole (1.6%)
trans-sabinene hydrate (0.1%)
menthone (3.5%)
menthofuran (46.1%)
isomenthone (0.7%)
menthyl acetate (24.5%)
neomenthol (1.7%)
β-caryophyllene (0.3%)
menthol (11.4%)
pulegone (0.9%)
piperitone (0.1%)

These same authors examined the change in oil composition as the peppermint plant growing in Indiana (United States) matured. These results can be found in Table 7.3j.

Voronina et al. (1990) analyzed the composition of oils produced from eight cultivars of so-called peppermint grown in Russia. The oils were found to vary in menthol content from 32.1% to 83.2% as can be seen in Table 7.3k. Based on these results, it must be postulated that the high menthol containing oils probably originated from hybrid plants not $2n = 72$ *M. piperita*.

When peppermint oil is produced by steam distillation a small amount of the oil dissolves in the distillation water that is separated from the oil. Fleisher and Fleisher (1991) examined the composition of this oil and found that it contained the following components:

dimethyl sulfide (0.1%)
isobutanal (0.2%)
valeraldehyde (2.5%)
2,5-diethyltetrahydrofuran* (0.1%)
isobutanol (0.1%)
hexanal (0.2%)
isoamyl alcohol (0.1%)
1,8-cineole (15.7%)
(E)-2-hexenal (0.5%)
2-pentenol* (0.3%)
3-methylcyclohexanone (0.3%)
hexanol (0.3%)
(Z)-3-hexenol (0.7%)
3-octanol (0.4%)
(E)-2-hexenol (0.4%)
1-octen-3-ol (0.2%)
menthone (16.6%)

*Correct isomer not identified.

TABLE 7.3i
Comparative Percentage Composition of French Peppermint Oil

Compound	Auvergne Preflowering Oil	Peppermint Flowering Oil	Mitcham Peppermint Oil
α-Pinene	0.6	0.7	0.7
β-Pinene	0.9	1.1	1.0
Sabinene	0.5	0.6	0.6
Myrcene	0.3	0.3	0.3
α-Terpinene	0.2	0.2	0.2
Limonene	1.8	2.2	1.9
1,8-Cineole	5.3	6.6	5.6
(Z)-β-Ocimene	0.5	0.6	0.2
α-Terpinene	0.3	0.4	0.3
(E)-β-Ocimene	0.1	0.1	—
p-Cymene	0.1	0.1	0.2
Terpinolene	0.1	0.1	0.1
Octyl acetate	—	0.1	—
3-Octanol	0.3	0.4	0.4
1-Octen-3-ol	0.1	—	—
trans-Sabinene hydrate	2.0	2.4	2.4
Menthone	26.2	15.4	15.7
Menthofuran	1.1	1.5	1.8
Isomenthone	3.0	2.2	8.5
β-Bourbonene	0.4	0.4	0.3
Neomenthyl acetate	0.2	0.3	—
Linalool	0.3	0.3	0.3
Linalyl acetate[t]	0.1	0.1	—
Menthyl acetate	2.1	4.2	4.4
Isopulegol	0.1	0.1	—
Bornyl acetate[t]	0.1	0.2	—
Isobornyl acetate[t]	0.1	<0.1	—
Neomenthol	3.3	4.1	7.1
Terpinen-4-ol	2.2	1.8	0.5
β-Caryophyllene	0.6	0.7	0.6
a Dihydrocarvone*	0.1	0.1	—
Neoisomenthol	0.7	1.0	—
Menthol	39.9	46.2	38.2
Pulegone	0.6	0.6	0.5
Isomenthol	0.2	0.3	—
Isoborneol[t]	0.2	0.2	—
α-Humulene	0.1	0.1	—
α-Terpineol	0.2	0.2	0.3
Germacrene D	2.5	1.9	3.7
Piperitone	0.7	0.6	0.7
Carvone	0.4	0.4	—
Viridiflorol	0.9	0.7	0.7

[t]Tentative identification.
*Correct isomer not identified.

TABLE 7.3j
Percentage Composition of the Oils Produced from Different Development Stages of Peppermint Plants

Compound	1	2	3	4	5	6
α-Pinene	0.4	0.7–0.9	0.5–0.8	0.6	0.2–0.9	0.1–0.5
β-Pinene	1.0	1.8–2.2	1.4–2.3	1.8	1.0–2.0	0.3–1.0
Limonene	1.6	1.3–2.0	1.6–1.9	2.2	1.5–2.4	0.6–2.0
1,8-Cineole	3.5	6.1–6.9	5.4–7.6	5.9	4.7–6.1	1.9–4.0
Menthone	30.6	16.3–37.2	14.0–20.8	21.8	13.0–15.1	3.3–6.1
Menthofuran	2.0	1.9–2.0	2.1–2.6	7.7	3.4–7.6	5.4–7.0
Isomenthone	3.7	3.5–4.1	3.5–4.3	3.6	2.1–3.5	0.7–1.4
Menthyl acetate	4.7	3.9–13.1	6.5–10.9	3.4	5.5–12.1	18.8–27.8
Neomenthol	4.2	4.1–5.5	4.7–5.3	3.7	4.2–4.5	4.8–5.0
Menthol	40.7	30.3–39.3	36.8–43.1	36.8	43.4–48.2	47.4–49.0
Pulegone	0.6	0.7–1.0	0.6–1.4	2.8	0.7–1.3	1.3–1.9
Piperitone	0.8	0.5–0.8	0.6–0.7	0.6	0.3–0.5	0.2–0.5

1, juvenile plants (5 cm); 2, immature plants; 3, just before flowering (normal harvest time for oil production); 4, flowering stage; 5, postflowering plants; 6, senescent plants.

TABLE 7.3k
Comparative Percentage Composition of the Peppermint Oils of Russian Cultivars

Compound	1	2	3	4	5	6	7	8
α-Thujene	0.1	0.4	0.2	0.1	—	0.1	—	0.1
α-Pinene	0.2	0.8	0.2	0.1	0.4	0.1	0.1	0.1
Camphene	0.3	0.7	0.3	0.4	—	0.3	—	0.3
Sabinene	0.4	0.6	0.5	0.5	—	0.2	—	0.4
Myrcene	0.4	0.3	0.1	0.4	—	*t*	—	*t*
1,8-Cineole	3.0	9.7	1.1	2.9	4.7	1.9	0.1	0.7
(Z)-β-Ocimene	0.3	0.9	—	0.4	—	0.1	—	0.2
Linalool	*t*	*t*	*t*	*t*	*t*	0.1	0.1	0.1
Menthone	27.1	31.8	8.1	27.5	42.2	9.3	5.8	18.3
Isomenthone	4.1	5.5	18.0	4.1	8.8	4.1	0.9	5.4
Menthofuran	—	—	—	—	—	2.6	0.1	—
Neomenthol	4.5	2.4	1.5	4.3	4.7	1.1	1.2	1.6
Menthol	50.1	38.0	44.9	53.3	32.1	76.0	83.2	71.3
Isomenthol	—	0.7	2.4	0.8	1.2	0.2	0.2	0.3
Carvotanacetone	1.4	1.0	0.2	1.1	—	1.8	0.1	—
Piperitone	1.1	0.8	1.2	1.2	0.8	0.9	2.0	0.9
Menthyl acetate	2.8	1.7	19.3	1.9	3.1	1.7	3.9	0.2
β-Caryophyllene	0.6	1.9	0.8	0.4	—	0.1	1.4	0.1
α-Humulene	0.4	1.2	0.6	0.4	—	0.1	1.1	0.3

1, Prilukskaya-6; 2, Krasnodarskaya-2; 3, Kubanskaya-6; 4, Prilukchanka; 5, Chernigovska; 6, Simpferoloskaya-200; 7, Vniienk; 8, Reznikova.

isomenthone (4.7%)
benzaldehyde (0.2%)
linalool (0.4%)
isopinocamphone (0.1%)
terpinen-1-ol (0.3%)
menthyl acetate (0.2%)
isopulegol (0.4%)
isopulegone*(0.1%)
terpinen-4-ol (0.1%)
neomenthol (6.2%)
trans-dihydrocarvone (0.1%)
neoisomenthol (2.2%)
menthol (36.7%)
pulegone (0.5%)
α-terpineol (0.7%)
borneol (0.1%)
piperitone (1.4%)
piperitenone (0.1%)
(Z)-jasmone (0.1%)
anisaldehyde (0.2%)
eugenol (0.1%)

*Correct isomer not identified.

Trace amounts (<0.1%) of 2-ethylfuran, tiglic aldehyde, *p*-cymene, (E)-2-pentenal, amyl alcohol, a 4-heptenal isomer, isoamyl isovalerate, 2-heptanol, veratraldehyde, methyl salicylate, myrtenol, dihydrocarveol, *cis*-carveol, *trans*-carveol, benzyl alcohol, 2-phenethyl alcohol, thymol, and carvacrol were also found in the same oil isolated from the distillation water.

Spencer (1992) also analyzed the oil that dissolved in the distillation water during the steam distillation of peppermint on the commercial scale. Spencer measured the level of each component obtained in fractions of distillation water that were taken every 5 min. As a result, the range in the components found in these fractions was as follows:

2-ethylfuran (*t*%–0.1%)
isoamyl alcohol (0.1%–0.2%)
2-methylbutanol (0.1%–0.3%)
hexanal (*t*%–0.1%)
(E)-hexenal (0%–0.4%)
menthyl butyrate (0%–*t*%)
(Z)-3-hexenol (0.2%–0.6%)
(E)-2-hexenol (0%–0.4%)
hexanol (*t*%–0.1%)
heptanal (0.1%)
α-thujene (0.2%)
α-pinene (0.1%–0.2%)
sabinene (0%–0.6%)
α-pinene (0%–0.3%)
1-octen-3-ol (0.4%–0.6%)
3-octanol (0.3%–0.6%)
α-terpinene (0.2%–0.3%)

limonene + 1,8-cineole (7.6%–15.1%)
trans-sabinene hydrate (0.4%–0.7%)
cis-linalool oxide* (0.2%–0.3%)
linalool (0.7%–1.0%)
3-octyl acetate (0.2%–0.3%)
menthone (7.7%–12.9%)
isomenthone (4.0%–4.7%)
menthofuran (0.5%–0.8%)
neomenthol (2.1%–2.4%)
menthol (42.2%–51.8%)
terpinen-4-ol (4.9%–6.4%)
neoisomenthol (0.2%–2.2%)
α-terpineol (0.8%–1.4%)
pulegone (1.7%–2.1%)
piperitone (3.0%–6.1%)
menthyl acetate (0.1%–1.5%)
β-bourbonene (0.1%–0.3%)
β-caryophyllene (*t*%–0.3%)
viridiflorol (*t*%–0.3%)

*Correct isomer not identified.
t = trace (<0.1%).

Spencer also noted that when distillation first commenced, the oil content of the water was 0.28% and by the end of the distillation, the oil content had dropped to 0.14% with an average content for the complete distillation of 0.27%. Italian peppermint oils that were produced commercially in different regions of the Piedmont Valley were analyzed by Chialva and Ariozzi (1992). The results of these analyses are found in Table 7.3l.

A large number of samples of peppermint oil produced commercially in different regions of the United States such as Midwest, Willamette Valley, and Madras Valley (Oregon), Idaho and Yakima Valley (Washington) were analyzed by Lawrence (1993c). The range of components found in each oil can be seen in Table 7.3m.

Court et al. (1993b) analyzed peppermint oil produced from plants grown in southern Ontario (Canada) that were harvested at monthly intervals between July (preflowering) and October (senescent plants). They showed that as the plant flowered and postflowering, the menthone content decreased whereas the menthofuran and menthyl acetate contents increased, as can be seen in Table 7.3n.

Lawrence (2000) analyzed 16 samples of peppermint oil produced commercially in the state of Punjab, and in Bareilly and Badaun (Uttar Pradesh, India). A summary of the oil compositions is shown in Table 7.3o.

The minor constituents that have been characterized in peppermint oil over the past 45 years can be seen categorized according to the class of compounds such as hydrocarbons, alcohols, esters, aldehydes, ketones, acids, phenols, nitrogen heterocyclic compounds, and miscellaneous compounds.

7.3.3.1 Hydrocarbons

The monoterpene hydrocarbons routinely found in peppermint oil in measurable quantities are α-pinene, β-pinene, sabinene, myrcene, α-terpinene, limonene, (E)-β-ocimene, γ-terpinene, and p-cymene. In addition, minor amounts (often less than 0.1%) of camphene, (Z)-β-ocimene, and terpinolene have been characterized as components of peppermint oil by

TABLE 7.3l
Percentage Composition of Peppermint Oils of Italian Origin

Compound	1*	2*	3*	4*
α-Pinene	0.7–0.9	0.8–0.9	0.7–0.9	0.8–0.9
β-Pinene	1.1–1.2	1.1–1.2	1.1–1.2	1.1–1.2
Sabinene	0.5–0.6	0.5–0.6	0.5–0.6	0.5–0.6
Myrcene	0.2–0.3	0.3	0.2–0.3	0.3
α-Terpinene	0.2–0.5	0.2–0.4	0.3–0.4	0.3–0.4
Limonene	1.6–2.4	1.6	1.5–2.0	1.7–2.0
1,8-Cineole	4.8–5.6	5.3–5.8	5.1–5.8	5.0–5.4
(Z)-β-Ocimene	0.2–0.3	0.2–0.3	0.2–0.4	0.2–0.3
γ-Terpinene	0.4–0.8	0.5–0.6	0.5–0.8	0.5–0.7
p-Cymene	0.1–0.3	0.1	0.1	0.1–0.2
3-Octanol	*t*–0.2	0.3–0.4	0.2–0.3	0.3–0.3
1-Octen-3-ol	0–0.1	0.1	0.1	0.1–0.2
trans-Sabinene hydrate	0.6–0.9	0.8–1.3	0.7–0.9	0.6–1.0
Menthone	14.9–21.8	16.1–19.1	16.0–20.5	18.8–21.1
Menthofuran	3.6–8.9	5.8–7.9	2.4–7.4	4.8–8.9
Isomenthone	2.8–3.5	2.8–3.0	3.0–3.1	3.1–3.3
β-Bourbonene	0.2–0.4	0.2–0.3	0.2–0.4	0.2–0.3
Linalool	0.3–0.5	0.4	0.4–0.4	0.3–0.4
Menthyl acetate	3.5–5.5	3.6–4.4	3.6–5.0	2.8–3.1
Neomenthol	2.8–3.9	3.1–3.3	2.9–3.7	2.6–2.8
Terpinen-4-ol	0.7–1.2	1.0–1.3	1.1–1.2	0.9–1.1
β-Caryophyllene	1.7–2.5	1.4–1.7	1.8–2.6	1.8–2.3
Isomenthol	0.6–0.8	0.7–0.8	0.7–0.8	0.6–0.7
Menthol	36.1–40.7	39.9–40.8	37.5–42.0	35.8–39.5
Pulegone	1.8–4.3	2.2–2.7	1.2–3.0	2.8–3.8
α-Terpineol	0.2–0.3	0.3–0.3	0.2	0.2–0.3
Germacrene D	2.1–3.2	1.9–2.4	2.2–3.3	2.1–2.8
Piperitone	0.4–0.5	0.5–0.5	0.5	0.5
Viridiflorol	0.3–0.5	0.3–0.5	0.3–0.5	0.2–0.5

*Numbers refer to different locations in the Piedmont Valley.
t = trace (<0.1%).

numberous research groups. The sesquiterpene hydrocarbons that have been routinely found in peppermint in measurable quantities are β-bourbonene, β-caryophyllene, and germacrene D. Since 1960, 49 other aliphatic monoterpene and sesquiterpene hydrocarbons have been occasionally identified in this commercially important oil as can be seen in Table 7.3p. Based on the data included in this section, it would appear that a total of 64 hydrocarbons have been characterized in peppermint oil.

7.3.3.2 Alcohols

The alcohols that have been routinely found in measurable quantities as components of peppermint oil are 3-octanol, *trans*-sabinene hydrate, linalool, terpinen-4-ol, α-terpineol, menthol, neomenthol, neoisomenthol, and viridiflorol. In addition, minor amounts (often less than 0.1%) of (Z)-3-hexenol, 1-octen-3-ol, and isomenthol have been characterized as components of peppermint oil by numerous research groups. Since 1960, 58 other aliphatic

TABLE 7.3m
Percentage Composition of Peppermint Oils Produced in Different Regions of the United States

Compound	Midwest	Willamette	Madras	Idaho	Yakima
α-Pinene	0.72–0.83	0.58–0.83	0.61–0.69	0.59–0.70	0.71–0.75
β-Pinene	1.04–1.13	0.87–0.96	0.86–0.95	0.84–0.96	0.97–1.04
Sabinene	0.53–0.66	0.41–0.61	0.46–0.63	0.46–0.58	0.54–0.66
Myrcene	0.23–0.32	0.19–0.26	0.05–0.23	0.21–0.29	0.05–0.30
α-Terpinene	0.35–0.46	0.36–0.42	0.37–0.40	0.35–0.51	0.36–0.39
Limonene	1.42–1.74	1.27–1.48	1.58–1.82	1.71–1.96	1.52–1.84
1,8-Cineole	5.57–6.16	4.93–5.47	4.52–5.27	5.08–5.77	4.73–5.65
(E)-β-Ocimene	0.24–0.29	0.32–0.46	0.28–0.48	0.34–0.51	0.28–0.43
γ-Terpinene	0.50–0.82	0.51–0.59	0.51–0.62	0.51–0.72	0.51–0.57
p-Cymene	0.05–0.17	*t*–0.05	*t*–0.05	*t*–0.05	*t*–0.05
Terpinolene	0.22–0.27	0.23–0.25	0.22–0.25	0.22–0.29	0.22–0.26
3-Octanol	0.22–0.29	0.25–0.30	0.17–0.21	0.23–0.27	0.23–0.28
1-Octen-3-ol	0.16–0.19	0.16–0.22	0.05–0.16	0.16–0.21	0.15–0.19
Menthone	24.47–29.92	17.75–23.34	13.94–22.64	14.77–25.32	11.19–19.46
trans-Sabinene hydrate	0.59–0.91	0.66–1.03	0.78–1.28	0.71–1.36	0.82–1.06
Menthofuran	1.55–2.97	1.38–2.01	1.13–2.32	1.65–3.59	4.29–6.71
Isomenthone	3.79.4.31	2.81–3.13	2.03–3.11	2.41–3.52	2.05–3.08
β-Bourbonene	0.33–0.43	0.41–0.55	0.56–0.72	0.43–0.55	0.44–0.63
Linalool	0.38–0.51	0.26–0.35	0.23–0.24	0.28–0.49	0.27–0.46
Menthyl acetate	2.91–3.58	4.31–5.68	3.73–7.49	2.77–6.36	5.25–8.39
Neoisomenthyl acetate + isopulegol	2.08–2.63	1.47–1.99	1.89–2.14	1.80–2.38	1.69–2.50
Neomenthol + isomenthyl acetate	2.38–3.33	3.47–3.93	3.21–4.53	3.15–3.83	3.17–3.80
Terpinen-4-ol + β-caryophyllene	1.02–1.22	0.99–1.09	0.89–1.15	0.87–1.51	0.96–1.09
Neoisomenthol + isomenthol	0.48–0.67	0.84–0.98	0.76–1.13	0.68–0.96	0.75–1.04
Pulegone	0.53–4.04	0.51–1.06	0.74–2.17	0.42–2.77	0.53–3.95
Menthol	33.16–37.52	41.27–45.39	40.61–47.44	38.06–46.59	40.27–44.37
α-Terpineol	0.22–0.26	0.15–0.17	0.12–0.50	0.15–0.22	0.15–0.22
Germacrene D + carvone	2.28–2.61	1.96–2.41	1.89–2.58	2.09–2.72	0.74–2.85
Piperitone	0.45–0.59	0.62–0.68	0.52–0.65	0.55–0.61	0.49–0.52
Viridiflorol	0.20–0.28	0.20–0.28	0.22–0.34	0.16–0.29	0.23–0.52
No. of samples	26	48	31	34	34

t = trace (<0.01%).

monoterpene and sesquiterpene alcohols have been occasionally identified in this commercially important oil (see Table 7.3q). From a summary of the data here, it would appear that a total of 70 alcohols have been characterized in peppermint oil. Earlier, it was noted that caryophyllene alcohol, clovanediol, and β-betulenol were reported as constituents of cornmint oil by Gildemeister and Hoffman (1961). These identifications have not been confirmed in any of the post-1961 studies.

7.3.3.3 Esters

The esters that have been routinely found in measurable quantities as components of peppermint oil are menthyl acetate and neoisomenthyl acetate. In addition, minor amounts (often less than 0.1%) of neomenthyl acetate and isomenthyl acetate have been characterized as components of peppermint oil by numerous research groups. Since 1960, 48 other aliphatic

TABLE 7.3n
Percentage Composition of Peppermint Oil Produced from Plants Harvested over a 4-Month Period

Compound	July	August	September	October
α-Pinene	0.6–1.0	0.6–0.9	0.5–0.9	0.4–0.8
β-Pinene	0.9–1.3	0.9–1.3	0.8–1.3	0.7–1.2
Sabinene	0.5–0.7	0.3–0.6	0.3–0.6	0.3–0.6
Myrcene	0.2–0.4	0.2–0.4	0.2–0.3	0.1–0.3
α-Terpinene	0.4–0.5	0.2–0.5	0.2–0.5	0.2–0.4
Limonene	0.2–2.5	0.2–2.1	0.2–1.6	0.2–1.6
1,8-Cineole	4.9–7.0	5.0–6.8	5.3–6.9	4.5–6.3
γ-Terpinene	0.7–0.8	0.5–0.9	0.5–0.8	0.4–0.8
Terpinolene	0.1–0.2	0.1–0.2	0.1–0.2	0.1
3-Octanol	0.5–0.7	0.6–0.8	0.7–0.8	0.5–0.7
Menthone	16.6–32.7	10.9–25.9	2.3–12.3	1.6–4.6
Menthofuran	1.7–3.3	2.1–8.9	7.0–8.7	5.4–9.5
Isomenthone	3.3–4.2	2.1–3.8	1.2–2.4	1.0–1.5
Linalool	0.2–0.4	0.3–0.5	0.2–0.9	0.1–2.1
Menthyl acetate	1.8–6.8	3.5–8.6	5.8–14.1	9.4–22.5
Neomenthol	3.2–4.2	3.1–4.7	3.4–5.0	3.8–5.0
Terpinen-4-ol	0.6–1.4	0.7–1.3	0.7–1.1	0.8–1.2
β-Caryophyllene	1.4–3.7	1.6–3.1	1.5–2.3	1.4–3.5
Pulegone	0.7–1.6	0.9–2.9	0.6–2.9	0.5–1.0
Menthol	31.8–44.6	35.9–48.0	38.5–57.0	41.4–54.7
α-Terpineol	0.2–0.4	0.3–0.4	0.2–0.3	0.1–0.3
Germacrene D	1.7–4.3	1.8–3.5	1.7–2.6	1.2–3.2
Piperitone	0.5–0.8	0.5–0.7	0.3–0.5	0.3–0.5

and monoterpene esters have been occasionally identified in this commercially important oil as shown in Table 7.3r. A summary of the data presented here reveals that a total of 52 esters have been characterized in peppermint oil. The characterization of methyl octenoate (Gildemeister and Hoffman, 1961) as a component of cornmint oil could not be confirmed in the post-1961 literature.

7.3.3.4 Aldehydes

To date, no aldehydes have been found in measurable quantities in peppermint oil. Since 1960, 34 aliphatic, aromatic, and monoterpene aldehydes have been identified in total in this commercially important oil as can be seen in Table 7.3s.

7.3.3.5 Ketones

The ketones that have been routinely identified in measurable quantities in peppermint oil are menthone, isomenthone, pulegone, piperitone, and carvone. Since 1960, 19 other aliphatic, aromatic, and monoterpene ketones have been occasionally identified in this commercially important oil (see Table 7.3t). A summary of the published data reveals that 24 ketones have been characterized in peppermint oil. Acetone, which was reported as a component of peppermint oil by Gildemeister and Hoffman (1961) could not be confirmed in the post-1961 literature.

TABLE 7.3o
Percentage Composition of Indian Peppermint Oil Produced in Three Different Regions

Compound	Punjab Oil	Bareilly Oil	Badaun Oil
α-Pinene	1.0–1.3	1.3–1.4	1.3–1.4
β-Pinene	1.3–1.4	1.4–1.5	1.3–1.5
Sabinene	0.6	0.6–0.7	0.6–0.7
Myrcene	0.4	0.4	0.4
α-Terpinene	0.2–0.3	0.2	0.2–0.3
Limonene	2.8–3.2	3.5–3.7	3.2–3.7
1,8-Cineole	4.5–4.9	4.4–4.6	4.6
(Z)-β-Ocimene	0.2	0.2	0.2
γ-Terpinene	0.4–0.5	0.3	0.3–0.4
p-Cymene	0.1–0.2	0.1–0.2	0.1
Terpinolene	0.2	0.2	0.2
3-Octanol	0.2–0.3	0.2	0.2
trans-Sabinene hydrate	0–2.7	—	0–2.9
Menthone	25.2–32.3	30.8–31.3	25.2–31.3
Menthofuran	4.7–6.9	9.2–10.8	6.3–9.0
Isomenthone	4.4–5.3	5.1–5.3	5.2–5.3
Menthyl acetate	3.8–4.8	3.1–3.5	3.5–4.8
Neoisomenthyl acetate	2.2–2.9	1.4–1.8	2.0–2.2
Neomenthol	1.8–3.4	2.4–2.7	2.3–2.5
Terpinen-4-ol	0.3–0.8	0.3–0.4	0.3–0.4
Neoisomenthol	0.4–0.5	0.4	0.4–0.5
Pulegone	6.3–9.8	7.7–9.5	6.3–7.8
Menthol	20.4–26.6	18.6–20.1	20.2–24.6
Germacrene D	1.0–1.6	0.9–1.2	1.0–1.2
Piperitone	0.5–0.7	0.5–0.6	0.5–0.7
Viridiflorol	0.1	<0.1–0.1	<0.1–0.1

7.3.3.6 Acids

Although no acids have been found in measurable quantities in peppermint oil, since 1960, 25 aliphatic, aromatic, and monoterpene acids have been identified in total in this commercially important oil as shown in Table 7.3u. The previous characterization of octenoic acid as a component of cornmint oil (Gildemeister and Hoffman, 1961) could not be confirmed in the post-1961 literature.

7.3.3.7 Phenols

No phenols have been found in measurable quantities in peppermint oil. Since 1960, a total of 19 phenols have been identified in this commercially important oil (see Table 7.3v).

7.3.3.8 Nitrogen Heterocyclic Compounds

As one might expect, no nitrogen heterocyclic compounds have been found in amounts exceeding 100 ppm in peppermint oil. Nevertheless, since 1960, 21 aliphatic and aromatic

TABLE 7.3p
Additional Hydrocarbons Found in Peppermint Oil

Compound	References
ε-Cadinene, α-calacorene, guaiazulene	Katsuhara et al. (1967)
α-Bourbonene, α-ylangene, α-maaliene, ε-bulgarene	Vlahov et al. (1967a, 1967b)
β-Cadinene	Goryaev et al. (1967)
δ-3-Carene	Von Schantz and Norri (1968)
Tetradecane, pentadecane, hexadecane, octadecane, nonadecane, eicosane, heneicosane, α-ylangene, viridiflorene, α-gurjunene, α-cadinene, β-Copaene	Lawrence et al. (1972).
p-Mentha-1(7), 8-diene	Sheldon et al. (1972)
β-Gurjunene, γ-elemene	Reverchon et al. (1994)
p-Cymenene	Nanmoku (1995)
Italicene	Zheljazkov et al. (1996)
ω-Cadinene	Joulain and König (1998)
Tricyclene	Moyler and Moss (1998)
trans-β-bergamotene, β-bisabolene	Oberhofer et al. (1999)
epi-Zonarene	Mimica-Dukic et al. (2003)
ε-Muurolene	Vlahov et al. (1967a); Nano et al. (1972)
α-Morphene, α-muurolene	Lawrence et al. (1972); Graven (1988)
Bicycloelemene	Vlahov et al. (1967a); Joulain and König (1998)
(Z,E)-Allo-ocimene	Sheldon et al. (1972); Nanmoku (1995)
1,(3Z,5Z)-Undecatriene	Mookherjee et al. (1989); Güntert et al. (2000)
β-Cubebene	Charles et al. (1990); Mimica-Dukic et al. (2003)
Aromadendrene	Katsuhara et al. (1967); Vlahov et al. (1967a); Nano et al. (1972)
α-Cubebene	Vlahov et al. (1967a); Reverchon et al. (1994); Nanmoku (1995)
γ-Muurolene	Lawrence et al. (1972); Graven (1988); Reverchon et al. (1994)
γ-Cadinene	Katsuhara et al. (1964); Goryaev et al. (1967); Lawrence et al. (1972); Reverchon et al. (1994)
α-Phellandrene	Röthbacher et al. (1967); Sacco and Gallino (1976); Nanmoku (1995); Lawrence (2000)
Bicyclogermacrene	Zheljazkov et al. (1996); Benn (1998); Oberhofer et al. (1999); Güntert et al. (2000)
trans-Calamenene	Vlahov et al. (1967b); Katsuhara et al. (1967); Lawrence et al. (1972); Belafi-Rethi et al. (1973a); Reverchon et al. (1994)
α-Copaene	Wenninger et al. (1967); Lawrence et al. (1972); Bicchi et al. (1989); Nanmoku (1995); Güntert et al. (2000)
β-Phellandrene	Lawrence et al. (1972); Formacek and Kubezka (1982); Birillo (1989); Nanmoku (1995); Moyler and Moss (1998)
δ-Cadinene	Katsuhara et al. (1964); Vlahov et al. (1967a); Lawrence et al. (1972); Chialva et al. (1982); Nanmoku (1995); Benn (1998)
(E)-β-Farnesene	Lawrence et al. (1972); Wong (1972); Hefendehl and Ziegler (1975); Zheljazkov et al. (1996); Oberhofer et al. (1999); Güntert (2000)
β-Elemene	Katsuhara et al. (1964); Vlahov et al. (1967a); Wenninger et al. (1967); Goryaev et al. (1967); Bicchi et al. (1989); Reverchon et al. (1994); Zheljazkov et al. (1996); Benn (1998); Güntert et al. (2000); Mimica-Dukic et al. (2003)
α-Humulene	Derying et al. (1962); Vlahov et al. (1967b); Wenninger et al. (1967); Chialva et al. (1982); Carnat and Lamaison (1987); Bicchi et al. (1989); Voronina et al. (1990); Reverchon et al. (1994); Nanmoku (1995); Zheljazkov et al. (1996); Benn (1998); Moyler and Moss (1998); Güntert et al. (2000)

TABLE 7.3q
Additional Alcohols Found in Peppermint Oil

Compound	References
Methanol, butanol	Hefendehl (1962)
p-Menthane-1,3-diol, *p*-menthane-2,3-diol, *p*-menth-2-ene-1,4-diol	Katsuhara et al. (1966)
Propanol, 2-propanol, undecanol, dodecanol, 1-penten-3-ol, *p*-cymen-8-ol, *p*-menth-1-en-9-ol	Sheldon et al. (1972)
p-Menthan-9-ol	Lawrence et al. (1972)
2,6-Dimethyl-2-octanol, 3-7-dimethyl-1, 7-octadien-3-ol	Belafi-Rethi et al. (1973b)
2-Ethylcyclohexanol, *cis*-piperitol, perillyl alcohol, *cis*-sabinol, (E)-nerolidol, cyclopentanol, cumin alcohol	Takahashi et al. (1980)
Terpinen-1-ol, *cis*-carveol, trans-carveol, amyl alcohol, 2-heptanol	Fleisher and Fleisher (1991)
T-Cadinol	Reverchon et al. (1994)
Aromadendrol*	Ohloff (1994)
trans-*p*-Mentha-2,8-dien-1-ol	Nanmoku (1995)
trans-Pinocarveol, α-cadinol	Zheljazkov et al. (1996).
γ-3,4-Dehydro-7,8-dihydro-α-ionol, (Z)-8-mycenol, (Z,Z)-8-ocimenol, (E,Z)-8-ocimenol	Güntert et al. (2000)
T-Muurolol	Mimica-Dukic et al. (2003)
3-Heptanol	Sheldon et al. (1972); Takahashi et al. (1980)
Citronellol	Lawrence et al. (1972); Nanmoku (1995)
(Z)-2-Pentenol, myrtenol	Takahashi et al. (1980); Fleisher and Fleisher (1991)
Lavandulol	Takahashi et al. (1980); Nanmoku (1995)
δ-Terpineol	Nanmoku (1995); Güntert et al. (2000)
Octanol	Nanmoku (1995); Shahi et al. (1999)
Ethanol	Hefendehl (1962); Lawrence et al. (1972); Nanmoku (1995)
trans-*p*-Menth-2-en-1-ol	Lawrence et al. (1972); Graven (1988); Güntert et al. (2000)
cis-*p*-Menth-2-en-1-ol	Zheljazkov et al. (1996); Shahi et al. (1999); Güntert et al. (2000)
Borneol	Hefendehl (1962); Fleisher and Fleisher (1991); Nanmoku (1995); Iscan et al. (2002)
trans-Piperitol	von Schantz and Norri (1968); Takahashi et al. (1980); Zheljazkov et al. (1996); Moyler and Moss (1998)
Isobutanol	Sheldon et al. (1972); Fleisher and Fleisher (1991); Benn (1998); Lawrence (2000)
2-Methylbutanol	Sheldon et al. (1972); Spencer (1992); Benn (1998); Lawrence (2000)
cis-Sabinene hydrate	Handa et al. (1964); Lawrence et al. (1972); Nanmoku (1995); Zheljazkov et al. (1996); Güntert (2000)
Isoamyl alcohol	Hefendehl (1962); Lawrence et al. (1972); Hefendehl and Ziegler (1975); Fleisher and Fleisher (1991); Nanmoku (1995); Benn (1998); Lawrence (2000)
(E)-2-Hexenol	Hefendehl (1962); Lawrence et al. (1972); Mookherjee et al. (1989); Fleisher and Fleisher (1991); Spencer (1992); Moyler and Moss (1998); Benn (1998)
Neoiso(iso)pulegol	Porsch and Farnow (1962); Spencer et al. (1997); Benn (1998); Moyler and Moss (1998); Lawrence (2000)

continued

TABLE 7.3q (continued)
Additional Alcohols Found in Peppermint Oil

Compound	References
Spathulenol	Reverchon et al. (1994); Nanmoku (1995); Zheljazkov et al. (1996); Benn (1998); Shahi et al. (1999); Güntert et al. (2000); Mimica-Dukic et al. (2003)
Hexanol	Hefendehl (1962); Lawrence et al. (1972); Sacco and Gallino (1976); Mookherjee et al. (1989); Topolov and Zheljazkov (1991); Fleisher and Fleisher (1991); Spencer (1992); Nanmoku (1995); Benn (1998)
Isopulegol	Sheldon et al. (1972); Carnat and Lamaison (1987); Fleisher and Fleisher (1991); Lawrence (1993); Nanmoku (1995); Zheljazkov et al. (1996); Spencer et al. (1997); Moyler and Moss (1998); Benn (1998); Güntert et al. (2000); Kubeczka and Formacek (2002).

*Correct isomer not identified.

nitrogen heterocyclic compounds have been identified in this commercially important oil as can be seen in Table 7.3w.

7.3.3.9 Miscellaneous Compounds

Menthofuran (I) and 1,8-cineole have been routinely identified in measurable quantities in peppermint oil. Since 1960, 51 other miscellaneous compounds have been characterized as minor constituents (mostly $<0.1\%$). Many of these minor components have been characterized as the subject of limited studies. In total, 53 miscellaneous compounds have been identified as constituents of peppermint oil as shown in Table 7.3x. The structures of some of the more unusual lactones and furans can be seen in Figure 7.1.

7.3.3.10 Chirality

The enantiomeric distribution of the main constituents of peppermint oil can be seen in Table 7.3y. Vlahov et al. (1967a) determined that the main enantiomers of the sesquiterpene hydrocarbons found in a sample of Bulgarian peppermint oil were as follows:

(−)-bicycloelemene
(−)-α-cubebene
(+)-α-ylangene
(+)-β-elemene
(+)-α-bourbonene
(−)-β-bourbonene
(−)-β-caryophyllene
(+)-aromadendrene
(+)-α-maaliene
(+)-ε-muurolene
(+)-γ-muurolene

TABLE 7.3r
Additional Esters Found in Peppermint Oil

Compound	References
Amyl formate	Ognyanov and Vlahov (1965)
p-Menth-1-en-9-yl acetate, 2-phenethyl isovalerate, 2-methylbutyl 2-methylbutyrate	Lawrence et al. (1972)
Ethyl acetate, isoamyl acetate, 2-methylbutyl acetate, 1-octen-3-yl acetate, hexyl isovalerate, 2-phenethyl 2-methylbutyrate	Sheldon et al. (1972)
Menthyl valerate	Hefendehl and Ziegler (1975)
Ethyl 3,6-dimethyl-7-oxo-octanoate, dihydro-α-terpinyl acetate,*isopulegyl acetate, 2-phenethyl acetate, 2-phenethyl butyrate, 2-phenethyl isobutyrate, 2-phenethyl valerate, isoamyl phenylacetate, methyl jasmonate, methyl salicylate	Takahashi et al. (1980)
α-Terpinyl isovalerate	Maurer and Hauser (1988)
(Z)-3-Hexenyl acetate, (Z)-3-hexenyl propionate	Bicchi et al. (1989)
Methyl butyrate	Spencer (1992)
Linalyl acetate	Reverchon et al. (1994)
Menthyl formate, ethyl phenylacetate	Nanmoku (1995)
Isoamyl 2-methylbutyrate, neoiso(iso)pulegyl acetate	Zheljazkov et al. (1996)
Amyl 2-methylbutyrate, amyl isovalerate, octyl acetate, ethyl isovalerate	Benn (1998)
(Z)-8-Myrcenyl acetate, (Z,Z)-8-ocimenyl acetate, (E,Z)-8-ocimenyl acetate	Güntert et al. (2000)
2-Methylbutyl isovalerate	Sheldon et al. (1972); Takahashi et al. (1980)
Geranyl acetate	Takahashi et al. (1980); Nanmoku (1995)
Octyl isovalerate	Takahashi et al. (1989); Benn (1998)
Methyl 2-methylbutyrate	Takahashi et al. (1980); Coleman et al. (2002)
Bornyl acetate	Reverchon et al. (1994); Mimica-Dukic (2003)
Menthyl isovalerate	Hefendehl (1962); Röthbacher et al. (1970); Takahashi et al. (1980)
(Z)-3-Hexenyl isovalerate	Sheldon et al. (1972); Nanmoku (1995); Benn (1998).
Ethyl 2-methylbutyrate	Sheldon et al. (1972); Benn (1978); Moyler and Moss (1998)
p-Mentha-1,8-dien-9-yl acetate	Lawrence et al. (1972); Nanmoku (1995); Güntert et al. (2000)
Isoamyl isovalerate	Takahashi et al. (1980); Fleisher and Fleisher (1991); Nanmoku (1995); Zheljazkov et al. (1996); Shahi et al. (1999); Güntert et al. (2000)
3-Octyl acetate	Vlahov and Ogyanov (1964); Sheldon et al.(1972); Carnat and Lamaison (1987); Spencer (1992); Nanmoku (1995); Zheljazkov et al. (1996); Benn(1998); Lawrence (2000); Güntert et al. (2000)

*Correct isomer not identified.

(−)-α-muurolene
(−)-ε-bulgarene
(+)-δ-cadinene
(−)-calamenene isomer

TABLE 7.3s
Aldehydes Found in Peppermint Oil

Compound	References
Furfural	Hefendehl (1962)
5-Methyl-2-hexenal, *p*-methoxybenzaldehyde	Sheldon et al. (1972)
Butanal	Lawrence et al. (1972)
2-Methyl-2-butenal, nonanal, (E)-2-octenal, geranial, salicylaldehyde, 3,4-dimethyloxybenzaldehyde, 4-methyl-2-phenyl-2-pentenal, 5-methyl-2-phenyl-2-pentenal, 2-methoxycinnamaldehyde	Takahashi et al. (1980)
(Z)-3-Hexenal, 2,4-hexadienal*	Mookherjee et al. (1989)
Tiglic aldehyde, veratraldehyde	Fleisher and Fleisher (1991)
Octanal, 2,4-nonadienal*, (E,Z)-2,6-nonadienal	Benn (1998)
Myrtenal	Sheldon et al. (1972); Takahashi et al. (1980)
Benzaldehyde	Hefendehl (1962); Fleisher and Fleisher (1991)
Heptanal	Sheldon et al. (1972); Spencer (1992)
Neral	Takahashi et al. (1980); Nanmoku (1995)
Anisaldehyde	Fleisher and Fleisher (1991); Benn (1998)
Valeraldehyde	Fleisher and Fleisher (1991); Coleman et al. (2002)
Phenylacetaldehyde	Ognyanov and Vlahov (1962); Sheldon et al. (1972); Benn (1998)
(Z)-4-Heptenal	Sheldon et al. (1972); Fleisher and Fleisher (1991); Benn (1998)
Hexanal	Mookherjee et al. (1989); Fleisher and Fleisher (1991); Spencer (1992)
Acetaldehyde	Ognyanov and Vlahov (1962); Hefendehl (1962); Wong (1972); Coleman et al. (2002)
2-Methylbutanal	Lawrence et al. (1972); Benn (1998); Coleman et al. (2002)
Isovaleraldehyde	Ognyanov and Vlahov (1962); Hefendehl (1962); McCarthy et al. (1963); Wong (1972); Nanmoku (1995); Benn (1998); Moyler and Moss (1998); Lawrence (2000)
(E)-2-Hexenal	Ognyanov and Vlahov (1965); Lawrence (1972); Graven (1988); Mookherjee et al. (1989); Fleisher and Fleisher (1991); Spencer (1992); Kubeczka and Protzen (1997); Coleman et al. (2002)
Isobutanal	Wong (1972); Hefendehl and Ziegler (1975); Fleisher and Fleisher (1991); Nanmoku (1995) Moyler and Moss (1998); Lawrence (2000); Coleman et al. (2002)

*Correct isomer not identified.

Emberger and Hopp (1985), Werkoff and Hopp (1986), and Faber et al. (1994) determined that the other menthol isomers found in peppermint oil were (1R,3S,4R)-(+)-isomenthol and (1R,3R,4R)-(+)-neoisomenthol. Takahashi et al. (1980) found that the absolute configurations of the mint lactones were (6R, 7aR)-(−)-mintlactone(4-hydroxy-2-methyl-2-cyclohexen-1-one) and (6R,7aS)-(+)-isomintlactone(3,6-dimethyl-5,6,7,7a-tetrahydro-2(4H)-benzofuranone). Derbesy et al. (1991) determined that (4R)-(+)-α-terpineol was a minor component of peppermint oil. König et al. (1997) reported that (1R,3R,4S)-(−)-isopulegol and (1R,4S,5S)-(+)-*trans*-sabinene hydrate were also found in peppermint oil. Galle-Hoffman

TABLE 7.3t
Additional Ketones Found in Peppermint Oil

Compound	References
2,3-Butanedione	Sheldon et al. (1972)
trans-Isopulegone	Lawrence (1978)
2-Butanone, 2-heptanone, 6-methyl-5-hepten-2-one, 5-methyl-3-heptanone, cryptone, *p*-methoxyacetophenone	Takahashi et al. (1980)
Isopinocamphone, *trans*-dihydrocarvone	Fleisher and Fleisher (1991)
Carvotanacetone	Voronina et al. (1990)
1-Octen-3-one	Moyler and Moss (1998)
Geranyl acetone	Sheldon et al. (1972); Benn (1998)
(E)-β-Damascenone	Moyler and Moss (1998); Güntert et al. (2000)
β-Ionone*	Takahashi et al. (1980); Benn (1998)
Piperitenone	Röthbacher et al. (1970); Sheldon et al. (1972); Fleisher and Fleisher (1998)
3-Octanone	Ognyanov and Vlahov (1962); Hefendehl and Ziegler (1975); Nanmoku (1995); Benn (1998); Moyler and Moss (1998)
(Z)-Jasmone	Sheldon et al. (1972); Fleisher and Fleisher (1991); Ohloff (1994); Nanmoku (1995); Benn (1998); Güntert (2000)

and König (1998) confirmed that the previous enantiomeric assignments for menthone, isomenthone, menthofuran, isopulegol, neomenthol, neoisomenthol, isomenthol, menthol, pulegone, and *trans*-sabinene hydrate were correct using chiral GC.

Güntert et al. (2001) determined that (6R)-(+)-menthofuran, (6R,7aR)-(−)-mintlactone, and (6R)-(+)-2,3-dehydromintlactone were identified as enantiomerically pure in peppermint oil.

7.4 PENNYROYAL OIL ex *Mentha pulegium* L.

7.4.1 Introduction

Mentha pulegium (pennyroyal) can be found throughout southwest and central Europe from Ireland and Central Poland to Ukraine (Tutin et al., 1972). It can be found in abundance in the Iberian Peninsula and North African countries that border the Mediterranean such as Algeria, Morocco, and Tunisia.

7.4.2 Cultivation and Oil Production

Pennyroyal oil is produced only from wild plants that are harvested when they are in full flower. The main countries where oil is produced are Spain and Morocco, where the annual production is estimated to be 3 tonnes and 7 tonnes to 16 tonnes respectively, depending on demand. Baser (1994) reported that Turkish production of pennyroyal oil was ca. 3 tonnes.

7.4.3 Oil Composition

A review of the early published literature (Gildemeister and Hoffman, 1961) shows that the components that have been characterized in pennyroyal oil are as follows:

TABLE 7.3u
Acids Found in Peppermint Oil

Compound	References
Heptanoic, decanoic	Lawrence et al. (1972)
Isobutyric, 3,6-dimethyl-7-oxo-octanoic, 3,7-dimethyl-6-oxo-octanoic, phenylacetic	Takahashi et al. (1980)
cis-2-Pentylcyclopropane-1-carboxylic, 3-isopropylpentane-1,5-dioic, salicylic	Tsuneya et al. (1998)
2,6-Dimethylheptanoic, 2-isopropyl-5-menthyl-5-hexenoic, 3,7-dimethyloctanoic, 2-isopropyl-5-oxo-hexanoic, 2,6-dimethyl-5-oxo-heptanoic, 2-(4-methyl-2-oxo-cyclohexyl)-propionic, 3,7-dimethyl-6-oxo-octanoic	Näf and Velluz (1998)
Hexanoic, octanoic	Lawrence et al. (1972); Tsuneya et al. (1998)
(E)-2-Hexenoic, benzoic	Takahashi et al. (1980); Tsuneya et al. (1998)
Citronellic, geranic acid	Takahashi et al. (1980); Näf and Velluz (1998)
Isovaleric acid	Derying et al. (1962); Lawrence et al. (1972); Tsuneya et al. (1998)
2-Methylbutyric acid	Lawrence et al. (1972); Benn (1998); Tsuneya et al. (1998)

α-pinene
limonene
3-octanol
3-octyl acetate
1-methyl-3-cyclohexanol*
1-methyl-3-cyclohexanone*
1-methyl-3-cyclohex-1-enone
1,1,3-trimethyl-4-cyclopentanone*
menthone
isomenthone
piperitone
piperitenone
isopiperitenone
menthol
thymol
carvacrol
eugenol

Examination of the composition of the commercial pennyroyal oil reveals that it is always rich in pulegone (Lawrence, 1998). The changes in oil composition of *M. pulegium* grown in Japan during its various development stages were examined by Fujita and Fujita (1970). As can be seen in Table 7.4a, oils produced from vegetative and flowering plants were richer in pulegone than oils produced from plants harvested at their post flowering stage.

An oil of *M. pulegium* produced from plants collected in the Guadalajara province (Spain) was reported (de Gavina Mugica and Ochoa, 1974) to contain the following components:

TABLE 7.3v
Phenols Found in Peppermint Oil

Compound	References
5-Methyl-2-(2′-oxo-3′-butyl)-phenol 5-methyl-2-(3′-oxo-2′-pentyl)-phenol	Sakurai et al. (1983a)
3-(2-hydroxy-4-methylphenyl)-ethanone, 1-(2-hydroxy-4-methylphenyl)-ethanone, 2-(2-hydroxy-4-methylphenyl)-3-pentanone, 4-ethylguaiacol, phenol, 4-(2-propenyl)-phenol, 4-isopropylphenol, 8,9-dehydrothymol, 8,9-dehydrocarvacrol	Näf and Velluz (1998)
o-Cresol, *p*-cresol, vanillin	Takahashi et al. (1980); Näf and Velluz (1998)
Guaiacol	Takahashi et al. (1980); Benn (1998)
4-Vinylguaicol	Benn (1998); Näf and Velluz (1998)
Carvacrol	Hefendehl (1962); Fleisher and Fleisher (1991); Näf and Velluz (1998)
Eugenol	Lawrence et al. (1972); Fleisher and Fleisher (1991); Nanmoku (1995); Benn (1998); Tsuneya et al. (1998); Näf and Velluz (1998)
Thymol	Hefendehl (1962); Lawrence et al. (1972); Hefendehl and Ziegler (1975); Fleisher and Fleisher (1991); Nanmoku (1995); Benn (1998); Näf and Velluz (1998); Shahi et al. (1999); Güntert et al. (2000)

α-pinene (1.5%)
camphene (0.1%)
β-pinene + sabinene (0.5%)
limonene + 1,8-cineole + β-ocimene (0.8%)
p-cymene (0.1%)
3-octanol (1.2%)
menthone (15.6%)
isomenthone + menthofuran (2.2%)
neomenthol (0.1%)
pulegone + isomenthol (72.4%)
piperitone (0.5)

Trace amounts (<0.1%) of α-terpinene, α-phellandrene, 1-octen-3-ol, neoisomenthol, and menthol were also found in this same oil.

Velasco-Negueruela et al. (1987) analyzed oils produced from *M. pulegium* that were collected from their natural habitats in the Iberian Peninsula. The components identified in these oils and their range in composition can be seen as follows:

α-pinene (0.2%–0.3%)
camphene (0%–0.1%)
β-pinene (0.2%–0.3%)
α-phellandrene (<0.1%)
α-terpinene (0.1%–0.5%)
limonene +1,8-cineole (0.7%–1.4%)
p-cymene (0.1%)

TABLE 7.3w
Nitrogen Heterocyclic Compounds Found in Peppermint Oil

Compound	References
Benzyl cyanide	Takahashi et al. (1980)
2,6-Dimethylpyridine, 5-ethyl-2-methylpyridine	Sakurai et al. (1983b)
2-Methylpyridine, 2,5-dimethylpyrazine. 2-acetylpyridine, 2-isopropyl-2-methylpyridine, 4-isopropyl-2-methylpyridine, 3-[(Z)-1-buten-1-yl]pyridine, 3-[(E)-1-buten-1-yl]pyridine, 2-ethyl-4-isopropylpyridine, quinoline, 5[(Z)-1-buten-1-yl]-2-propylpyridine, 5[(Z)-1-buten-1-yl]-4-propylpyridine, 5[(E)-1-buten-1-yl]-2-propylpyridine, 5[(E)-1-buten-1-yl]-4-propylpyridine	Ishihara et al. (1992)
Pyridine	Takahashi et al. (1980); Ishihara et al. (1992)
4-Methylquinoline	Takahashi et al. (1980); Benn (1998)
3-Phenylpyridine, 5-phenyl-2-propylpyridine, 3-phenyl-4-propylpyridine	Sakurai et al. (1983b); Ishihara et al. (1992)

terpinolene (0%–0.1%)
3-cotanol (0.5%–0.8%)
1-octen-3-ol (0%–0.1%)
3-octanone (0%–0.1%)
allo-ocimene*+ fenchone† (0.2%–0.4%)
α-thujone† (0%–0.1%)
trans-sabinene hydrate + sabinyl acetate† (0%–0.1%)
menthone (1.7%–7.5%)
menthofuran (0.1%)
isomenthone (0.8%–4.5%)
isopulegol (1.8%–2.2%)
neomenthol + neoiso(iso)pulegol (0.5%–0.6%)
menthol + neoisomenthol (0.7%–1.3%)
pulegone + isopulegone* (76.0%–81.2%)
piperitone (0.3%–0.4%)
cis-piperitone oxide (0.3%–0.7%)
nerol (<0.1%–0.3%)
piperitenol* (0.9%–1.6%)
piperitenone (1.1%–2.6%)
piperitenone oxide (0%–0.1%)
thymol (0.1%)
δ-cadinene (0.2%–0.6%)

*Correct isomer not identified,
†Questionable identity.

Stengele (1994) determined that the composition of a commercial oil of pennyroyal was as follows:

α-pinene (0.4%)
β-pinene (0.3%)

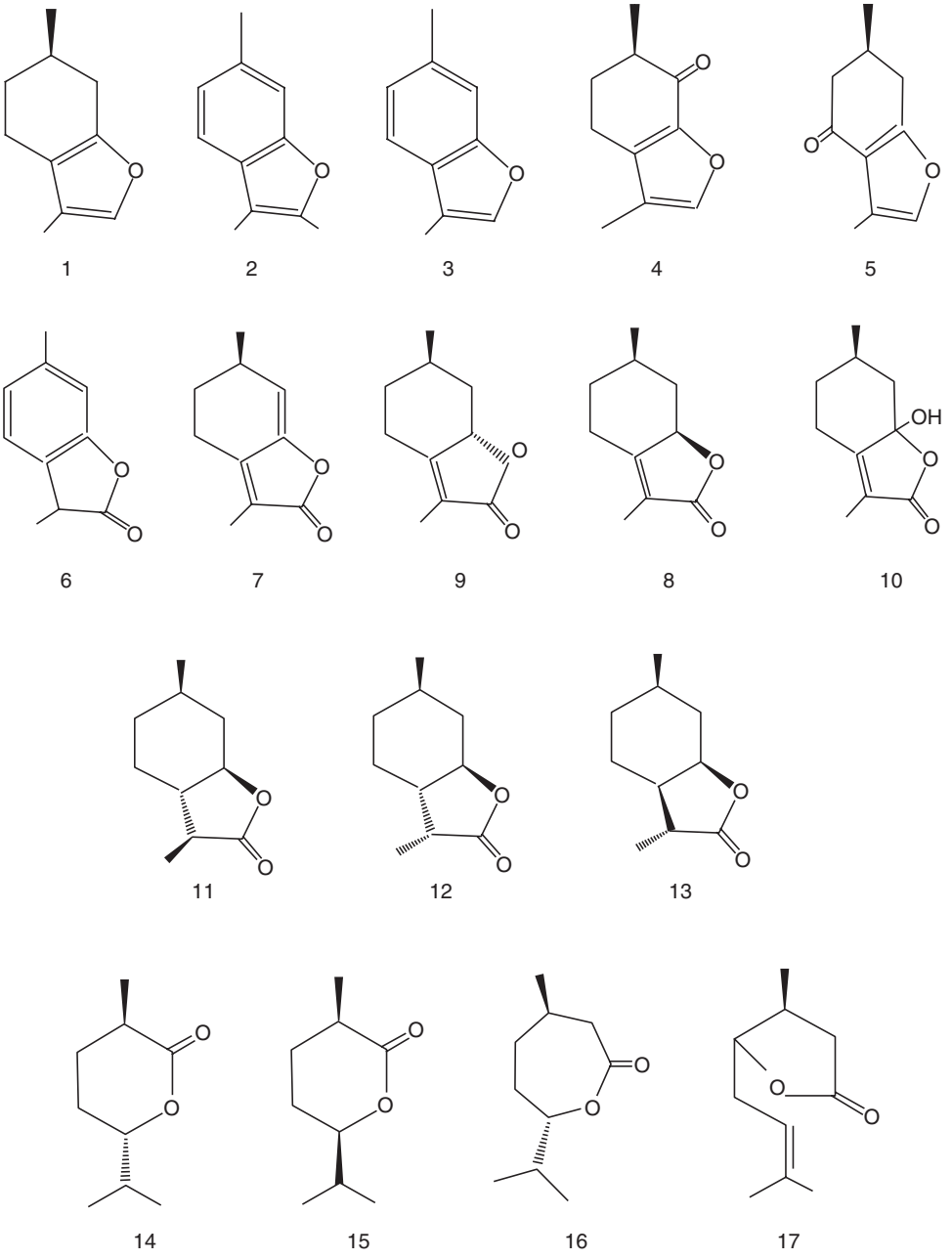


FIGURE 7.1 Miscellaneous furans and lactones found in peppermint oil.

TABLE 7.3x
Additional Miscellaneous Compounds Found in Peppermint Oil

Compound	References
2-Methylfuran	Lawrence et al. (1972)
Menthofurolactone (3,6-dimethyl-4,5,6,7-tetrahydro-benzo-[b]-furan-2(3H)-one	Sheldon et al. (1972)
(E)-Anethole, bovolide, hydroxybovolide, 1,4-dimethoxybenzene, dimethyl sulphoxide, *cis*-rose oxide, *trans*-rose oxide, diosphenol, 4-hydroxy-2-cyclohex-1-one, γ-jasmin lactone, δ-jasmin lactone	Takahashi et al. (1980)
3-(5′,5′-dimethyltetrahydrofuran-2′-yl)-(Z)-2-butenol	Sakurai et al. (1983a)
p-Menthane-3,8,9-triol or the ester	Köpsel et al. (1986)
cis-Linalool oxide (furanoid)	Spencer (1992)
1,4-Cineole, limonene oxide*	Nanmoku (1995)
Methional	Benn (1998)
2,3-6-Trimethylbenzo-[b]-furan (**2**) 5,6-dihydro-3,6-dimethylbenzo-[b]-furan-2(4H)-one (**4**) evodone (**5**), γ-octalactone (4-octanolide), *trans*-2,6-dimethyl-5-heptanolide (**14**) *cis*-2,6-dimethyl-5-heptanoilide (**15**) 3,7-dimethyl-4-oct-6-enolide (**17**) (3S,3aS,6R,7[a]R)perhydro-3,6-dimethylbenzo-[b]-furan-2-one (**11**) (3R,3aS,6R, 7[a]R)perhydro-3,6-dimethylbenzo-[b]-furan-2-one (**12**) (3R,3aS,6R,7[a]R)perhydro-3,6-dimethylbenzo-[b]-furan-2-one (**13**) 5,6,7,7[a]-tetrahydro-3,7-dimethyl-7[a]-hydroxy-benzo-[b]-furan-2(4H)-one (**10**) 5-hydroxy-3,4-dimethyl-5-pentyl-2-(5H)-furanone 4-dec-(7Z)-enolide, 5-dec-(7Z)-enolide, *trans*-menthone lactone (**16**), 7-methoxycoumarin, δ-undecalactone(5-undecanolide), 2(5H)-furanone	Näf and Velluz (1998)
Coumarin, γ-decalactone(4-decanolide) δ-dodecalactone(5-dodecanolide)	Takahashi et al. (1980); Näf and Velluz (1998)
Dihydroedulan	Zheljazkov et al. (1996); Näf and Velluz (1998)
Mint sulfide	Takahashi et al. (1981); Güntert et al. (2000)
3,6-Dimethylbenzo-[b]-furan (**3**) (6R)-5,6-dihydro-3,6-dimethylbenzo-[b]-furan-2(4H)-one (dehydromintlactone) (**7**) 3,6-dimethylbenzo-[b]-furan-2(3H)-one (furamintone) (**6**) perhydro-3,6-dimethylbenzo-[b]-furan-2-one	Näf and Velluz (1998); Güntert et al. (2000)
Piperitone oxide*	Handa et al. (1964); Nanmoku (1995); Benn (1998)
Mintlactone (9), isomintlactone (8)	Takahashi et al. (1980); Benn (1998); Güntert et al. (2000)
cis-2,5-Diethyltetrahydrofuran	Sheldon et al. (1972); Fleisher and Fleisher (1991); Nanmoku (1995); Benn (1998); Lawrence (2000); Coleman et al. (2002)
Dimethyl sulfide	Peyron (1961); Fleisher and Fleisher (1991); Nanmoku (1995); Benn (1998); Moyler and Moss (1998); Lawrence (2000); Coleman et al. (2002)
2-Ethylfuran	Lawrence et al. (1972); Hefendehl and Ziegler (1975); Fleisher and Fleisher (1991); Spencer (1992); Nanmoku (1995); Benn (1998); Moyler and Moss (1998); Coleman et al. (2002)
Caryophyllene oxide	Lawrence et al. (1972); Reverchon et al. (1994); Nanmoku (1995); Zheljazkov et al. (1996); Benn (1998); Shahi et al. (1999); Lawrence (2000); Güntert et al. (2000)

*Correct isomer not identified.

TABLE 7.3y
Enantiomeric Distribution of the Main Constituents of Peppermint Oil

Enantiomeric Ratio	References
(1R,5R)-(+)-α-pinene (30.0%–54.9%):	
(1S,5S)-(−)-α-pinene (45.1%–70.0%)	Mosandl et al. (1991); Coleman and Lawrence (2000)
(1R,5R)-(+)-β-pinene (40.0%–75.%):	
(1S,5S)-(−)-β-pinene (24.5%–60.0%)	Mosandl et al. (1991); Coleman and Lawrence (2000)
(4R)-(+)-limonene (1.7%–20.0%):	
(4S)-(−)-limonene (79.9%–98.3%)	Mosandl et al. (1991); Coleman and Lawrence (2000)
(1S,4R)-(+)-menthone (0%–1.0%):	
(1R,4S)-(−)-menthone (99.0%–100%)	Werkoff and Hopp (1986); Ravid et al. (1994b); Casabianca et al. (1996)
(1R,4R)-(+)-isomenthone (<1.0%):	
(1S,4S)-(−)-isomenthone (>99%)	Werkoff and Hopp (1986); Ravid et al. (1994b)
(1R)-(+)-pulegone (95.0%–99.0%):	
(1S)-(−)-pulegone (1.0%–5.0%)	Ravid et al. (1994c)
(4S)-(+)-piperitone (76.0%–99.0%):	
(4R)-(−)-piperitone (<1.0%–24.0%)	Burbott et al. (1983); Ravid et al. (1994a)
(6R)-(+)-menthofuran (>99.9%):	
(6S)-(−)-menthofuran (<0.1%)	Wüst and Mosandl (1999)
(1S,3S,4R)-(+)-menthol (0–1.0):	
(1R,3R,4S)-(−)-menthol (99.0%–100%)	Emberger and Hopp (1985); Werkoff and Hopp (1986); Casabianca et al. (1996)
(3R)-(+)-3-octanol (94.1%–100%):	
(3S)-(−)-3-octanol (0%–5.9%)	Casabianca et al. (1996)
(1S,3S,4R)-(+)-menthyl acetate (0%):	
(1R,3R,4S)-(−)-menthyl acetate	Kreis et al. (1990b); Casabianca et al. (1996)

TABLE 7.4a
Comparative Percentage Composition of *Mentha pulegium* Oil Produced from Plants at Various Stages of Maturity

Compound	Vegetative Stage	Preflowering Stage	Full Flowering Stage	Postflowering Stage
α-Pinene	0.1	0.1	0.1	0.1–0.3
β-Pinene	0.1	0.1	0.1–0.2	0.2–0.3
Limonene	0.5–3.4	0.2–0.4	0.1	0.1
3-Octanone	0.4–1.0	0.2–0.6	0.2–0.4	0.2
p-Cymene	t–0.2	0.1	0.1	0.1
3-Octanol	0.8–2.0	2.2–2.3	1.8–2.2	1.4–1.8
1-Octen-3-ol	*t*–0.1	*t*	0.1	0.1
Menthone	3.4–8.8	6.0–7.2	13.8–14.6	21.8–41.8
Isomenthone	0.4–0.7	0.7	0.7	2.2–6.3
Isopulegone*	1.5–2.4	1.5–1.9	1.4–2.0	1.1–1.2
Menthol	0.4–0.9	0.6–0.7	0.2–0.4	0.2
Pulegone	81.0–85.5	85.0	76.0–78.0	43.5–69.5
Piperitone	0–5	*t*	*t*–0.4	0.6–1.0
Piperitenone	0.4–3.5	2.0–2.2	1.2–2.5	2.2–3.2

*Correct isomer not identified.
t = trace (<0.1%).

limonene (1.0%)
5-methyl-3-heptanone (0.5%)
3-octanol (0.7%)
menthone (2.3%)
isomenthone (0.8%)
cis-isopulegone (0.6%)
trans-isopulegone (1.2%)
β-caryophyllene (0.6%)
neomenthol (0.4%)
pulegone (86.7%)
α-humulene (1.1%)
germacrene D (0.2%)
piperitenone (1.8%)

In addition, trace amounts (<0.1%) of α-thujene, sabinene, myrcene, (E)-2-hexenal, octyl acetate, β-bourbonene, neomenthyl acetate, linalool, isopulegol, terpinen-4-ol, neoisomenthol, isomenthol, α-terpineol, piperitone, δ-cadinene, and isopiperitenone were found in the same oil.

A summary of the literature on the composition of the commercial oil of *M. pulegium* published between 1961 and 2004 reveals that it has been the subject of analysis by Handa et al. (1964); Nigam and Levi (1964); Fujita and Fujita (1970), de Gavina Mugica and Ochoa (1974); Lawrence (1978); Srinivas (1986); Velasco-Negueruela et al. (1987); and Stengele

TABLE 7.4b
Hydrocarbons Found in Pennyroyal Oil

Compound	References
(E)-β-Ocimene (*t*)	de Gavina Mugica and Ochoa (1974) Lawrence (1978)
Terpinolene (*t*), allo-ocimene (*t*), α-phellandrene (*t*)	Velasco-Negueruela et al. (1987)
β-Phellandrene	de Gavina Mugica and Ochoa (1974)
(Z)-β-Ocimene	Lawrence (1978)
α-Thujene (*t*)	Stengele (1994)
Myrcene (*t*–0.1%), β-bourbonene (*t*), α-humulene (1.1%–1.5%), germacrene D (*t*–0.4%)	Lawrence (1978); Stengele (1994)
Camphene (*t*), α-terpinene (*t*)	de Gavina Mugica and Ochoa (1974); Velasco-Negueruela et al. (1987)
δ-Cadinene (*t*–0.6%)	Velasco-Negueruela et al. (1987); Stengele (1994)
p-Cymene (0.1%–0.2%)	Lawrence (1978); de Gavina Mugica and Ochoa (1974); Velasco-Negueruela et al. (1987)
Sabinene (*t*–0.3%)	Lawrence (1978); de Gavina Mugica and Ochoa (1974); Stengele (1994)
β-Caryophyllene (*t*–0.5%)	Lawrence (1978); Srinivas (1986); Stengele (1994)
α-Pinene (*t*–1.5%), β-pinene (0.3%–0.8%), limonene (0.1%–3.4%)	Handa et al. (1964); Fujita and Fujita (1970); de Gavina Mugica and Ochoa (1974); Lawrence (1978); Velasco-Negueruela et al. (1987); Stengele (1994)

*Correct isomer not identified.
t = trace (<0.1%)

TABLE 7.4c
Alcohols and Esters Found in Pennyroyal Oil

Compound	References
trans-Sabinene hydrate (*t*–0.1%), Isopulegol (1.8%–2.2%), nerol (*t*–0.3%), neoiso(iso)pulegol (*t*), piperitenol*(*t*)	Velasco-Negueruela et al. (1987)
Terpinen-4-ol (*t*)	Stengele (1994)
α-Terpineol (0.1%–0.4%), linalool (*t*–0.3%)	Lawrence (1978); Stengele (1994)
Isomenthol (*t*)	de Gavina Mugica and Ochoa (1974); Stengele (1994)
Neomenthol (0.4%)	Velasco-Negueruela et al. (1987); Stengele (1994)
Menthol (*t*–0.7%)	Handa et al. (1964); de Gavina Mugica and Ochoa (1974); Velasco-Negueruela et al. (1987)
1-Octen-3-ol (*t*–0.1%)	Lawrence (1978); de Gavina Mugica and Ochoa (1974); Velasco-Negueruela et al. (1987)
Neoisomenthol (*t*–0.3%)	Lawrence (1978); de Gavina Mugica and Ochoa (1974); Velasco-Negueruela et al. (1987); Stengele (1994)
3-Octanol (0.5%–1.2%)	Handa et al. (1964); Lawrence (1978); de Gavina Mugica and Ochoa (1974); Velasco-Negueruela et al. (1987); Stengele (1994)
Linalyl acetate (1.7%–4.4%)	Handa et al. (1964)
Sabinyl acetate* (*t*)	Velasco-Negueruela et al. (1987)
Neomenthyl acetate (*t*)	Stengele (1994)
Neoisomenthyl acetate (*t*–0.3%)	Lawrence (1978); Stengele (1994)
3-Octyl acetate (0.1%–0.4%)	Handa et al. (1964); Lawrence (1978); Stengele (1994)

*Correct isomer not identified.
t = trace (<0.1%).

(1994). The components characterized according to the class of compounds such as hydrocarbons, alcohols and esters, aldehydes and ketones, and miscellaneous compounds can be seen summarized in Table 7.4b, Table 7.4c, Table 7.4d, and Table 7.4e. A review of these data reveals that 20 hydrocarbons, 14 alcohols, 6 esters, 1 aldehyde, 13 ketones, and 5 miscellaneous compounds have been found in pennyroyal oil. It should be noted, however, that other constituents (not included in this section) have been found in oils of *M. pulegium* that were collected from their natural habitats in countries where pennyroyal oil is either not produced or the oils analyzed were not produced from plants that were normally used for commercial production.

7.4.4 Chirality

The enantiomeric distribution of the main oxygenated components found in pennyroyal oil can be seen in Table 7.4f.

7.5 NATIVE SPEARMINT OIL ex *Mentha spicata* L.

7.5.1 Introduction

Spearmint has been known for many centuries for its curative properties. It is thought that the first record of spearmint cultivation was a garden plant of large European convents and

TABLE 7.4d
Aldehydes and Ketones Found in Pennyroyal Oil

Compound	References
(E)-2-Hexenal (*t*)	Stengele (1994)
3-Methylcyclohexanone (0.7%)	Lawrence (1978)
Fenchone[†], α-thujone[†], 3-octanone (0%–0.1%)	Velasco-Negueruela et al. (1987)
3-Methyl-5-heptanone (0.5%)	Stengele (1994)
cis-Isopulegone (0.6%), *trans*-isopulegone (1.2%–1.6%), isopiperitenone (*t*–0.1%)	Lawrence (1978); Stengele (1994)
Piperitenone (1.1%–2.6%)	Handa et al. (1964); Lawrence (1978); Velasco-Negueruela et al. (1987); Stengele (1994)
Piperitone (*t*–1.7%), menthone (0.4%–16.5%), isomenthone (0.8%–4.5%)	Handa et al. (1964); de Gavina Mugica and Ochoa (1974); Lawrence (1978); Velasco-Negueruela et al. (1987); Stengele (1994)
Pulegone	Handa et al. (1964); de Gavina Mugica and Ochoa (1974); Lawrence (1978); Srinivas (1986); Velasco-Negueruela et al. (1987); Stengele (1994)

t = trace (<0.1%)
[†]Questionable identity.

monasteries in the 9th century. In addition to its use in herbal medicine, spearmint became popular in England in the 18th century as a component of mint sauce, a traditional sauce served with lamb or mutton.

Spearmint can also be found discussed in Greek mythology. It is believed that Pluto had a favorite mistress who was radiantly beautiful. Pluto was jealous of the way in which the other gods revered the beauty of this woman, so to protect her and to demonstrate his love for her he changed her into a mountain, Mt. Minthe (Kokkini, 1992). The sterile *M. spicata* ($2n = 36$) is thought to originate from a cross between *M. spicata* ($2n = 48$) and *M. longifolia* ($2n = 24$). According to Kokkini, Mt. Minthe is the only place on earth where the two species can be found in close proximity to each other, where such a natural hybridization could take place. This genetic origin of *M. spicata* is generally accepted by cytotaxonomists, although where this natural hybridization took place remains unknown at this time. It is hoped that the future research of Kokkini will shed some light on this matter and at least give credence to her hypothesis.

TABLE 7.4e
Miscellaneous Compounds Found in Pennyroyal Oil

Compound	References
cis-Piperitone oxide (*t*), piperitenone oxide (0%–0.1%), thymol (*t*)	Velasco-Negueruela et al. (1987)
1,8-Cineole (*t*)	de Gavina Mugica and Ochoa (1974); Velasco-Negueruela et al. (1987)
Menthofuran (0.2%–0.8%)	Handa et al. (1964); Nigam and Levi (1964); Lawrence (1978); de Gavina Mugica and Ochoa (1974); Velasco-Negueruela et al. (1987)

t = trace (<0.1%).

TABLE 7.4f
Enantiomeric Distribution of Some Pennyroyal Oil Constituents

Enantiomeric Ratio	References
(1S,4R)-(+)-menthone (0%): (1R,4S)-(−)-menthone (100%) (1R,4R)-(+)-isomenthone (100%): (1S,4S)-(−)-isomenthone (0%)	Stengele (1994); Ravid et al. (1994b)
(1R)-(+)-pulegone (100%): (1S)-(−)-pulegone (0%)	Stengele (1994); Ravid et al. (1994b)
(1R,3S,4S)-(+)-neomenthol (100%): (1S,3R,4R)-(−)-neomenthol (0%)	—
(1R,3R,4R)-(+)-neoisomenthol (100%): (1S,3S,4S)-(−)-neoisomenthol (0%)	—
(1R,3S,4R)-(+)-isomenthol (100%): (1S,3R,4S)-(−)-isomenthol (0%)	Stengele (1994)

7.5.2 Cultivation and Oil Production

Throughout the 18th and 19th centuries, spearmint cultivation proliferated mainly as a garden plant, although some commercial cultivation took place in Mitcham in Surrey and a few other southeastern and central counties in England. Currently, there is limited spearmint cultivation for mint sauce production and negligible quantities for oil production. The botanical origin of the spearmint used for oil production was *Mentha spicata* L.

Native spearmint was brought to the United States as a garden plant by the British, who settled in New England in the mid-15th century (Landing, 1969). By the early 17th century, spearmint had become established as an important drug and flavoring crop in western Massachusetts. Over the next 100 years, spearmint like peppermint cultivation moved through New York state to the Midwest (Ohio, Michigan, Indiana, and eventually Wisconsin). According to Landing (1969), by 1911 spearmint cultivation had increased to 700 ha in Indiana and Michigan, which increased to 830 ha the following year. By the 1930s, production of a large portion of Native spearmint had moved from the Midwest to the farwestern states of Idaho, Oregon, and more important, Washington.

In 1980, a group of spearmint growers from Washington, Idaho, and Oregon approached the United States Department of Agriculture (USDA) and, as a result of a vote by the spearmint growers, a spearmint marketing order was established and a Spearmint Marketing Board was established (Christensen, 1995). This board establishes an annual base amount and price of Native spearmint oil that can be produced in the western states (Washington, Montana, Oregon, Idaho, California, Nevada, and Utah). As a result of the production and price controls for Native spearmint oil, the level of production and price has been stable since its inception. A summary of production levels in the Midwest and Farwest of the United States for the years 1990 and 2000 can be seen in Table 7.5a. More recent data can be found in Chapter 9.

Spearmint was first introduced into India during the British occupation, presumably to supply the raw material for the mint sauce to accompany roast lamb dishes. As the result of selective breeding practices, two clones (MSS-1 and MSS-5) of superior oil yield were developed from the original Native spearmint that was introduced into India from the United States (Hussain et al., 1988). These were introduced into the mint growing regions of Uttar Pradesh as an additional crop to cornmint and peppermint. By 1986, 1987, and 1988, oil production from these clones had increased to 40 tonnes, 80 tonnes, and 100 tonnes, respectively (Singh et al., 1989).

Spearmint oil production in India has been increasing over the years. There are three popular cultivars grown namely MSS-5, Arka, and Neera. According to Patra et al. (2000), the annual yield of oil per 16 m × 5 m plot for MSS-5, Arka, and Neera was 125 kg, 139 kg,

TABLE 7.5a
Native Spearmint Oil Production (Tonnes) from 1990 to 2000 in the United States

Year	Midwest	Farwest
1990	37.2	518.0
1991	74.8	537.9
1992	60.3	728.0
1993	62.1	592.4
1994	38.6	466.3
1995	40.8	468.1
1996	13.2	516.2
1997	14.5	399.6
1998	17.7	548.4
1999	35.8	484.9
2000	46.3	492.6

and 65 kg, respectively. Furthermore, the authors reported that a hybrid (Neera–Kalka) had an annual yield of 271 kg/plot and, as a result, has been released to growers. To date, no data could be found on the difference in oil composition (if any) between the cultivars and this new hybrid.

A summary of the characteristics, yield, and origin of the currently grown spearmint cultivars in India (as *M. spicata* and *M. viridis*) according to Bahl et al. (2000) can be seen in Table 7.5b. In addition, Bahl and colleagues reported that India annually produces ca. 2000 tonnes of both Native and Scotch spearmint oil, whereas the rest of the world is estimated to produce an additional 2500 tonnes. It would appear that this estimate for both India's production and the worldwide production of spearmint oil is too large. A more accurate estimate of the annual Indian production for Native spearmint oil is ca. 200 tonnes to 250 tonnes. An equivalent amount of spearmint oil of unknown *M. spicata* cultivar origin is produced in China. In addition, it is estimated that some quantities (<10 tonnes) are produced in Argentina, France, and Japan.

7.5.3 Oil Composition

A review of the pre-1960 literature reveals that, in addition to (−)-carvone, the major constituent of Native spearmint oil, other compounds found in the oil were a pinene isomer, (−)-limonene, a phellandrene isomer, 1,8-cineole, linalool, and some dihydrocuminyl and dihydrocarveol esters (Gildemeister and Hoffmann, 1961).

Shimizu and Ikeda (1962) obtained oils from three clonally reproduced spearmint cultivars; one produced commercially in Okayama (Japan), the other in the U.S., and the third of unknown commercial origin. They found that the carvone contents of the oils were 51.4%, 68.8%, and 73.0%, respectively.

Smith et al. (1963) examined the composition of Native spearmint oil produced commercially in the United States and Taiwan and oils produced experimentally in the Netherlands. This study is summarized in Table 7.5c.

Burks and Gjerstad (1964) determined that an oil of Native spearmint produced in the United States contained α-pinene, 1,8-cineole, and linalool as minor constituents.

Averill (1968) analyzed a commercial sample of North American Native spearmint oil and characterized the presence of α-pinene, β-pinene, sabinene, myrcene, α-terpinene, limonene,

TABLE 7.5b
Characteristics, Yield, and Origin of Indian Spearmint Cultivars

Characteristics	MSS-5	Arka	Neera	Neera–Kalka	Supriya[a]
Plant structure	Medium tall erect	Medium tall erect	Medium tall semierect	Medium tall erect	Dwarf semierect
Stems	Medium hard	Hard	Medium hard	Medium hard	Soft
Color	Mainly green lower portion magenta	Mainly green lower portion magenta	Mainly green lower portion magenta	Mainly green purple pigment at base	Orchid-purple
Leaf/stem ratio	1.0:1	1.1:1	1.5:1	0.6:1	1.2:1
Leaves and size	Green elliptic-ovate 6.4 cm^2	Green elliptic-ovate 6.8 cm^2	Green elliptic 4.5 cm^2	Green ovate-elliptic 6.9 cm^2	Dark green crinkled 11.8 cm^2
Flower color	White Medium fertile	White (early flowering) medium fertile	Purplish white low fertile	Pinkish-white fertile	White sterile
Oil yield	Moderate (0.55%)	Moderate (0.60%)	Low (0.40%)	High (0.80%)	Low (0.35%)
Origin	Clonal selection of MSS-1	Clonal selection of MSS-5	Unknown	F_1 hybrid between *M. canadensis* cv. Kalka and *M. spicata* cv. Neera	A northern Himalayan accession

[a] *M. spicata* ssp. *spicata* (as *M. viridis*).

1,8-cineole, a β-ocimene isomer, γ-terpinene, terpinolene, 3-octanol, menthol, isomenthone, terpinen-4-ol, a dihydrocarvone isomer, α-terpineol, dihydrocarvyl acetate, carvone, a carvyl acetate isomer, and a 2-phenethyl isovalerate.

Murray et al. (1972) compared the major components found in the oils of Native spearmint produced from plants harvested at different development times. The results of this study are shown in Table 7.5d.

Examination of the various Native spearmint oils analyzed by Murray et al. (1972), Clark and Menary (1983), Srinivas (1986), Maffei et al. (1986), Surburg and Köpsel (1989), and Kubeczka and Formacek (2002) reveals that the oil has been found to possess the following composition:

isovaleraldehyde (0%–0.1%)
α-pinene (0.2%–0.9%)
α-thujene (0%–0.7%)
trans-2,6-diethyltetrahydrofuran (0%–0.1%)
β-pinene (0.7%–0.8%)
sabinene (0%–0.5%)
myrcene (1.5%–4.4%)
α-terpinene (0%–0.2%)

TABLE 7.5c
Comparative Percentage Composition of Native Spearmint Oil Produced in Different Geographic Regions

Compound	United States	Taiwan	Netherlands
α-Pinene	0.5–1.2	0.5	0.7–0.8
β-Pinene	1.8–5.2	0.9	4.4–4.5
Limonene	13.3–20.5	16.4	10.9–16.2
1,8-Cineole	2.9–7.5	5.0	6.0–6.8
3-Octanol	0.6–1.4	0.3	0.6–0.7
Linalool	0–0.4	—	0.1–0.2
Menthone	0.1–0.5	0.3	0.2
Isomenthone	0–0.1	0.2	—
Carvomenthone†	0.4–1.3	0.5	0.2
Dihydrocarvone*	3.4–13.1	3.8	3.5–4.6
Pulegone†	0.1–3.7	1.6	1.7–1.9
Dihydrocarvyl acetate	0.5–8.1	3.9	4.4–7.7
Carvone	46.3–68.1	64.4	58.2–62.1
cis-Carvyl acetate	0.5–2.6	1.1	0.9–1.7
cis-Carveol	0–0.7	0.1	0.2

*Correct isomer not identified.
†Questionable identity.

limonene (8.7%–11.6%)
1,8-cineole (0%–2.2%)
(Z)-β-ocimene (0%–0.2%)
γ-terpinene (0%–0.5%)
(E)-β-ocimene (0%–0.1%)
p-cymene (0%–0.4%)
terpinolene (0%–0.1%)
3-octyl acetate (0%–0.4%)
(Z)-3-hexenol (0%–0.1%)
3-octanol (0%–1.5%)
1-octen-3-ol (0%–0.9%)
menthone (0%–1.2%)
trans-sabinene hydrate (0.4%–2.2%)
isomenthone (0%–0.1%)
β-bourbonene (0%–1.9%)
α-copaene (0%–0.1%)
linalool (0%–0.1%)
cis-sabinene hydrate (0%–0.1%)
β-caryophyllene (0%–1.9%)
terpinen-4-ol (0%–1.4%)
cis-dihydrocarvone (0%–2.5%)
carvomenthone† (0%–1.9%)
trans-dihydrocarvone (0%–0.2%)
γ-muurolene (0%–0.4%)
(E)-β-farnesene (0%–0.7%)

TABLE 7.5d
Comparative Percentage Composition of Selected Constituents of Native Spearmint Oil Produced from Plants Harvested at Different Development Stages

Compound	1	2	3	4	5
α-Pinene	0.6	0.6–0.7	0.6–0.8	0.3–0.9	0.2–0.8
β-Pinene	0.8	1.0–1.3	0.9–1.3	0.7–1.4	0.5–1.2
Myrcene	4.0	3.1–3.4	2.8–3.5	2.7–4.1	2.8–3.6
Limonene	8.4	6.8–11.4	7.4–9.4	6.0–8.2	4.6–7.1
1,8-Cineole	0.9	1.5–2.2	1.6–2.3	1.6–2.5	0.6–0.8
3-Octanol	1.0	0.6–1.0	0.7–0.9	0.6–0.8	0.5–0.7
trans-Sabinene hydrate	1.6	2.0–3.0	1.7–2.3	1.3–2.1	1.0–2.2
Menthone	0.5	0.3–0.6	0.1–0.3	0–0.2	0–0.2
Dihydrocarvone*	3.0	2.3–4.7	2.9–5.0	3.3–8.4	4.6–9.3
Carvone	68.4	60.5–67.0	58.1–68.2	58.9–62.8	45.5–64.4

Development stages: 1, juvenile plants; 2, immature plants; 3, mature plants (normal harvest time with full flowering); 4, over mature plants; 5, senescent plants.
*Correct isomer not identified.

dihydrocarvyl acetate (0%–3.0%)
α-terpineol (0%–0.9%)
germacrene D (0%–1.5%)
neodihydrocarveol (0%–0.6%)
carvone (59.3%–70.0%)
dihydrocarveol (0%–2.4%)
neoisodihydrocarveol (0%–1.6%)
cis-carvyl acetate (0%–0.6%)
trans-carvyl acetate (0%–0.7%)
trans-carveol (0%–0.4%)
cis-carveol (0%–0.2%)
(Z)-jasmone (0%–0.3%)
viridiflorol (0%–0.4%)

[†]Questionable identity.

The more pertinent studies will be discussed in more detail. For example, Lawrence (1978) reported that Native spearmint produced in the Willamette Valley (Oregon, United States) possessed the following composition:

α-thujene (0.1%)
α-pinene (0.7%)
2-methylbutanal (0.1%)
trans-2,5-diethyltetrahydrofuran (0.1%)
β-pinene (0.7%)
sabinene (0.6%)
myrcene (2.5%)
α-terpinene (0.3%)
limonene (9.3%)

1,8-cineole (2.6%)
(Z)-β-ocimene (0.3%)
(E)-β-ocimene (0.2%)
p-cymene (0.2%)
terpinolene (0.1%)
3-octyl acetate (0.3%)
3-octanol (0.7%)
1-octen-3-yl acetate (0.1%)
trans-sabinene hydrate (1.3%)
isomenthone (0.1%)
β-bourbonene (1.4%)
linalool (0.1%)
cis-sabinene hydrate (0.1%)
terpinen-4-ol (0.2%)
β-caryophyllene (0.9%)
cis-dihydrocarvone (1.5%)
menthol (0.2%)
δ-terpineol (0.2%)
dihydrocarvyl acetate (1.4%)
α-terpineol (0.2%)
neodihydrocarveol (0.3%)
germacrene D (0.2%)
carvone (67.1%)
dihydrocarveol (0.5%)
cis-carvyl acetate (2.0%)
trans-carveol (0.5%)
cis-carvone oxide (0.1%)
cis-carveol (0.3%)
(Z)-jasmone (0.3%)
viridiflorol (0.2%)

In addition, the oil contained trace (<0.1%) amounts of dimethyl sulfide, isobutanal, butanal, isovaleraldehyde, methanol, ethanol, 2-ethylfuran, methyl 2-methylbutyrate, γ-terpinene, ethyl 2-methylbutyrate, isobutanol, 1-penten-3-ol, isoamyl alcohol, 2-methylbutanol, (E)-2-hexenal, 2-methylbutyl 2-methylbutyrate, pinol, hexanol, (E)-2-hexenol, (Z)-3-hexenol, hexyl 2-methylbutyrate, *p*-cymenene, (Z)-3-hexenyl isovalerate, menthone, menthofuran, 3-nonanol, *cis*-*p*-menth-2-en-1-ol, *trans*-*p*-menth-2-en-1-ol, β-copaene, β-ylangene, β-elemene, (E)-β-farnesene, δ-cadinene, γ-cadinene, cadina-1,4-diene, *trans*-dihydrocarvone, *cis*-verbenol, *trans*-verbenol, *cis*-*p*-menth-1(7),8-dien-2-ol, *trans*-carvyl acetate, and eugenol.

Fraisse et al. (1985) compared the composition of American and Chinese commercial spearmint oils presumably of *M. spicata* origin. A summary of two compositions can be seen in Table 7.5e. In addition, the authors also determined that the oils could be differentiated by the minor components of the two oils. They found that the discriminant minor components of American spearmint oil were as follows:

sabinene (0.2%)
α-terpinene (*t*)
β-phellandrene (2.2%)
1,8-cineole (1.4%)
(Z)-β-ocimene (*t*)

TABLE 7.5e
Comparative Main Component Composition of North American and Chinese Spearmint Oils

Compound	American Oil	Chinese Oil
α-Pinene	0.4	*t*
β-Pinene	0.4	*t*
Myrcene	1.6	*t*
Limonene	5.0	1.2
3-Octanol	1.0	0.1
p-Cymenene	0.1	*t*
β-Bourbonene	1.2	0.9
Linalool	0.2	0.1
β-Caryophyllene	0.4	0.2
trans-Dihydrocarvone	1.1	2.0
cis-Dihydrocarvone	0.1	0.3
Menthol	0.2	3.4
Allo-aromadendrene	*t*	*t*
γ-Muurolene	*t*	*t*
α-Terpineol + α-humulene	—	0.1
Carvone	76.6	86.0
Carvyl acetate*	0.1	—
trans-Carveol	0.5	0.8
cis-Piperitone oxide	*t*	0.2
cis-Carveol	0.2	0.3
trans-Piperitone oxide	*t*	—
(Z)-Jasmone	*t*	0.1
Caryophyllene oxide	0.1	0.4
Eugenol	*t*	0.1

*Correct isomer not identified.
t = trace (<0.1%).

γ-terpinene (*t*)
(E)-β-ocimene (*t*)
p-cymene (0.5%)
terpinolene (0.1%)
6-methyl-5-hepten-2-one (0.4%)
1-octen-3-ol (0.1%)
trans-sabinene hydrate (1.4%)
(Z)-3-hexenyl isovalerate (0.1%)
p-menth-2-en-1-ol* (*t*)
δ-terpineol (0.8%)
p-cymen-8-ol (*t*)
2-phenethyl isovalerate (*t*)
humulene oxide* (0.3%)
thymol (*t*)

*Correct isomer not identified.
t = trace (<0.1%).

In contrast, they found that the discriminant components of Chinese spearmint oil were as follows:

hexyl isovalerate (*t*)
menthone (1.1%)
δ-elemene (*t*)
isomenthone (0.4%)
octanol (0.1%)
isomenthyl acetate (0.1%)
menthyl acetate (0.1%)
β-elemene (*t*)
pulegone (0.1%)
methyl chavicol (*t*)
neral (0.8%)
α-terpinyl acetate (0.1%)
methyl salicylate (*t*)
calamenene* (*t*)
piperitenone (*t*)
piperitenone oxide (*t*)
isocaryophyllene oxide (0.1%)
cubenol (*t*)
spathulenol (*t*)
α-cadinol (*t*)
eugenyl acetate (*t*)

*Correct isomer not identified.

Mookherjee et al. (1990) compared the major components of Native spearmint oil with those found in the headspace of a living spearmint plant. A summary of their results can be seen in Table 7.5f. From these results, it can be concluded that spearmint oil is very different from the headspace of the living plant and the harvested plant. As this study was conducted using a dynamic headspace procedure with Tenax trapping followed by solvent elution, the quantitative data presented may not necessarily be truly representative of the living plant headspace. Also, plant material at different stages of development is known to produce

TABLE 7.5f
Major Components (%) Found in the Headspace of Spearmint

Compound	Headspace Live Plant	Headspace Picked Plant	Spearmint Oil
Hexanal	0.5	*t*	—
Hexanol	—	2.3	0.1
Limonene	17.7	1.8	21.4
Dihydrocarvone*	0.7	2.6	0.1
Carvone	24.0	70.0	63.0
Menthone/isomenthone	—	—	1.2
Menthol*	—	—	1.7
Undeca-1,3,5-triene*	0.5	—	—

*Correct isomer not identified.
t = trace (<0.1%).

secondary metabolites at a different rate. In addition, across a 24-h period, there are additional changes in secondary metabolite production. Finally, *M. spicata,* like all aromatic plants, contains its own genetic code for secondary metabolite production. Unless the living plants, the harvested plants, and the oil are all produced from clonally reproduced plants, at the same stage of development and at the same time of day, only then can the intrinsic influence on secondary metabolite production be minimized.

Lawrence (1993a) compared the composition of a number of samples of Native spearmint oil production in the Midwest and Farwest of the United States As the Farwest (particularly Washington) has a much longer season than the Midwest, a portion of the oil produced is from a second harvest. The results of the analyses of these oils are summarized as the average percentage composition of both Midwest and two types of Farwest oil (first harvest and second harvest) is presented in Table 7.5g.

Mohan Rao (2000) compared the oil yield and composition of fresh, shade-dried, and sun-dried *M. spicata* leaves obtained from plants grown in India (Table 7.5h). As can be seen, sun drying the leaves caused a reduction in oil content (ca. 45%) and the lower boiling monoterpenes and a corresponding increase in the higher boiling components, particularly carvone. As spearmint leaves are dried prior to commercial oil production by steam distillation, the amount of sun drying will undoubtedly affect the oil composition.

The effect of development stage on the composition of selected major components of the various spearmint cultivars grown in India can be seen in Table 7.5i (Bahl et al., 2000). The typical carvone contents of oils produced from each of the cultivars was reported as ''MSS-5'': (65%), ''Arka'': (68%); ''Neera'': (58%), and ''Neera–Kalka'': (72%). A summary of the main components of the ''Supriya'' cultivar of *M. spicata* ssp. *subspicata* (as *M. viridis*) is shown in Table 7.5j. The typical carvone content of the oil produced from ''Supriya'' was reported to be 65%.

Coleman et al. (2002) examined the headspace of more than 20 samples of freshly distilled Native spearmint oil using a nonequilibrated solid phase microextraction procedure combined with gas chromatography–mass spectrometry (GC/MS). The components found in the headspace of these oils are as follows:

dimethyl sulfide (0.1%)
isobutanal (*t*%–0.1%)
2-methylbutanal (0.3%–0.5%)
2-ethylfuran (0.1%–0.2%)
methyl 2-methylbutyrate (0.1%–0.2%)
α-pinene (3.8%–4.0%)
α-thujene (0.4%–0.5%)
trans-2,6-diethyltetrahydrofuran (0.2%–0.6%)
β-pinene (3.0%–3.2%)
sabinene (2.0%–2.4%)
myrcene (8.5%–9.6%)
α-terpinene (1.1%–1.3%)
limonene (31.6%–35.1%)
1,8-cineole (7.3%–8.9%)
(E)-β-ocimene (0.4%–0.6%)
(Z)-β-ocimene (0.4%–0.6%)
γ-terpinene (1.5%–1.7%)
(E)-β-ocimene (0.2%–0.3%)
p-cymene (0.3%)
3-octanol (1.5%–1.8%)

TABLE 7.5g
Average Percentage Composition of Native Spearmint Oil Produced in the United States

Compound	Midwest Oil (15)[a]	Farwest Oil	
		1st harvest (50)	2nd harvest (20)
α-Pinene	0.80	0.75	0.71
Camphene	0.08	0.07	0.05
β-Pinene	0.72	0.65	0.68
Sabinene	0.54	0.51	0.53
Myrcene	2.68	3.53	3.04
α-Terpinene	0.33	0.37	0.30
Limonene	9.98	10.84	11.16
1,8-Cineole	1.79	1.80	1.82
(Z)-β-Ocimene	0.13	0.11	0.09
(E)-β-Ocimene	0.21	0.31	0.24
γ-Terpinene	0.56	0.61	0.52
3-Octanone	0.13	0.14	0.13
p-Cymene	0.16	0.15	0.15
Terpinolene	0.17	0.19	0.16
3-Octyl acetate	0.25	0.37	0.36
3-Octanol	0.89	0.63	0.91
1-Octen-3-yl acetate	0.09	0.06	0.03
trans-Sabinene hydrate	0.14	0.12	0.08
Menthone	0.89	1.22	1.23
β-Bourbonene	2.15	2.22	1.69
Linalool	0.16	0.20	0.17
cis-Sabinene hydrate	0.18	0.15	0.15
β-Elemene	0.28	0.27	0.20
Terpinen-4-ol	0.84	0.79	0.58
cis-Dihydrocarvone	2.92	3.57	3.14
trans-Dihydrocarvone	0.14	0.26	0.20
Menthol	0.20	0.21	0.15
Dihydrocarvyl acetate	1.09	1.20	0.90
Germacrene D	0.24	0.27	0.38
Carvone	66.00	62.53	67.43
cis-Carvyl acetate	0.39	0.50	0.28
trans-Carveol	0.98	0.74	0.44
cis-Carveol	0.35	0.36	0.38
Viridiflorol	0.19	0.27	0.19

[a]Number of samples analyzed.

trans-sabinene hydrate (1.0%–1.9%)
neomenthol[†] (0.4%–0.9%)
terpinen-4-ol[†] (0.7%–0.9%)
carvone (26.7%–33.5%)

[†]Questionable identity.
t = trace (<0.1%).

The minor constituents that have been found in Native spearmint oil since 1960 can be seen categorized according to the class of compounds such as hydrocarbons, alcohols, esters, aldehydes, ketones, acids, phenols, nitrogen heterocyclic compounds, and miscellaneous compounds.

TABLE 7.5h
Comparative Percentage of the Oil Obtained from Fresh, Shade-Dried and Sun-Dried *Mentha spicata* Leaves

Compound	Fresh Leaf Oil	Shade-Dried Leaf Oil	Sun-Dried Leaf Oil
α-Pinene	0.8	0.8	*t*
β-Pinene	1.0	0.9	*t*
Myrcene	2.8	1.5	0.3
Limonene	28.8	27.0	3.8
β-Ocimene*	0.5	0.3	0.3
3-Octanol	0.4	0.2	0.3
1-Octen-3-ol	0.4	0.2	0.3
β-Bourbonene	0.4	0.5	1.1
Linalool	0.6	0.6	1.2
trans-Dihydrocarvone	0.8	0.6	1.8
Germacrene D	0.1	0.3	0.5
Dihydrocarveol	0.1	1.0	1.1
Carvone	59.6	62.8	82.9
trans-Carvyl acetate	4.3	0.9	0.7
cis-Carvyl acetate	0.2	0.1	0.3
Eucarvone†	0.1	0.2	0.2
(Z)-Jasmone	0.1	0.2	0.2
Cubenol†	0.2	0.2	0.4
α-Cadinol	0.3	0.2	0.4
Oil yield	1.48	1.28	0.66

*Correct isomer not identified.
†Questionable identity.

7.5.3.1 Hydrocarbons

The monoterpene hydrocarbons that have routinely been found as constituents of Native spearmint oil in amounts greater than 0.1% are α-thujene, α-pinene, β-pinene, sabinene, myrcene, α-terpinene, limonene, (Z)-β-ocimene, (E)-β-ocimene, γ-terpinene, *p*-cymene, and terpinolene. In addition, the sesquiterpene hydrocarbons regularly found as constituents (>0.1%) in Native spearmint oil are β-bourbonene, β-elemene, β-caryophyllene, and germacrene D.

Since 1960, 13 other monoterpene and sesquiterpene hydrocarbons have been occasionally characterized as constituent of this commercially important oil (see Table 7.5k). In summary, it would appear that 30 hydrocarbons have been identified in Native spearmint oil.

7.5.3.2 Alcohols

The alcohols found as constituents in amounts greater than 0.1% of Native spearmint oil are 3-octanol, *trans*-sabinene hydrate, *cis*-sabinene hydrate, linalool, terpinen-4-ol, α-terpineol, menthol, *trans*-carveol, *cis*-carveol, and viridiflorol. Since 1960, 26 aliphatic, monoterpene, and sesquiterpene alcohols have been occasionally found as components of this commercially important oil (Table 7.5l). In summary, it appears that a total of 36 alcohols have been characterized in Native spearmint oil.

TABLE 7.5i
Effect of Development Stage on Selected Major Component Amounts of Four Indian *Mentha spicata* Cultivars

Cultivar/Compound	1	2	3	4	5	6	7
"MSS-5"							
Limonene	0.6	0.4	1.2	18.9	22.8	24.7	15.1
1,8-Cineole	0.7	0.6	0.6	2.2	2.0	2.0	0.8
trans-Sabinene hydrate	5.2	0.3	0.8	0.1	0.6	1.5	0.9
β-Bourbonene	1.1	1.5	1.2	0.5	1.8	1.2	1.0
Menthol	6.9	0.2	—	0.1	0.1	0.1	1.5
Carvone	38.3	65.9	80.2	58.3	60.2	57.8	59.7
"Arka"							
Limonene	1.5	0.6	8.7	16.2	19.4	17.2	16.2
1,8-Cineole	0.6	0.3	3.1	2.0	2.2	2.8	0.5
trans-Sabinene hydrate	—	1.5	0.7	1.5	0.8	3.1	1.6
β-Bourbonene	0.8	1.6	1.2	0.9	0.4	0.3	0.5
Menthol	—	—	—	—	—	—	—
Carvone	66.5	78.0	72.3	62.1	62.2	54.1	59.0
"Neera"							
Limonene	1.0	4.3	4.9	25.4	27.7	27.1	11.3
1,8-Cineole	0.3	0.3	4.7	6.5	6.8	3.7	0.9
Menthol	1.9	1.5	0.7	0.1	0.1	0.1	0.4
Carvone	58.7	64.1	67.5	44.2	44.9	48.2	58.6
"Neera–Kalka"							
Limonene	6.6	4.5	9.6	35.4	39.0	37.0	26.9
1,8-Cineole	0.5	0.1	0.2	0.4	0.2	0.2	0.1
Menthol	9.7	0.4	0.9	0.5	0.2	0.2	0.3
Carvone	19.5	76.4	71.6	47.4	45.9	48.3	50.2

Development stages: 1, early sucker production; 2, late sucker production; 3, main crop early; 4, main crop midpoint; 5, main crop late; 6, early (2nd) ratoon crop; 7, late (2nd) ratoon crop.

7.5.3.3 Esters

The esters that have been characterized as constituents (>0.1%) of Native spearmint oil are 3-octyl acetate, 1-octen-3-yl acetate, dihydrocarvyl acetate, and *cis*-carvyl acetate. Since 1960, 11 aliphatic, aromatic, and monoterpene esters have been occasionally found as components of Native spearmint oil, as can be seen in Table 7.5m. To date, it would appear that 15 esters have been identified in Native spearmint oil. A survey of the recent literature (1960–2004) could not find any confirmation of dihydrocuminyl esters as constituents of Native spearmint oil, as reported by Gildemeister and Hoffmann (1961).

7.5.3.4 Aldehydes

Although no aldehydes have been routinely found as constituents of Native spearmint oil, a total of nine have been occasionally found as trace components (<0.1%) of the oil (see Table 7.5n).

TABLE 7.5j
Effect of Development Stage on the Amounts of Select Components of the "Supriya" Cultivar of *Mentha spicata* ssp. *subspicata*

	Stages of Development						
Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Limonene	0.6	1.1	4.7	29.2	13.6	17.4	23.5
1,8-Cineole	0.6	2.1	3.4	3.0	1.4	0.4	0.8
3-Octanol	0.1	0.5	0.3	0.2	0.2	0.1	0.4
trans-Sabinene hydrate	1.8	—	—	—	—	—	—
Isomenthone	0.5	0.3	0.5	0.1	0.8	0.7	—
β-Bourbonene	7.3	0.4	0.4	0.4	0.6	0.3	0.5
Menthol	11.0	5.7	—	0.1	0.4	0.2	1.9
Carvone	58.3	64.7	77.1	56.9	53.3	70.7	58.3

For stages of development description, see Table 7.5i.

7.5.3.5 Ketones

The ketones that have been routinely found as constituents in Native spearmint oil are *cis*-dihydrocarvone, *trans*-dihydrocarvone, carvone, and (Z)-jasmone. Since 1960, eight other monoterpene ketones have been characterized in this commercially important oil as shown in Table 7.5o. To date, 12 ketones have been identified in Native spearmint oil.

7.5.3.6 Acids

The ten acids, which were identified as trace (<0.1%) constituents of Native spearmint oil by Tsuneya et al. (1998) are 2-methylbutyric acid, isovaleric acid, hexanoic acid, (E)-2-hexenoic acid, octanoic acid, *cis*-2-pentylcyclopropane-1-carboxylic acid, 3-isopropenyl-6-oxo-heptanoic acid, 3-isopropylpentane-1,5-dioic acid, benzoic acid, and salicylic acid.

TABLE 7.5k
Additional Hydrocarbons Found in Native Spearmint Oil

Compound	**References**
β-Copaene, β-ylangene, δ-cadinene, γ-cadinene, cadina-1,4-diene	Lawrence (1978)
β-Phellandrene, δ-elemene, allo-aromadendrene, α-humulene, calamenene*	Fraisse et al. (1985)
Undeca-1,3,5-triene	Mookherjee et al. (1990)
p-Cymene	Lawrence (1978); Fraisse et al. (1985)
(E)-β-Farnesene	Lawrence (1978); Kubeczka and Formacek (2002)
γ-Muurolene	Fraisse et al. (1985); Kubezcka and Formacek (2002)

*Correct isomer not identified.

TABLE 7.5l
Additional Alcohols Found in Native Spearmint Oil

Compound	References
Methanol, ethanol, isobutanol, 1-penten-3-ol, 2-methylbutanol, isoamyl alcohol, (E)-2-hexenol, 3- nonanol, *cis*-*p*-menth-2-en-1-ol, *cis*-verbenol, *trans*-verbenol, *cis*-*p*-menth-1(7), 8-dien-2-ol	Lawrence (1978)
3-(5′,5′-Dimethyltetrahydrofuran-2-yl)-(Z)-2-butenol	Sakurai et al. (1983)
Spathulenol, *p*-cymen-8-ol, octanol	Fraisse et al. (1985)
1-Octen-3-ol, neoisodihydrocarveol	Maffei (1986)
δ-Terpineol, *trans*-*p*-menth-2-en-1-ol	Lawrence (1978); Fraisse et al. (1985)
Hexanol	Lawrence (1978); Mookherjee et al. (1990)
(Z)-3-Hexenol, neodihydrocarveol	Lawrence (1978); Kubeczka and Formacek (2002)
α-Cadinol, cubenol	Fraisse et al. (1985); Mohan Rao (2000)
Menthol	Lawrence (1978); Fraisse et al. (1985); Mookherjee et al. (1989); Bahl et al. (2000); Coleman et al. (2002)

7.5.3.7 Phenols

Eugenol has been found as a trace constituent (<0.1%) in Native spearmint oil by Lawrence (1978), Fraise et al. (1985), Chien (1988), and Tsuneya et al. (1998). Fraise et al. (1985) also characterized thymol as a trace constituent in the oil. As a result, two phenols have been identified in Native spearmint oil.

7.5.3.8 Nitrogen Heterocyclic Compounds

Nitrogen heterocyclic compounds have been found as components of Native spearmint oil only in trace amounts, whereas 22 compounds have been identified in the ppm level in the oil as shown in Table 7.5p. Tsuneya et al. (1993) determined that 2-acetyl-4-isopropylpyridine, 2,4-diisopropenylpyridine, 2-acetyl-4-isopropenylpyridine, and 4-acetyl-2-isopropenylpyridine were unique to both Native and Scotch spearmint oils but not found in peppermint

TABLE 7.5m
Additional Esters Found in Native Spearmint Oil

Compound	References
trans-Sabinene hydrate acetate	Nigam and Levi (1963)
Ethyl 2-methylbutyrate, 2-methylbutyl 2-methylbutyrate, hexyl 2-methylbutyrate, *trans*-carvyl acetate	Lawrence (1978)
Hexyl isovalerate, α-terpinyl acetate, methyl salicylate	Fraisse et al. (1985)
Menthyl acetate[†]	Coleman et al. (2002)
2-Phenethyl isovalerate	Averill (1968); Fraisse et al. (1985)
(Z)-3-Hexenyl isovalerate	Lawrence (1978); Fraisse et al. (1985)
Methyl 2-methylbutyrate	Lawrence (1978); Coleman et al. (2002)

[†]Questionable identity.

TABLE 7.5n
Aldehydes in Native Spearmint Oil

Compound	References
Butanal	Lawrence (1978)
Neral	Fraisse et al. (1985)
Hexanal	Mookherjee et al. (1990)
Acetaldehyde, valeraldehyde, (E)-2-hexenal	Coleman et al. (2002)
Isobutanal	Lawrence (1978); Coleman et al. (2002)
Isovaleraldehyde, 2-methylbutanal	Lawrence (1978); Kubeczka and Formacek (2002)

oil. They further reported that 2-acetyl-4-isopropenylpyridine was the major nitrogen heterocyclic compound in the oil. It was determined to have a grassy, sweet, minty, and somewhat amber-like odor.

7.5.3.9 Miscellaneous Compounds

1,8-cineole is the only miscellaneous compound routinely found as a constituent of Native spearmint oil. Since 1960, 16 other miscellaneous compounds have been identified in this commercially important oil as can be seen in Table 7.5q. As a result, a total of 17 miscellaneous compounds have been found in Native spearmint oil.

7.5.3.10 Chirality

The enantiomeric distribution of some of the constituents of Native spearmint can be seen in Table 7.5r. In addition, Nagasawa et al. (1976) determined that the major enantiomers also found in spearmint oil were (1S,4S)-(+)-*cis*-dihydrocarvone, (1R,4S)-(+)-*trans*-dihydrocarvone, (1R,2S,4S)-(−)-dihydrocarveol, (1R,2R,4S)-(+)-neodihydrocarveol, (1R,2S,4S)-(−)-dihydrocarvyl acetate, (2S,4S)-(−)-*cis*-carvyl acetate, and (4R,4S)-(−)-*trans*-carvyl acetate.

TABLE 7.5o
Additional Ketones Found in Native Spearmint

Compound	References
6-Methyl-5-hepten-2-one, piperitenone	Fraisse et al. (1985)
6-Hydroxycarvone	Tsuneya et al. (1998)
Eucarvone[†]	Mohan Rao (2000)
Carvomenthone[†]	Smith et al. (1963); Handa et al. (1964); Srinivas (1986)
Pulegone	Smith et al. (1963); Handa et al. (1964); Coleman et al. (2002)
Isomenthone	Smith et al. (1963); Handa et al. (1964); Averill (1968); Lawrence (1978); Mookherjee et al. (1990)
Menthone	Smith et al. (1963); Handa et al. (1964); Murray et al. (1972); Lawrence (1978); Mookherjee et al. (1990)

[†]Questionable identity.

TABLE 7.5p
Nitrogen Heterocyclic Compounds Found in Native Spearmint Oil

Compound	References
Pyridine, 2-methylpyridine, 2,5-dimethylpyrazine, 2-acetylpyridine, 2-isopropyl-4-methylpyridine, 4-isopropyl-2-methylpyridine, 4-isopropenyl-2-methylpyridine, 3-[(Z)-1-buten-1-yl]pyridine, 3-[(E)-1-buten-1-yl]pyridine, 2-ethyl-4-isopropenylpyridine, quinoline, 2,4-diisopropenylpyridine, 2-acetyl-4-isopropylpyridine, 2-acetyl-4-isopropenylpyridine, 4-acetyl-2-isopropenylpyridine, 5-[(Z)-1-buten-1-yl]-2-propylpyridine, 5-[(Z)-1-buten-1-yl]-4-propylpyridine 5-[(E)-1-buten-1-yl]-2-propylpyridine 5-[(E)-1-buten-1-yl]-4-propylpyridine	Tsuneya et al. (1993)
3-Phenylpyridine, 5-phenyl-2-propylpyridine, 3-phenyl-4-propylpyridine	Sakurai et al. (1983b); Tsuneya et al. (1993)

TABLE 7.5q
Additional Miscellaneous Compounds Found in Native Spearmint Oil

Compound	References
Pinol, *cis*-carvone oxide	Lawrence (1978)
Mint lactone	Takahashi et al. (1980)
Mint sulfide	Takahashi et al. (1981)
1,5-Epoxysalvial-4(14)-ene	Surburg and Köpsel (1989)
cis-Piperitone oxide, *trans*-piperitone oxide, piperitenone oxide, caryophyllene oxide, isocaryophyllene oxide, humulene epoxide*, eugenyl acetate	Fraisse et al. (1985)
Menthofuran	Nigam and Levi (1964); Coleman et al. (2002)
Dimethylsulfide 2-ethylfuran	Lawrence (1978); Coleman et al. (2002)
trans-2,5-Diethyltetrahydrofuran	Lawrence (1978); Kubeczka and Formacek (2002); Coleman et al. (2002)

*Correct isomer not identified.

TABLE 7.5r
Enantiomeric Distribution of Constituents of Native Spearmint Oil

Enantiomeric Ratio	References
(2R,4S)-(+)-*trans*-carveol (15%):(2S,4R)-(−)-*trans*-carveol (85%) (2S,4S)-(+)-*cis*-carveol (4%):(2R,4R)-(−)-*cis*-carveol (96%)	Nakamoto et al. (1996)
(3R)-(+)-camphene (>99.9%):(3S)-(−)-camphene (<0.1%) (4R)-(+)-limonene (1.1%–19.0%):(4S)-(−)-limonene (81.0%–98.8%)	Coleman et al. (2002)
(1R,5R)-(+)-α-pinene (37.6%–57.0%):(1S,5S)-(−)-α-pinene (43.0–62.4)	Mosandl et al. (1991)
(1R,5R)-(+)-β-pinene (40.0%–48.7%):(1S,5S)(−)-β-pinene (51.3%–60.0%)	Coleman et al. (2002)
(4S)-(+)-carvone (<1.0%–4.0%):(4R)-(−)-carvone (>96.0%)	Casiabianca et al. (1996); Ravid et al. (1992b); König et al. (1997)

7.6 SCOTCH SPEARMINT OIL ex *Mentha gracilis* SOLE

7.6.1 Introduction

From the early 1800s, when spearmint was first introduced into the United States, all commercial oil production was from *M. spicata*. In 1908, a Michigan mint grower found a spearmint that was mint-odored in a Wisconsin garden (Landing, 1969). This mint originated from Scotland and, as a result, became known as Scotch spearmint. It was formerly described as *Mentha cardiaca* Ger. In 1990, it was determined that Scotch spearmint should be classified as *M. gracilis* Sole (Tucker and Fairbrothers, 1990).

7.6.2 Cultivation and Oil Production

Like Native spearmint, Scotch spearmint has been and is still grown in the Midwest and Farwest of the United States. In addition, like Native spearmint, Scotch spearmint produced in the western states falls under the price and quota controls of the Spearmint Marketing Board. A summary of the production levels of this oil between 1990 and 2000 in North America can be seen in Table 7.6a. (Lawrence, 2000). For more recent production levels, see Chapter 9.

Commercial production of Scotch spearmint in Canada commenced in 1990 in Alberta with a production level of less than 1 tonne. By 1997, production in Alberta had increased to ca. 107 tonnes and production had commenced in Saskatchewan with a production level of ca. 23 tonnes (Thacker, 1997).

At one time a cultivar of Scotch spearmint (as *M. cardiaca*) known as MCA-1 was the main taxon grown in India for the commercial production of oil. By 1995, production of Scotch spearmint oil in India had reached 2 to 3 tonnes (Kothari and Singh, 1995). More recently, the MCAS-2, which was a clonal selection of the selfed progeny of MCA-1, has now become the main cultivar used for oil production (Bahl et al., 2000).

The cultivar MCAS-2 is a medium, tall, erect, vigorous growing, hard-stemmed plant, which is pubescent, largely green in color with a reddish tinge at the nodes. It possesses ovate leaves with a leaf and stem ratio of 1.5:1 and white flowers. It produces a moderate oil yield of 0.5%. Current Indian production of Scotch spearmint oil is estimated to be ca. 10 tonnes. Limited quantities of oil are also produced in France (Feraud, 1997) and Argentine (Romito, 1990).

TABLE 7.6a
Scotch Spearmint Production (Tonnes) in North America 1990–2000

Year	Midwest	Farwest	Canada
1990	196.0	498.5	6.8
1991	332.0	620.3	11.8
1992	187.3	677.2	18.1
1993	184.6	508.5	32.7
1994	171.9	326.1	60.8
1995	175.5	360.2	76.2
1996	102.5	440.0	94.8
1997	120.2	474.9	168.8
1998	136.5	630.9	114.3
1999	88.5	551.1	157.9
2000	89.4	420.9	167.9

7.6.3 Oil Composition

A survey of the literature reveals that the first detailed analysis of Scotch spearmint oil was performed by Smith et al. (1963). These authors examined the composition of a number of oils produced commercially in the United States and compared them with oils produced experimentally in Japan and China. As can be seen from the results of this study presented in Table 7.6b, it would appear that the Chinese oil had been redistilled because of the reduced level of monoterpene hydrocarbons and the increased level of carvone.

Commercial samples of Scotch spearmint oil (as *M. cardiaca*) were reported (Handa et al., 1964) to possess the following major components:

α-pinene (0.5%–1.0%)
β-pinene (1.3%–2.6%)
limonene (17.7%–29.7%)
1,8-cineole (1.8%–2.5%)
3-octanol (1.6%–2.6%)
3-octyl acetate (0.3%)
trans-sabinene hydrate + *cis*-sabinene hydrate (0.1%–0.4%)
linalool (0%–0.4%)
menthofuran (<0.1%)
menthone (0.9%–2.2%)
isomenthone (0%–0.3%)
carvomenthone† (0.1%–0.6%)
dihydrocarvone* (2.5%–6.9%)
pulegone (0%–0.9%)
dihydrocarvyl acetate (0.4%–1.3%)

TABLE 7.6b
Percentage Composition of Scotch Spearmint Oil of Various Origins

Compound	United States	Japan	China
α-Pinene	0.3–1.0	0.6	—
β-Pinene	1.3–2.6	1.6	0.1
Limonene	17.7–29.7	20.0	3.2
1,8-Cineole	1.8–2.6	1.8	0.1
3-Octanol	1.6–2.5	2.6	0.1
Linalool	0–0.3	0.1	0.4
Menthone	0.9–2.2	1.1	1.0
Isomenthone	0–0.3	0.3	0.3
Carvomenthone†	0.1–0.6	0.3	0.5
Dihydrocarvone*	2.5–6.9	2.9	3.0
Pulegone	0–0.7	0.2	0.9
Dihydrocarvyl acetate	0.3–1.3	1.2	1.3
Carvone	53.9–67.4	66.7	86.0
cis-Carvyl acetate	0–1.6	0.3	2.0
cis-Carveol	0–0.9	0.1	1.1

*Correct isomer not identified.
†Questionable identity.

dihydrocarveol (0%–0.1%)
carvone (53.9%–68.2%)
trans-carvyl acetate (0.7%–0.9%)
cis-carvyl acetate (0%–1.6%)
cis-carveol (0%–0.9%)

*Correct isomer not identified.
†Incorrect identification.

Over the past 25 years, Scotch spearmint oil has been the subject of analysis by Murray et al. (1972); Umemoto and Nagasawa (1980); Srinivas (1986); Surburg and Köpsel (1989); Tucker et al. (1991); Platin et al. (1994); Shi et al. (1996); Lawrence (2000); and Kubeczka and Formacek (2002). The components identified in amounts greater than 0.1% can be seen as follows:

isovaleraldehyde (0%–0.1%)
α-pinene (0%–1.2%)
cis-2,5-diethyltetrahydrofuran (0%–0.1%)
β-pinene (0.4%–4.2%)
sabinene (0%–1.0%)
myrcene (0%–1.5%)
limonene (11.9%–33.7%)
1,8-cineole (0%–2.8%)
γ-terpinene (0%–1.4%)
3-octyl acetate (0%–0.6%)
(Z)-3-hexenol (0%–0.1%)
3-octanol (0%–2.4%)
menthone (0%–2.4%)
trans-sabinene hydrate (0%–1.0%)
isomenthone (0%–0.2%)
β-bourbonene (0%–1.1%)
carvomenthone† (0%–0.8%)
linalool (0%–0.1%)
cis-sabinene hydrate (0%–0.1%)
β-caryophyllene (0%–1.2%)
terpinen-4-ol (0%–1.1%)
cis-dihydrocarvone (0%–5.0%)
trans-dihydrocarvone (0%–0.6%)
menthol (0%–4.5%)
γ-muurolene (0%–0.4%)
(E)-β-farnesene (0%–0.7%)
dihydrocarvyl acetate (0%–2.0%)
α-terpineol (0%–0.3%)
germacrene D (0%–0.7%)
neodihydrocarveol (0%–1.6%)
carvone (49.6%–77.2%)
dihydrocarveol (0%–0.3%)
δ-cadinene (0%–0.1%)
γ-cadinene (0%–0.1%)
β-cadinene† (0%–0.5%)
trans-carveol (0.3%–5.8%)
cis-carveol (0%–1.7%)

(Z)-jasmone (0%–0.3%)
viridiflorol (0%–0.2%)

[†]Questionable identity.

The more pertinent studies are discussed in greater detail. For example, Murray et al. (1972) examined the effect of development stage on oil composition of Scotch spearmint grown in Indiana and Michigan. The results shown in Table 7.6c revealed that there was only a slight change in oil composition as the plant developed.

Hogg and Lawrence (1972) described the isolation and characterization of 1-vinyl-3,5,-dimethyl [2.1.1.]bicyclohexane, a new bicyclic monoterpene hydrocarbon from *M. gracilis* (as *M. ×gentilis* nm. *cardiaca*). Although the authors discussed the ease with which this compound could be made by pyrolyzing myrcene, they felt that as myrcene was only present in the oil at a level of 0.8%, it would seem unlikely that 1-vinyl-5,5-dimethyl[2.1.1.]bicyclohexane was an artifact; however, this origin should not be discounted.

Canova (1972) reported the results of a 15-year study on the composition of Scotch spearmint oil. Unfortunately, he did not present any quantitative data, although he did report that of the more than 190 compounds characterized, 32 were present in amounts greater than 0.1%, 65 (0.1% to 0.01%), 60 (0.01% to 0.001%), and 37 (<0.001%). The components identified were 12 monoterpene hydrocarbons, 13 sesquiterpene hydrocarbons, 3 aromatic alcohols, 24 monoterpene alcohols, 1 sesquiterpene alcohol, 22 aliphatic esters, 8 aromatic esters, 11 monoterpene esters, 17 aliphatic aldehydes, 2 aromatic aldehydes, 2 monoterpene aldehydes, 10 aliphatic and aromatic ketones, 14 monoterpene ketones, 10 mono- and sesquiterpene oxides, and 21 miscellaneous compounds.

Lawrence (1978) analyzed an oil of Scotch spearmint oil that was produced in the Willamette Valley (Oregon, United States) and found that it possessed the following composition:

α-pinene (0.7%)
trans-2,5-diethyltetrahydrofuran (0.1%)
β-pinene (0.7%)

TABLE 7.6c
Comparative Percentage Composition of Select Constituents of Scotch Spearmint Oil Produced from Plants Harvested at Different Plant Development Stages

Compound	1	2	3	4
α-Pinene	0.6	0.6–0.8	0.5–1.0	0.1–0.9
β-Pinene	0.9	1.1–1.5	1.0–1.7	0.3–1.4
Myrcene	1.5	0.9–1.2	0.8–1.2	0.2–0.8
Limonene	11.6	12.2–17.7	12.8–17.8	6.7–15.5
1,8-Cineole	1.2	1.9–2.2	1.7–2.0	0.8–2.0
3-Octanol	1.9	1.9–2.3	1.9–2.4	1.6–2.5
trans-Sabinene hydrate	0.5	0.2–0.4	0.3	0.3
Menthone	1.0	1.5–1.7	1.4–1.5	1.1
Dihydrocarvone*	2.3	1.8–3.4	2.2–3.4	4.0–4.1
Carvone	69.9	59.1–69.3	60.3–71.8	66.9–78.3

Development stages: 1, juvenile plants; 2, immature plants; 3, mature plants (normal harvest time with full flowering); 4, over mature plants.
*Correct isomer not identified.

sabinene (0.5%)
myrcene (0.5%)
limonene (15.3%)
1,8-cineole (1.8%)
3-octyl acetate (0.3%)
3-octanol (1.9%)
1-octen-3-yl acetate (0.1%)
1-octen-3-ol (0.2%)
trans-sabinene hydrate (0.3%)
menthone (1.0%)
menthofuran (0.1%)
isomenthone (0.1%)
β-bourbonene (1.0%)
linalool (0.2%)
cis-sabinene hydrate (0.1%)
cis-*p*-menth-2-en-1-ol (0.1%)
terpinen-4-ol (0.1%)
cis-dihydrocarvone (1.0%)
trans-dihydrocarvone (0.1%)
menthol (0.2%)
trans-pinocarveol (0.1%)
dihydrocarvyl acetate (0.2%)
α-terpineol (0.2%)
neodihydrocarveol (0.2%)
germacrene D (0.3%)
carvone (68.5%)
dihydrocarveol (0.7%)
cis-carvyl acetate (0.1%)
cadina-1,4-diene (0.1%)
trans-carveol (0.6%)
cis-carveol (0.2%)
(Z)-jasmone (0.3%)

Trace amounts (<0.1%) of butanal isovaleraldehyde, 2-methylbutanal, 2-ethylfuran, isobutanol, methyl 2-methylbutyrate, α-thujene, 1-vinyl-5,5-dimethyl[2.1.1.]bicyclohexane, ethyl 2-methylbutyrate, camphene, 1-penten-3-ol, isoamyl alcohol, α-terpinene, (E)-2-hexenal, (Z)-β-ocimene, (E)-β-ocimene, γ-terpinene, *p*-cymene, 2-methylbutyl 2-methylbutyrate, terpinolene, 3-heptanol, hexanol, (Z)-3-hexenol, (E)-2-hexenol, hexyl 2-methylbutyrate, *p*-cymenene, (Z)-3-hexenyl isovalerate, benzaldehyde, (Z)-3-hexenyl 2-methylbutyrate, 3-nonanol, octanol, α-copaene, β-copaene, β-ylangene, β-elemene, β-caryophyllene, (E)-β-farnesene, α-muurolene, δ-cadinene, γ-cadinene, ar-curcumene, α-cadinene, α-calacorene, *trans*-*p*-menth-2-en-1-ol, *trans*-*p*-mentha-2,8-dien-1-ol, carvotanacetone, *trans*-carvyl acetate, benzyl alcohol, 2-phenethyl alcohol, *p*-menth-1-en-9-ol (dihydrolimonen-10-ol), viridiflorol, and eugenol were found in this same oil.

Starting with 48 kg of Scotch spearmint oil that was produced commercially in the Midwest (United States), Ishihara et al. (1992) isolated a basic fraction (2 to 8 g), which they subjected to analysis. A total of 38 nitrogen-containing compounds were identified, all of which were present in the original oil in amounts less than 1 ppm except 1-methylpyridine (1.25 ppm) and 2-acetyl-4-isopropenylpyridine (3.34 ppm). This latter compound was found to possess a grassy, sweet, minty, slightly amber-like aroma.

Spencer (1992) analyzed the oil (0.2% to 0.4%) isolated from the distillation waters of a commercial steam distillation of Scotch spearmint with samples taken every 5 min for the 90-min duration of distillation. Water sampling began as soon as the condensate was formed. The range in composition of the oils isolated from the water samples was determined to be as follows:

isoamyl alcohol (*t*%–0.2%)
2-methylbutanol (*t*%–0.1%)
butanal (*t*%–0.1%)
(E)-2-hexenal (*t*%–0.5%)
(Z)-3-hexenol (*t*%–0.3%)
(E)-2-hexenol (*t*%–0.2%)
hexanol (0%–0.1%)
heptanal (0.1%–0.3%)
α-pinene (*t*%–0.1%)
sabinene (*t*%–0.1%)
β-pinene (*t*%–0.1%)
3-octanol (0.2%–3.2%)
α-terpinene (0.1%)
limonene + 1,8-cineole (0.2%–2.3%)
cis-linalool oxide[†] (*t*%–0.1%)
linalool (0.1%–0.2%)
3-octyl acetate (*t*%–0.2%)
menthone (*t*%–0.4%)
isomenthone (*t*%–0.1%)
terpinen-4-ol (0.1%–0.5%)
trans-dihydrocarvone (0.4%–1.8%)
cis-dihydrocarvone (0.1%–0.3%)
trans-carveol (0.6%–1.1%)
carvone (85.5%–93.3%)
piperitone (0.3%–0.4%)
(Z)-jasmone (0.2%–0.6%)
β-bourbonene (*t*%–0.1%)
β-caryophyllene (*t*%–0.1%)

t = trace (<0.1%).
[†]Furanoid form.

A trace (<0.1%) of ethyl 2-methylbutyrate was also characterized in this oil.

A comparative composition of Scotch spearmint oil produced in the Midwest and Farwest of the United States was reported by Lawrence (1993b). The results of a number of analyses can be seen in Table 7.6d.

Tsuneya et al. (1993) examined the composition of a basic fraction of Scotch spearmint oil starting with 49 kg, from which they obtained a fraction (2.8 g) containing the nitrogen heterocyclic compounds. Analysis of this fraction combined with the structural elucidation and synthesis of unknown compounds led to the identification of numerous nitrogen-containing components present in amounts less than 1 ppm in the original oil, except for 2-acetyl-4-isopropenylpyridine.

Lawrence (2000) analyzed a number of samples of Scotch spearmint oil produced both in the United States (Midwest and Farwest) and Alberta (Canada). A summary of the variability of selected major constituents can be seen in Table 7.6e.

TABLE 7.6d
Comparative Percentage Composition of Scotch Spearmint Oil

Compound	Midwest Oil	Farwest Oil
α-Pinene	0.51–0.72	0.55–0.78
Camphene	0.05–0.08	0.05–0.08
β-Pinene	0.58–0.76	0.56–0.79
Sabinene	0.43–0.56	0.42–0.60
Myrcene	0.82–1.13	0.86–1.08
Limonene	13.65–17.27	13.76–21.18
1,8-Cineole	1.40–1.57	1.00–1.56
(Z)-β-Ocimene	0.06–0.14	0.07–0.14
(E)-β-Ocimene	*t*–0.07	*t*–0.04
γ-Terpinene	0.07–0.14	0.04–0.09
3-Octanone	*t*–0.07	*t*–0.01
p-Cymene	*t*–0.04	*t*–0.05
Terpinolene	0.04–0.07	0.04–0.06
3-Octyl acetate	0.13–0.23	0.10–0.27
3-Octanol	2.09–2.66	1.75–2.41
1-Octen-3-yl acetate	0.05–0.12	0.05–0.10
trans-Sabinene hydrate	0.95–1.43	0.74–1.42
Menthone	0.08–0.26	0.08–1.14
β-Bourbonene	0.91–1.24	0.83–1.34
Linalool	0.10–0.51	0.08–0.13
cis-Sabinene hydrate	0.06–0.08	0.05–0.08
β-Elemene	0.06–0.23	0.10–0.24
Terpinen-4-ol	0.33–0.45	0.26–0.46
cis-Dihydrocarvone	0.92–2.20	1.11–4.36
trans-Dihydrocarvone	0.12–0.43	0.11–0.43
Menthol	0.08–0.11	0.08–0.12
Dihydrocarvyl acetate	0.34–0.43	0.24–0.44
Germacrene D	0.09–0.30	0.09–0.41
Carvone	65.79–71.62	62.84–69.79
cis-Carvyl acetate	0.03–0.12	0.02–0.05
trans-Carveol	0.11–0.17	0.10–0.18
Viridiflorol	*t*–0.2	*t*–0.1

t = trace (<0.01%).

The effect of development stage on the amounts of selected constituents found in oils produced from Scotch spearmint (MCAS-2) grown commercially in India can be seen in Table 7.6f (Bahl et al., 2000).

Finally, Coleman et al. (2002) used nonequilibrated solid phase microextraction coupled with GC/MS to examine the volatiles of freshly distilled Scotch spearmint oils. The compounds identified in the headspace were as follows:

dimethyl sulfide (*t*%–0.2%)
isobutanol (*t*%–0.1%)
2-methylbutanal (0.1%–0.4%)
2-ethylfuran (*t*%–0.1%)
methyl 2-methylbutyrate (*t*%–0.2%)
α-pinene (3.7%–4.0%)

TABLE 7.6e
Variability of Selected Components of North American Scotch Spearmint Oil

Compound	Midwest	Farwest	Canadian
Myrcene	0.8–1.1	0.9–1.4	0.5–1.3
Limonene	13.7–17.3	12.8–21.7	8.6–21.4
1,8-Cineole	1.4–1.6	1.0–2.1	0.7–2.0
3-Octyl acetate	0.1–0.2	0.1–0.3	0.1–0.2
3-Octanol	2.1–2.7	1.7–4.3	1.7–3.4
trans-Sabinene hydrate	1.0–1.4	0.1–1.4	0.1–0.3
β-Bourbonene	0.9–1.2	0.5–1.4	0.6–1.4
Carvone	65.8–71.6	59.9–74.2	64.4–78.6
trans-Carveol	0.1–0.2	0.1–0.21	0.1–0.2
cis-Carveol	0.3–0.5	0.2–0.4	0.2–0.5
No. of samples	30	20	65

α-thujene (*t*%–0.2%)
trans-2,6-diethyltetrahydrofuran(*t*%–1.0%)
β-pinene (3.1%–3.3%)
myrcene (3.4%–3.6%)
α-terpinene (0.1%–0.2%)
limonene (42.2%–48.9%)
1,8-cineole (4.9%–6.3%)
(E)-β-ocimene (*t*%–0.1%)
(Z)-β-ocimene (*t*%–0.1%)
γ-terpinene (0.1%–0.3%)
(E)-2-hexenal (*t*%–0.5%)
p-cymene (*t*%–0.1%)
3-octanol (3.5%–4.4%)
menthone (1.2%–1.4%)
isomenthone (0.2%)
menthyl acetate† (*t*%–0.1%)
neomenthol† (0.3%)
terpinen-4-ol (*t*%–0.2%)

†Questionable identity.
t = trace (<0.1%).

The minor constituents that have either been routinely characterized in Scotch Spearmint oil as trace constituents (<0.1%) or occasionally identified as components in one or more studies can be seen characterized according to the class of compounds such as hydrocarbons, alcohols, esters, aldehydes, ketones, acids, phenols, nitrogen heterocyclic compounds, and miscellaneous compounds.

7.6.3.1 Hydrocarbons

The monoterpene hydrocarbons that have been routinely found as constituents of Scotch spearmint oil are α-pinene, β-pinene, sabinene, myrcene, limonene, and γ-terpinene. In addition, the sesquiterpene hydrocarbons routinely characterized in the oil are β-bourbonene, β-caryophyllene, and germacrene D. Since 1960, 32 other monoterpene and sesquiterpene hydrocarbons have been occasionally identified in this commercially important oil as can be seen in Table 7.6g.

TABLE 7.6f
Effect of Development Stage on the Contents of Selected Constituents of the MCAS-2 Cultivar of Scotch Spearmint Oil

	Development Stage					
Compound	**1**	**2**	**3**	**4**	**5**	**6**
Limonene	2.6	11.2	26.7	32.8	23.8	18.4
1,8-cineole	1.2	2.1	2.1	2.1	1.5	0.2
3-Octanol	—	1.8	0.8	1.3	0.8	—
Menthone	0.2	0.1	0.2	0.1	0.1	—
Isomenthone	0.1	0.1	0.4	0.2	0.3	0.4
Menthyl acetate	0.1	0.1	0.2	0.1	0.1	0.1
Terpinen-4-ol	3.3	0.2	0.3	0.1	0.7	0.2
Neomenthol	0.2	0.2	0.1	0.2	0.2	—
Menthol	0.2	0.2	0.1	0.1	0.1	4.2
Carvone	74.0	72.0	55.9	49.4	62.1	54.6

Development Stages: 1, late sucker production; 2, main crop early; 3, main crop midpoint; 4, main crop late; 5, early (2nd) ratoon crop; 6, late (2nd) ratoon crop.

TABLE 7.6g
Additional Hydrocarbons Found in Scotch Spearmint Oil

Compound	**References**
1-Vinyl-5,5-dimethyl[2.1.1.]bicyclohexane	Hogg and Lawrence (1971)
Toluene, α-fenchene, psi-limonene, isocaryophyllene, α-humulene, ε-bulgarene†, ε-muurolene†, bicycloelemene, farnesene*	Canova (1972)
α-Cadinene, α-muurolene, ar-curcumene, α-calacorene, cadina-1,4-diene	Lawrence (1978)
β-Cadinene†	Platin et al. (1994)
γ-Muurolene	Kubeczka and Formacek (2002)
p-Cymenene, β-elemene, α-copaene, β-copaene, β-yanglene	Canova (1972); Lawrence (1978)
γ-Cadinene	Lawrence (1978); Umemoto and Nagasawa (1980)
Terpinolene	Lawrence (1978); Kubeczka and Formacek (2002)
δ-Cadinene	Canova (1972); Lawrence (1978); Umemoto and Nagasawa (1980)
α-Thujene	Canova (1972); Lawrence (1978); Tucker et al. (1991)
(E)-β-Farnesene	Canova (1972); Lawrence (1978); Kubeczka and Formacek (2002)
(Z)-β-Ocimene	Lawrence (1978); Tucker et al. (1991); Coleman et al. (2002)
Camphene	Canova (1972); Lawrence (1978); Umemoto and Nagasawa (1980); Tucker et al. (1991)
(E)-β-Ocimene	Canova (1972); Lawrence (1978); Tucker et al. (1991); Coleman et al. (2002)
α-Terpinene	Lawrence (1978); Spencer (1992); Coleman et al. (2002); Kubezcka and Formacek (2002)

†Questionable identity.

Although Hogg and Lawrence (1972) believed that 1-vinyl-5,5-dimethyl[2.1.1.]bicyclohexane was a true constituent of Scotch spearmint oil, it should not be discounted that it could be an artifactual rearrangement product of myrcene. In summary, it would appear that a total of 41 hydrocarbons have been characterized in Scotch Spearmint oil.

7.6.3.2 Alcohols

The alcohols that have been regularly identified as constituents of Scotch spearmint oil are 3-octanol, *trans*-sabinene hydrate, linalool, terpinen-4-ol, α-terpineol, neodihydrocarveol, dihydrocarveol, *cis*-carveol, *trans*-carveol, and viridiflorol. Since 1960, 47 other aliphatic, aromatic monoterpene, and sesquiterpene alcohols have been occasionally identified in this commercially important oil as shown in Table 7.6h.

Surburg and Köpsel (1989) noted that viridiflorol and β-bourbonen-13-ol were found in 0.4% and 0.5% amounts, respectively. They also noted that β-bourbonen-13-ol had a mild woody aroma, whereas endo-1-bourbonanol (a trace constituent) had a powerful woody

TABLE 7.6h
Additional Alcohols Found in Scotch Spearmint Oil

Compound	References
Methanol, ethanol, amyl alcohol, (Z)-3-pentenol, (E)-3-hexenol, decanol, 3-decanol, *p*-cymen-8-ol, 1-Phenethyl alcohol, *cis*- and *trans*-*p*-mentha-1, 7(8)-dien-2-ol, *cis*-*p*-mentha-2,8-dien-1-ol, *trans*-verbenol, *trans*-3-pinen-2-ol, myrtenol, perillyl alcohol, *p*-mentha-1,8-dien-4-ol, *p*-menth-1(7)-en-8-ol	Canova (1972)
trans-*p*-Menth-2-en-1-ol, *p*-menth-1-en-9-ol	Lawrence (1978)
1-Penten-3-ol, 3-heptanol, 3-nonanol, octanol, *trans*-pinocarveol, benzyl alcohol, 2-phenethyl alcohol, *trans*-*p*-mentha-2,8-dien-1-ol	Canova (1972); Lawrence (1978)
cis-*p*-Menth-2-en-1-ol	Canova (1972); Lawrence (1993)
Neomenthol	Canova (1972); Coleman et al. (2002)
cis-Sabinene hydrate	Canova et al. (1972); Lawrence (1978); Shimizu et al. (1990); Kubeczka and Formacek (2002)
1-Octen-3-ol	Canova (1972); Lawrence (1978); Tucker (1991)
Borneol	Canova (1972);Umemoto and Nagasawa (1980); Shimizu et al. (1990)
2-Methylbutanol, isoamyl alcohol, (E)-2-hexenol, hexanol	Canova (1972); Lawrence (1978); Spencer (1992)
(Z)-3-Hexenol	Canova (1972); Lawrence (1978); Spencer (1992); Kubeczka and Formacek (2002)
Menthol	Canova (1972); Lawrence (1978); Umemoto and Nagasawa (1980); Tucker et al. (1991); Coleman et al. (2002)
Menthol	Canova (1972); Lawrence (1978); Umemoto and Nagasawa (1980); Tucker et al. (1991); Coleman et al. (2002)
3-(5′,5-dimethyltetrahydrofuran-2′-yl-butenol	Sakurai et al. (1983a)
β-Bourbonen-13-ol, endo-1-bourbonanol, T-cadinol, T-muurolol, spathulenol,(E)-nerolidol, caryophylla-2(12),6(15)-dien-5-ol	Surburg and Köpsel (1989)

TABLE 7.6i
Additional Esters Found in Scotch Spearmint Oil

Compound	References
Ethyl isobutyrate, ethyl valerate, propyl-2-methylbutyrate, isobutyl 2-methylbutyrate, 2-methylbutyl isovalerate, isoamyl 2-methylbutyrate, isoamyl isovalerate, hexyl isovalerate, (E)-2-hexenyl acetate, (Z)-3-hexenyl acetate, heptyl isovalerate, octyl formate, octyl 2-methylbutyrate, benzyl isobutyrate, benzyl 2-methylbutyrate, 2-phenethyl butyrate, 2-phenethyl 2-methylbutyrate, 2-phenethyl isovalerate, hexyl phenylacetate, (Z)-3-hexenyl phenylacetate, *cis*-carvyl formate, *trans*-carvyl formate, neodihydrocarvyl acetate, myrtenyl acetate, *p*-menth-1-en-9-yl acetate, lavandulyl butyrate	Canova (1972)
α-Terpinyl acetate	Shi et al. (1996)
2-Methylbutyl 2-methylbutyrate, hexyl 2-methylbutyrate (Z)-3-hexenyl 2-methylbutyrate	Canova (1972); Lawrence (1978)
Menthyl acetate	Canova (1972); Coleman et al. (2002)
Methyl salicylate	Canova (1972); Tsuneya et al. (1998)
1-Octen-3-yl acetate	Lawrence (1978, 1993b); Tucker et al. (1991)
(Z)-3-Hexenyl isovalerate	Canova (1972); Lawrence (1978); Umemoto and Nagasawa (1980)
Ethyl 2-methylbutyrate	Canova (1972); Lawrence (1978); Spencer (1992)
Methyl 2-methyl butyrate	Canova (1972); Lawrence (1978); Coleman et al. (2002)

aroma. In summary, it can be seen that 57 alcohols have been characterized in Scotch spearmint oil.

7.6.3.3 Esters

The esters that are routinely identified as constituents of Scotch spearmint oil are 3-octyl acetate, dihydrocarvyl acetate, and *cis*- and *trans*-carvyl acetate. Since 1960, 36 other aliphatic, aromatic, and monoterpene esters have been occasionally identified in this commercially important oil (see Table 7.6i). In summary, it would appear that 40 esters have been characterized in Scotch spearmint oil.

7.6.3.4 Aldehydes

No aldehydes have been found as constituents of Scotch spearmint oil in amounts greater than 0.1%. Since 1960, 22 aliphatic, aromatic, and monoterpene aldehydes have been occasionally characterized as constituents of Scotch spearmint oil (see Table 7.6j).

7.6.3.5 Ketones

Carvone (the major constituent), *cis*- and *trans*-dihydrocarvone, menthone, isomenthone, and (Z)-jasmone have routinely been found in measurable quantities in Scotch spearmint oil.

TABLE 7.6j
Aldehydes Found in Scotch Spearmint Oil

Compound	References
Furfural, propanal, hexanal, (Z)-4-heptenal, (E)-2-heptenal, octanal, 5-methyl-2-hexenal, tiglic aldehyde, nonanal, myrtenal, perillaldehyde, phenylacetaldehyde	Canova (1972)
Salicylaldehyde	Tsuneya et al. (1998)
Benzaldehyde	Canova (1972); Lawrence (1978)
Heptanal	Canova (1972); Spencer (1992)
Acetaldehyde, valeraldehyde	Canova (1972); Coleman et al. (2002)
Butanal	Canova (1972); Lawrence (1978); Spencer (1992)
(E)-2-Hexenal	Canova (1972); Lawrence (1978); Spencer (1992); Coleman et al. (2002)
2-Methylbutanal, isobutanal	Canova (1972); Lawrence (1978); Coleman et al. (2002); Kubeczka and Formacek (2002)
Isovaleraldehyde	Canova (1972); Lawrence (1978); Shi et al. (1996); Coleman et al. (2002); Kubeczka and Formacek (2002)

Since 1960, 20 other aliphatic and monoterpene ketones have been occasionally characterized as components of this commercially important oil. A survey of the data reveals that 25 ketones have been identified in Scotch spearmint oil as shown in Table 7.6k.

7.6.3.6 Acids

To date, no acids have been found as components of Scotch spearmint oil in amounts greater than 0.1%; however, a number of them have been characterized as occurring in much lesser amounts as can be seen in Table 7.6l.

TABLE 7.6k
Additional Ketones Found in Scotch Spearmint Oil

Compound	References
Acetone, acetophenone, 2,3-butanedione, 3-heptanone, 6-methyl-5-hepten-2-one, β-ionone*, pinocamphone, isopinocamphone, pinocarvone, (E)-jasmone, verbenone, *p*-mentha-5,8-dien-2-one (spicatone), geranyl acetone, hexahydrofarnesyl acetone	Canova (1972)
Carvotanacetone	Lawrence (1978)
3-Octanone, piperitenone	Canova (1972); Tucker et al. (1991)
Piperitone	Canova (1972); Spencer (1992)
Pulegone	Canova (1972); Coleman et al. (2002)
Carvomenthone†	Smith et al. (1963); Handa et al. (1964); Srinivas (1986)

*Correct isomer not identified.
†Questionable identity.

TABLE 7.6l
Acids Found in Scotch Spearmint Oil

Compound	References
Propionic, isobutyric, butyric, 2-methylbutyric, isovaleric, valeric, hexanoic, tiglic, (Z)-3-hexenoic, 2-ethylhexanoic, (E)-2-hexenoic, heptanoic, (Z)-4-heptenoic, (Z)-5-heptenoic, (Z)-3-heptenoic, octanoic, (Z)-5-octenoic, (Z)-3-octenoic, (E)-2-octenoic, nonanoic, *cis*- and *trans*-2-pentylcyclopropane-1-carboxylic, citronellic, decanoic, (E)-2-nonenoic, neric, geranic, perillic, 3-isopropenylpentane-1,5-dioic 3-isopropenyl-6-oxoheptanoic, benzoic, salicylic, phenylacetic	Tsuneya et al. (1998)
Acetic	Canova (1972); Tsuneya et al. (1998)

Of the 34 acids identified in the oil *cis*-2-pentylcyclopropane-1-carboxylic acid, 3-isopropenylpentane-1,5-dioic acid, and 3-isopropyl-6-oxoheptanoic acid were found to be present in higher levels in Scotch spearmint oil than found in either peppermint or cornmint oils. Tsuneya et al. (1998) also reported that the cyclopropane acid had an aroma reminiscent of the residual obtained from spearmint fractionation, which is diffusive, leathery, buttery, and patchouli-like. The dicarboxylic acid was found to be oily–acidic, whereas the oxoacid had a woody, fermented odor.

7.6.3.7 Phenols

Although no phenols have been found in amounts greater than 0.1% in Scotch spearmint oil, ten have been occasionally characterized as trace constituents in the oil as can be seen in Table 7.6m.

7.6.3.8 Nitrogen Heterocyclic Compounds

As expected, no nitrogen heterocyclic compounds have been characterized as constituents of Scotch spearmint oil in amounts exceeding 0.1%. To date, 39 compounds have been identified in the oil in ppm levels as shown in Table 7.6n. Ishihara et al. (1992) and Tsuneya et al. (1993) determined that, similar to Native spearmint, 2-acetyl-1-4-isopropylpryidine, 2,4-diisopropenylpyridine, 2-acetyl-2-4-isopropenylpyridine (major compound), and 4-acetyl-2-isopropenylpyridine were nitrogen heterocyclic compounds, unique to Scotch spearmint oil and not present in peppermint oil.

TABLE 7.6m
Phenols in Scotch Spearmint Oil

Compound	References
4-Vinylphenol, guaiacol, *o*-cresol, *m*-cresol, *p*-cresol	Tsuneya et al. (1998)
Eugenol, thymol, carvacrol, dehydrocarvacrol, vanillin	Canova (1972); Lawrence (1978)

TABLE 7.6n
Nitrogen Heterocyclic Compounds Found in Scotch Spearmint Oil

Compound	References
1,5-Dimethylpyrazine, 2-ethyl-6-methylpyrazine pyridine, 2-methylpyridine, 2-acetylpyridine, 2-ethylpyridine, 2-propylpyridine, 2-butylpyridine, 2-pentylpyridine, 3-methylpyridine, 3-ethylpyridine, 3-propylpyridine, 3-butylpyridine, 4-propylpyridine, 4-isopropenylpyridine, 4-butylpyridine, 2,4-diisopropenylpyridine, 2,6-dimethylpyridine, 2-ethyl-4-isopropenylpyridine, 2-acetyl-4-isopropylpyridine, 2-acetyl-4-isopropenylpyridine, 2-isopropyl-4-methylpyridine, 4-isopropyl-2-methylpyridine, 4-isopropenyl-2-methylpyridine, 4-acetyl-2-isopropenylpyridine, 5-ethyl-2-methylpyridine, 3-[(Z)-1-buten-1-yl]pyridine, 3-[(E)-1-buten-1-yl]pyridine 3-[(Z)-1-buten-1-yl]-4-propylpyridine 3-[(E)-1-buten-1-yl]-4-propylpyridine 5-[(Z)-1-buten-1-yl]-2-propylpyridine 5-[(E)-1-buten-1-yl]-2-propylpyridine	Ishihara et al. (1992)
Quinoline, 3-benzylpyridine, methyl anthranilate, 3-vinylpyridine	Tsuneya et al. (1993)
3-Phenylpyridine, 3-phenyl-4-propylpyridine 5-phenyl-2-propylpyridine	Sakurai et al. (1983b); Ishihara et al. (1992); Tsuneya et al. (1993)

7.6.3.9 Miscellaneous Compounds

Although only 1,8-cineole is routinely found as a constituent of Scotch spearmint oil, 27 other trace components have also been found in the oil as can be seen in Table 7.6o.

TABLE 7.6o
Miscellaneous Compounds Found in Scotch Spearmint Oil

Compound	References
cis-1,2-Epoxycarvone, *trans*-1,2-epoxycarvone, 8,9-epoxycarvone, 8,9-epoxylimonene, *cis*-1,2-expoxylimonene, *trans*-1,2-epoxylimonene, carvyl acetate-6,7-oxide, piperitenone oxide, 2-amylfuran, carvone-10-acetate, 3-hydroxy-2-butanone, 2-phenethyl methyl ether, 1-hydroxy-2-acetoxy-*p*-menth-8(9)-ene, 8-hydroxy-9-acetoxy-*p*-menth-1-ene 5-hydroxycarvone, 4-hydroxypiperitone, dimethyl sulphoxide, chloroform	Canova (1972)
1,5-Epoxysalvial-4(14)-ene, exo-1,5-epoxysalvial-4(14)-ene	Surburg and Köpsel (1989)
cis-Linalool oxide (furanoid)	Spencer (1992)
2-Hydroxycarvone	Tsuneya et al. (1998)
Caryophyllene oxide	Canova (1972); Surburg and Köpsel (1989)
Dimethylsulphide, 2-ethylfuran, 2,5-diethyltetrahydrofuran	Canova (1972); Lawrence (1978); Coleman et al. (2002)
Menthofuran	Smith et al. (1963); Nigam and Levi (1964); Handa et al. (1964); Canova (1972); Lawrence (1978); Coleman et al. (2002)

7.6.3.10 Chirality

Lawrence (2000) determined that the enantiomeric distribution for certain constituents of Scotch spearmint oil was as follows:

(1R,5R)-(+)-α-pinene (35.6%–37.6%):(1S,5S)-(−)-α-pinene (62.4%–64.4%)
(1R,5R)-(+)-β-pinene (45.4%–53.2%):(1S,5S)-(−)-β-pinene (46.8%–54.6%)
(3R)-(+)-camphene (<99.9%):(3S)-(−)-camphene (<0.1%)
(4R)-(+)-limonene (0.1%–1.0%):(4S)-(−)-limonene (99.0%–99.9%)
(4S)-(+)-carvone (<0.1%):(4R)-(−)-carvone (>99.9%)

REFERENCES

Adamovic, D.S. (1993). Variability of yield and essential oil in genotypes of cultivated species of the genus *Mentha. Arh. Farm.* **43(5/6)**, 105–114.

Anonymous. (1924). Russell's mint research portends much for perfumery industry. *Amer. Perfum.* **(Jan)**, 556.

Anonymous. (1988). New variety of *Mentha citrata. PAFAI J.* **(Oct/Nov)**, pp. 40.

Arumugam, R., and Kumar, N. (1980). Effect of leaves on rooting stem cuttings of bergamot mint (*Mentha citrata* Ehrh.). *Indian Perfum.,* **24**, 166–167.

Averill, W. (1968). Instrument news. Perkin-Elmer, Norwalk, CT, **19(2)**, 10–11.

Bahl, J.R., Bansal, R.P., Garg, S.N., Naqvi, A.A., Luthra, R., Kukreja, A.K., and Kumar, S. (2000). Quality evaluation of the essential oils of the prevalent cultivars of commercial mint species *Mentha arvensis, spicata, cardiaca, citrata* and *viridis* cultivated in Indo-Gangetic plains. *J. Med. Arom. Plant Sci*., **22**, 787–797.

Baser, K.H.C. (1994). Aromatic plant and essential oil production in Turkey. In N. Verlet Ed., 4th Recontres Techniques et Economiques. *Plantes Aromatiques et Medicinales*, Centre Formation Professionnelia Promotion Agricole, Centre de Rechierche et de diffusion sur L'economie des plantes à parfum, aromatiques det Médicinales, Nyons, France, pp. 241–245.

Baslas, R.K. and Baslas, K.K. (1968). Essential oils from some exotic plants raised in Kumaoa (*Mentha arvensis*), Part III. *Perfum. Essent. Oil Rec.,* **60**, 337–340.

Belafi-Rethy, K., Iglewski, S., Kerenyi, E., and Kolta, R. (1973a). Untersuchung der Zusammensetzung von einheimischen und Ausländischen ätherischen Ölen, I. Kombinierte methode zur analyse von ätherischen Ölen mittels wirksamer trennung de Gemische and instrumenteller Identifizierang der Komponenter, *Acta Chem. Akad. Sci. Hung*., **76**, 1–11.

Belafi-Rethy, K., Inglewski, S., Kerenyl, E., and Kolta, R. (1973b). Untersuchung der Zusammensetzung von einheimischen un Ausländischen ätherischen Ölen II. Analyse eines Ungarischen Pfefferminzöles. *Acta Chim. Acad. Sci. Hung.,* **76**, 1671–1677.

Benezet, M.L., Igolen, L.G., and Garnero, J. (1947). A contribution to our knowledge of Brazilian peppermint oil. *Perfum. Essent. Oil Rec*., **38**, 242.

Benn, S. (1998). Potent odorants in peppermint and cornmint oils characterized by CG-O and AEDA. *Perfum. Flavor*., **23(5)**, 5–16.

Bhagat, S.D., Upadhaya, D.N., and Singh, K.K. (1974). Oil of bergamot mint (*Mentha citrata* Ehrh.). *Indian Perfum*., **18(1)**, 31–33.

Bhardwaj, S.D. and Srivastava, L.J. (1984). Harvesting management on *Mentha citrata* Ehrh. in mid-hill condition of Himachal Pradesh. *Indian Perfum*., 28, 38–41.

Bhardwaj, S.D., Srivastava, L.J., and Kaushal, A.N. (1983). Studies on the rate and split applications of nitrogen on herb and oil yield of *Mentha citrata* Ehrh. in Himachal Pradesh. *Indian Perfum*., **27**, 99–103.

Bicchi, C., D'Amato, A., David, F., and Sandra, P. (1989). Capturing of volatiles emitted by living plants by means of thick film open tubular traps. *J. High Resol. Chromatogr*., **12**, 316–321.

Birillo, V.A. (1989). Composition of the essential oil of peppermint grown in Belorussia. *Vetsi Akad. Navuk. BSSR. Ser. Byal, Nuvyk*., **(4)**, 11–14.

Boelens, M.H. (1993). Chemical characterization of cornmint oils. *Perfum. Flavor*., **18(5)**, 27–32.

Burbott, A.J., Hennessey, J.P., Johnson, W.C., and Loomis, W.D. (1983). Configuration of piperitone from oil of *Mentha piperita. Phytochemistry*, **22**, 2227–2230.

Burks, T.F. and Gjerstad, G. (1964). Presence of three new ingredients in spearmint oil. *J. Pharm. Sci*., **53**, 964–965.

Canova, L. (1972). The composition of Scotch spearmint oil. *Ann. Acad. Brasil Cienc*., **44(Suppl.)**, 273–277.

Carnat, A.P. and Lamaison, J.L. (1987). La Menthe "Auvergne Tradition" *Mentha ×piperita* L.f. *rubescens* Camus source potentielle de l'huille essentielle officiale., *Plant Med., Phytother*., **21**, 242–251.

Casabianca. J., Graff, J.B., and Guillaumet, S. (1996). Analyses chirales et/ou isotopiques de quelques composées d'intérèt. *Rivista Ital. EPPOS,* **(Numero Speciale)**, 287–300.

Charles, D.D., Joly, R.J., and Simon, J.E. (1990). Effects of osmotic stress on the essential oil content and composition of peppermint. *Phytochemistry*, **9**, 2837–2840.

Chialva, F. and Ariozzi, A. (1992). Huile essentielle de Menthe poivrée Italienne Production 1990—approche chimiometrique. *Rivista Ital. EPPOS*, **(Numero Speciale)**, 533–550.

Chialva, F., Gabri, G., Liddle, P.O.P., and Ulian, F. (1982). Quantitative evaluation of aromatic herbs by direct headspace $(GC)^2$ status. Applications of method and comparison with the traditional analysis of essential oils. In N. Margaris, A. Koedam, and D. Vokou, Eds., *Aromatic Plants, Basic and Applied Aspects*. Martinus Nijhoff Publishers, The Hague, Netherlands, pp. 183–185.

Chien, M-J. (1988). A computer database for essential oils. In B.M. Lawrence, B.D. Mookherjee, and B.J. Willis, Eds., *Flavors and Fragrances: A World Perspective*. Elsevier Sci. Publ., Amsterdam, Netherlands, pp. 923–929.

Chomchalow, N., Buranasilpin, P., Pichitakul, N., Euraree, A., and Pangspa, A. (1976). Hill-tribe mint production and processing. *Thai J. Agric. Sci.,* **9**, 127–144.

Choudhury, S.N., Shahi, A.K., and Singh, A. (1979). Harvest management practices and oil quality of *Mentha citrata* Ehrh. at Jammu. *Indian Perfum.,* **23,** 103–106.

Christensen, R. (1995). The establishment and functionality of the Farwest spearmint marketing order. In N. Verlet, Ed., *4 émes recontres techniques et economiques Plantes Aromatiques et Medicinales*, Nyons, 1994. Office National Interprofessionel des Plantes à Parfum. Aromatiques et Medicinales, Nyons, France, pp. 180–197.

Clark, R.J. and Menary, R.C. (1983). The Tasmanian essential oil industry—production of steam distilled essential oil crops. *Proceedings of the 9th International Congress of Essential Oils, Essential Oil Technical Paper Book 1*, Singapore, pp. 18–29.

Coleman, W.M. and Lawrence, B.M. (2000). Examination of the enantiomeric distribution of certain monoterpene hydrocarbons in selected essential oils by automated solid-phase microextraction-chiral gas chromatography-mass selective detection. *J. Chromatogr. Sci*., **38**, 95–99.

Coleman, W.M., and Lawrence, B.M. (2001). Unpublished data.

Coleman, W.M., Lawrence, B.M., and Cole, S.K. (2002). Semi-quantitative determination of off-notes in mint oils by solid-phase microextraction. *J. Chromatogr. Sci*., **40**, 133–139.

Court, W.A., Roy, R.C., and Pocs, R. (1993a). Effect of harvest date on the yield and quality of the essential oil of peppermint. *Can. J. Plant Sci*., **73**, 815–824.

Court, W.A., Roy, R.C., Pocs, R., More, A.F., and White, P.H. (1993b). Optimum nitrogen fertilizer rate for peppermint (*Mentha piperita* L.) in Ontario, Canada. *J. Essent. Oil Res.,* **5**, 663–666.

de Gavina Mugica, M. and Ochoa, J.T. (1974). Aceite esencial de *Mentha pulegium* L. "poleo." In Contribucion al studio de los aceites esenciales Espanoles II. Aceites esenciales de la provincial de Guadalajara. *Minist. Agric. Instituto Nacional de Investigaciones,* Agrarias, Madrid, pp. 235–254.

Derbesy, M., Uzio, R.L., Boyer, D., and Cozon, V. (1991). Contribution to a study of essential mint oil of *Mentha piperita. Rivista Ital. EPPOS*, (**Numero Special**), 537–537; *Ann. Fals. Chim*., **84**, 205–216.

Emberger, R. and Hopp, R. (1985). Synthesis and sensory characterization of menthol enantiomers and their derivatives for the use in natural identical peppermint oils. In R.G. Berger, S. Nitz, and P. Schreier, Eds., *Topics in Flavor Research,* H. Eichhorn, Marzling-Hangenham, Germany, pp. 201–218.

Faber, B., Dietrich, A., and Mosandl, A. (1994). Chiral compounds of essential oil. XV. Stereodifferentiation of characteristic compounds of *Mentha* species by multi-dimensional gas chromatography. *J. Chromatogr*., **A666**, 161–165.

Feraud, J-P. (1997). Les huiles essentielles des les Alpes de Haute Provence. *Rivista Ital. EPPOS* (**Numero Speciale**), 511–512.

Fleisher, A. and Fleisher, Z. (1991). Water-soluble fractions of the essential oils. *Perfum. Flavor.,* **16(3)**, 37–41.

Formacek, K. and Kubeczka, K.H. (1982). *Essential Oil Analysis by Capillary Gas Chromatography and Carbon-^{13}NMR Spectroscopy*. J. Wiley & Sons, New York, pp. 197–209.

Fraisse, D., Suon, K.N., Scharff, C., Vernin, G., Zamkotsian, R.M., and Metzger, J. (1985). Huiles essentelles de *Menthe crèpue*. Analyse GC-SM-banque SPECM. *Parfum. Cosmet. Arom.,* **65**, 71–75.

Fujita, S.I. and Fujita, Y. (1970). Studies on the essential oils of the genus *Mentha*. Part V. Biochemical study of the essential oils of *Mentha pulegium* Linn. *Nippon Nogei Kagaku Kaishi*, **44**, 293–298.

Galle-Hoffman, U. and König, W.A. (1998). Pfefferminzöl. *Deutsch. Apoth. Ztg.,* **138**, 101–107.

Garnero, J., Benezet, L., and Igolen, G. (1951). Chemical composition of the peppermint oil of Brazil (*Mentha arvensis*) presence of free dL-borneol. *Bull. Soc. Chim*., France, 114–118.

Gasic, O., Mimica-Dukic, N., and Adamovic, D. (1992). Variability of content and composition of essential oils of different *Mentha arvensis* L. var. *piperascens* cultivars. *J. Essent. Oil Res*., **4**, 49–56.

Gildemeister, E. and Hoffman, F. (1961). Oils of *Mentha* species. In Die Ätherischen Öle. *Akademic Verlag*, Berlin, Germany, Vol. VII., pp. 246–421.

Goryaev, M.I., Sharipova, F.S., Tikhonova, K.K., and El'chibekova, L.A. (1967). Analysis of mint essential oil. *Maslozir. Prom*., **33(9)**, 27–30.

Graven, E.H. (1988). Personal communication.

Güntert, M., Krammer, G., Lambrecht, S., Sommer, H., Surburg, H., and Werkhoff, P. (2001), Flavor chemistry of peppermint oil (*Mentha piperita* L.). In G.A. Takeoka, M. Güntert, and K-H. Engel, Eds., *Aroma Active Compounds in Foods, Chemistry and Sensory Properties*. Amer. Chem. Soc., Washington, DC, ACS Symp. Series. No. 794, pp. 119–137.

Gupta, R. (2000). Developing models for corporate farming in aromatic crops sector in India. *J. Med. Arom. Plant Sci*., **22**, 179–185.

Handa, K.L., Smith, D.M., Nigam, I.C., and Levi, K. (1964). Essential oil and their constituents. XXIII. Chemotaxonomy of the genus *Mentha. J. Pharm. Soc.,* **53**, 1407–1409.

Hashizume, T. and Sakata, I. (1967). Constituents of Japanese peppermint oil, I. Isolation and identification of (−)-p-menthane-*trans*-2,5-diol from ryokubi. *Tetrahedron Lett*., **(35)**, 3355–3359.

Hashizume, T. and Sakata, I. (1970). Constituents of Japanese peppermint, Part II. Isolation and identification of (−)-β-caryophyllene epoxide from Ikako No. 6. *Agric. Biol. Chem*., **34**, 638–640.

Hawkes, S.J. and Wheaton, M.D. (1967). A statistical method for the analysis of peppermint oil and other complex mixtures. *J. Food. Sci*., **32**, 629–632.

Hefendehl, F.W. (1962). Die Anwendung der Gaschromatographie zur Analyse det ätherischen Öles von *Mentha piperita. Planta Med.,* **10,** 169–207.

Hefendehl, F.W. and Ziegler, E. (1975). Analytik von Pfefferminzölen, *Deutsch. Lebensmitt.-Rundsch*., **8**, 287–290.

Hiraizume, T. (1959). Essential oils and synthetic perfumes in Japanese mint oil. *Perfum. Essent. Oil Rec*., **50**, 582–588.

Hogg, J.W. and Lawrence, B.M. (1972). Essential oils and their constituents. VIII. A new bicyclic monoterpene from *Mentha cardiaca. Ger. Flav. Indust*., **3**, 321–323.

Hussain, A. (1993). Present and future agrotechnology of essential oil plants in India. In K.L. Dhar, R.K. Thappa, and S.G. Agarwal, Eds., *Newer Trends in Essential Oils and Flavours*, Tata McGraw-Hill Publ. Co., New Delhi, India, pp. 174–193.

Hussain, A., Virmani, O.P., Sharma, A., Kumar, A., and Misra, L.N. (1988). Major essential oil-bearing plants of India. *Central Inst. Med. Aromatic Plant.,* Lucknow, India, pp. 167–181.

Ikeda, N., Shimizu, S., Karasawa, D., Origasa, T., and Ono, S. (1971). *Mentha arvensis* L. var. *piperascens* Mal. which grows wild in the northeastern part of Japan. III. Studies on essential oils. *Sci. Rept. Fac. Agric.*, Okayama Univ., **(37)**, 1–8.

Ikeda, N., Shimizu, S., Udo, S., and Karasawa, D. (1966). Essential oils of artificial polyploids derived from Japanese mint, *Koryo*, **82**, 55–60.

Iscan, G., Kirimer, N., Kurkcuoglu, M., Baser, K.H.C., and Demirici, F. (2002). Antimicrobial screening of *Mentha piperita* essential oils. *J. Agric. Food Chem.*, **50**, 3943–3946.

Ishihara, M., Tsuneya, T., Shiga, M., Kawashima, S., Yamagishi, K., Yodshida, B., Sato, H., and Uneyama, K. (1992). New pyridine derivatives and basic components in spearmint oil (*Mentha gentilis* f. *cardiaca*) and peppermint oil (*Mentha piperita*). *J. Agric. Food Chem.,* **40**, 1647–1655.

Itahara, Y., Watanabe, H., and Katsuhara, J. (1970). The occurrence of (−)-(2R:5R)-*trans*-2,5-diethyltetrahydrofuran in "Hoyo" mint oil. *Bull. Chem. Soc*. Japan, **43**, 3947–3948.

Ito, M., Sagawa, S., Abe, K., and Onogaki, T. (1969). Isolation of menthofurolactone from *Mentha arvensis* and solvent effect of oil components on the formation. *Kitami Kogyo, Tanki, Daigaku,* Kenlayu Hokoku, **2**, 585–581.

Joulain, D. and König, W.A. (1998). *The Atlas of Spectral Data of Sesquiterpene Hydrocarbons*. E.B. Verlag, Hamburg.

Kak, S.N. and Kaul, B-L. (1978). Untersuchungen über induzierte Mutation be: *Mentha citrata* Ehrh. *Parfum. Kosmet*, **59**, 296–299.

Kak, S.N., and Kaul, B.L. (1980). Radiation induced useful mutants of Japanese mint (*Mentha arvensis* L.). *Zeit. Pflanzenzuch*., **85**, 170–174.

Kapil, V.B., Gulati, B.C., and Singh, G.K. (1974). Chemical studies of essential oil of *Mentha citrata*. I. Physicochemical study of different oil samples and water soluble constituents. *Indian Perfum*., **18(1),** 41–46.

Kartnig, Th. and Still, F. (1975). Die Zusammensetzung des ätherischen Öles verchiedener Menthasorten aus des Landesversuchsanlang für Spezialkulturen in Burgstall/Weiss (Stmk.). *Sci. Pharm*., **43**, 236–242.

Karasawa, D. and Shimizu, S. (1976). Sesquiterpene alcohols in the essential oils of *Mentha aquatica* L. and *Mentha piperita* L. *J. Fac. Agric*., Shinshu Univ., **(13)**, 89–97.

Katsuhara, J., Hashimoto, K., and Kobayashi, M. (1964). Sesquiterpene hydrocarbons in the oils of some *Mentha* species. *Kogyo Kagaku Zasshi*, **67**, 821–824.

Katsuhara, J., Ishibashi, K., Hasimoto, K., and Kobayashi, M. (1966). Monoterpenediols in the oil of Mitcham peppermint. *Kogyo Kagaku Zasshi*, **69**, 1170–1171.

Katsuhara, J., Yamasaki, H., Hashimoto, K., and Kobayashi, M. (1967). Sesquiterpene hydrocarbons in peppermint oil. *Kogyo Kagaku Zasshi*, **70**, 343–344.

Kirichenko, E.K. (1989). Essential oil composition of some Vietnamese mint varieties. *Byull. Gl. Bot. Sada*, **54**, 56–60.

Kobayashi, M., Yoshigi, H., and Katsuhara, J. (1960). The components of Japanese mint oil. *Koryo,* **60**, 27–35.

Kokkini, S. (1992). Essential oils as taxonomic markers in *Mentha*. In R.M. Harley and T. Reynolds, Eds., *Advances in Labiatae Science*, Royal Botanic Gardens, Kew, London, pp. 325–334.

Komatsu, A. (1960). Alcohols in dementholized peppermint oil. *Koryo,* **(55)**, 30–33; *Chem. Abs.,* **55**, 12778a.

König, W.A., Fricke, C., Saritas, Y., Momeni, B., and Hohenfeld, G. (1997). Adulteration or natural variability? Enantioselective gas chromatography in purity control of essential oils. *J. High Resol. Chromatogr*., **20**, 55–61.

Köpsel, M., Krempel, A., and Surburg, H. (1986). An unusual compound isolated from *Mentha piperita* oil. In E-J. Brunke, Ed., *Progress in Essential Oil Research*. Walter de Gruyter, New York, pp. 241–248.

Kothari, S.K. and Singh, U.B. (1995). The effect of row spacing and nitrogen fertilization on Scotch spearmint *Mentha gracilis* Sole. *J. Essent. Oil Res*., **7**, 287–297.

Kreis, P., Hener, U., and Mosandl, A. (1990a). Chirale Inhaltstoffe ätherischer Öle. III. Stereodifferenzierung von α-pinene, β-pinen und Limonen in ätherschen Ölen, Drogen und Fertigarzneimitteln. *Dtsch. Apoth. Ztg*., **130**, 985–988.

Kreis, P., Mosandl, A., and Schmarr, H.G. (1990b). *Mentha-Ölen. Dtsch. Apoth. Ztg.,* **130,** 2579–2581.

Kremers, R.E. (1926). Studies in the genus *Mentha*. VIII. The aldehydes of peppermint oil. *Amer. J. Pharm. Sci*., **98**, 86–91.

Krishnamoorthy, S., (1989). Indigenous essential oils—recent developments and perfumery applications. *Indian Perfum*., 215–218.

Krishnamoorthy, A. (1993). Country profile on Indian essential oils and related materials. Essential Oil Association of India, Bombay, pp. 1–12.

Kubeczka, K.H., and Protzen, J.A. (1997). Investigations of tea infusions and distillation waters using solid phase microextraction (SPME) technique. In Ch. Franz, A. Mathé, and G. Buchbauer, Eds., *Proceedings of the 27th International Symposium of Essential Oils*, Sept. 8–11, 1996, Vienna. Allured Publ., Carol Stream, IL, pp. 157–159.

Kubeczka, K.H. and Formacek, V. (2002). Essential oils analysis by capillary gas chromatography and carbon-13 NMR. 2nd Edn. J. Wiley and Sons, New York, pp. 239–253.

Kumar, N., Arumugam, R., Sambandamoorthy, S., and Kandasamy, O.S. (1980). A note on rooting of planting material of bergamot mint (*Mentha citrata* Ehrh.), *Indian Perfum*., **24**, 31–35.

Kumar, S., Bahl, J.R., Bansal, R.P., Kukreja, A.K., Garg, S.N., Naqvi, A.A., Luthra, R., and Sharma, S. (2000). Profiles of the essential oils of Indian menthol mint, *Mentha arvensis* cultivars at different stages of crop growth in northern plains. *J. Med. Arom. Plant. Sci*., **22**, 774–787.

Landing, J.E. (1969). *American Essence. A History of the Peppermint and Spearmint Industry in the United States*. Kalamazoo Public Museum, Kalamazoo, MI, pp. 1–244.

Lawrence, B.M. (1978). A study of the monoterpene interrelationships in the genus *Mentha* with special reference to the origin of pulegone and menthofuran. Ph. D. Thesis, State University, Groningen, Netherlands.

Lawrence, B.M. (1983). Progress in essential oils (cornmint oil). *Perfum. Flavor*., **8(2)**, 61–66.

Lawrence, B.M. (1993a). Progress in essential oils (Native spearmint oil). *Perfum. Flavor*., **18(2)**, 46–52.

Lawrence, B.M. (1993b). Progress in essential oils (Scotch spearmint oil). *Perfum. Flavor*., **18(2)**, 61–64.

Lawrence, B.M. (1993c). Progress in essential oils (peppermint oil). *Perfum. Flavor*., **18(4)**, 59–71.

Lawrence, B.M. (1998). Progress in essential oils (Pennyroyal oil). *Perfum. Flavor*., **23(3)**, 63–68.

Lawrence, B.M. (2000). Commercially important mint oils. Paper presented at 31st International Symp. Essential Oils. Hamburg, Germany.

Lawrence, B.M. (2001). Unpublished cornmint oil data.

Lawrence, B.M., Hogg, J.W., and Terhune, S.J. (1972). Essential oils and their constituents. X. Some new trace constituents in the oil of *Mentha piperita* L. *Flavor Indust*., **3**, 467–472.

Lawrence, B.M., Shu, C-K., and Harris, W.R. (1989). Peppermint oil differentiation. *Perfum. Flavor*., **14(6)**, 21–30.

Li, B-G., An, Q-R., and Guo, Z-F. (2001). Identification of isomer of volatile oils in peppermint leaves and its stalks by gas chromatography–mass spectrometry. *Fenxi Hauxue*, **29**, 530–533.

Lincoln, D.E., Murray, M.J., and Lawrence, B.M. (1986). Chemical composition and genetic basis for the isopinocamphone chemotype of *Mentha citrata* hybrids. *Phytochemistry*, **25**, 1857–1863.

Machale, K.W., Niranjan, K., and Pangar, V.G. (1997). Recovery of dissolved essential oils from condensate waters of basil and *Mentha arvensis* distillation. *J. Chem. Tech. Biotechnol*., **69**, 362–366.

Maffei, M., Codignola, A., and Fieschi, M. (1986). Essential oil from *Mentha spicata* L. (spearmint) cultivated in Italy. *Flav. Fragr. J*., **1**, 105–109.

Malla, S., Nigam, M.C., and Handa, K.L. (1962). Essential oil of *Mentha arvensis. Indian Oil Soap J*., **27**, 173–178.

Malingré, Th.M. (1969). Gas chromatographic investigation of the essential oil of *Mentha piperita* during development of the plant. *Pharm. Weekbl*., **104**, 381–406.

Mathur, P., Farooqi, A.H.A., Sharma, S., and Kumar, R. (2001). Effect of osmotic stress on growth of *Mentha arvensis. Indian Perfum*., **45**, 89–93.

Maurer, B. and Hauser, A. (1988). Identification de certains produits aromatisants dans des arômes. *Parfum. Cosmet. Arôm.*, **(82)**, 69–72.

McCarthy, W.C., Venkatramana Bhatt, K., Roscoe, C.W., and Fischer, L. (1963). Some low boiling constituents of peppermint oil. *J. Pharm. Sci.*, **52**, 1005.

Mimica-Dukic, N., Bozin, B., Sokovic, M., Mihajlovic, B., and Matavulj, M. (2003). Antimicrobial and antioxidant activities of three *Mentha* species essential oils. *Planta. Med.*, **69**, 413–419.

Mohan Rao, L.J. (2000). Quality of essential oils and processed materials of selected spices and herbs. *J. Med. Arom. Plant Sci.*, **23**, 808–816.

Mookherjee, B.D., Trenkle, R.W., and Wilson, R.A. (1990). The chemistry of flowers, fruits and species: live vs. dead—a new dimension in fragrance research. *Pure Appl. Chem.*, **62**, 1357–1364.

Mookherjee, B.D., Wilson, R.A., Trenkle, R.W., Zampino. M.J., and Sands, K.P. (1989). New dimensions in flavor research. Herbs and Spices. In R. Teranishi, R.G. Buttery, and F. Shahidi, Eds., *Flavor Chemistry Trends and Developments*. Amer. Chem. Soc., Washington, DC, ACS Symp. Series No. 338, pp. 176–187.

Mosandl, A., Fleisher, K., Hener, U., Kreis, P., Rettinger, K., Schubert, V., and Schmarr, H.G. (1991). Flavor compounds 48. Chirospecific analysis of natural flavors and essential oils using multidimensional gas chromatography. *J. Agric. Food Chem.*, **39**, 1131–1134.

Moyler, D. and Moss, N. (1998). Potential for standardizing profiles with natural flavoring substances. *Perfum Flavor.*, **23(2)**, 37–42.

Murray, M.J. (1969). A new essential oil plant, a hybrid *Mentha citrata. Reichstoff. Arom. Körperpflegemit.*, **19**, 533–540.

Murray, M.J. and Lincoln, D.E. (1970). The genetic basis of acidic oil constituents in *Mentha citrata* Ehrh. *Genetics*, **65**, 457–471.

Murray, M.J., Faas, W., and Marble, P. (1972). Effects of plant maturity on oil composition of several spearmint species grown in Indiana and Michigan. *Crop Sci.*, **12**, 723–728.

Murray, M.J., Marble, P., Lincoln, D., and Hefendehl, F.W. (1988). Peppermint oil quality differences and the reasons for them. In B.M. Lawrence, B.D. Mookherjee, and B.J. Willis, Eds., *Flavors and Fragrances: A World Perspective*. Elsevier Science Publ., Amsterdam, Netherlands, pp. 189–210.

Näf, R. and Velluz, A. (1998). Phenols and lactones in Italo-Mitcham peppermint oil (*Mentha ×piperita* L.), *Flav. Fragr. J.*, **13**, 203–208.

Nagasawa, T. (1959). Chemical studies on *Mentha*. II. On the odorous components of Japanese peppermint oil. *Shimane Daigtau Ronshu (Shizen Kagaku)*, **(9)**, 30–43.

Nagasawa, T., Umemoto, K., Tsuneya, T., and Shiga, M. (1976). Studies on essential oil *Mentha spicata* L. var. *crispa*. Benth. grown in Winter season. *Nippon Nogei Kagaku Kaishi*, **50**, 287–289.

Nakamoto, H., Ishihara, M., Matsuyama, A., and Kawai, T. (1996). Enantiomeric distributions of carveols in grapefruit, orange and spearmint oils. In *IFEAT Proceedings, International Conference of Aromas and Essential Oils*. IFEAT Secretariat, Tel Aviv, Israel, pp. 36–50.

Nakamura, Y., Shimomura, T., and Sawai, K. (1952). The components of the higher boiling fractions (tailings) of peppermint oil. III. Identification of Isocaryophyllene alcohol and presence of an azulene-type sesquiterpene. *J. Agric. Chem. Soc.* (Japan), **26**, 493–496.

Nakayama, T., Higashiyama, T., Sakata, I., and Hashizume, T. (1970). New Japanese peppermint (*Mentha arvensis piperascens*) variety Shubi developed in Okayama. *Koryo*, **(97)**, 47–66.

Nanmoku, T. (1995). Geographical production and features of peppermint oil. *Aromatopia*, **(13)**, 15–18.

Nano, G.M., Fundar, A., Calvino, R., and Cabella, P. (1972). Sesquiterpene components of *Mentha piperita* Ital-Micham cultivated in the Piemonte. *Essenz. Deriv. Agrum.*, **42**, 325–332.

Nigam, I.C. and Levi, L. (1963). Sabinene hydrate and sabinene acetate: two new constituents of American spearmint oil. *J. Agric. Food Chem.*, **11**, 276.

Nigam, I.C. and Levi. L. (1964). Essential oils and their constituents. XX. Detection and estimation of menthofuran in *Mentha arvensis* and other mint species by coupled gas-liquid-thin-layer chromatography. *J. Pharm. Sci.*, **53**, 1008–1013.

Nigam, I.C., Sahasrabudhe, M., and Levi. L. (1963). Coupled gas-liquid-thin layer-chromatography. Simultaneous determination of piperitone and piperitone oxide in essential oils. *Can. J. Chem.*, **41**, 1535–1539.

Nikolaeva, D.A. (1964). Variétè hybrid MC-51 Comme nouvelle source à haut rendement de L-Menthol. In *Compte rendu du Troisiemme Congrés International des huiles essentielles*, May, Plodiv, Bulgaria, pp. 169–171.

Oberhofer, B., Nikoforov, A., Buchbauer, G., Jirovetz, L., and Bicchi, C. (1999). Investigation of the alteration of the composition of various essential oils used in aroma lamp applications. *Flav. Fragr, J*., **14**, 293–299.

Ognyanov. I. and Vlahov, R. (1962). Non-terpene compounds in the terpene fractions of Bulgarian peppermint oil. *Compt. Rend. Acad. Bulg. Sci*., **15**, 395–397.

Ognyanov, I. and Vlahov, R. (1965). On the composition of Bulgarian peppermint oil. *Perfum. Essent. Oil Rec*., **56**, 370–373.

Ohloff, G. (1994). Scent and fragrances. The fascination of odors and their chemical perspectives. *Springer Verlag*, Berlin, Germany, pp. 153 and 188.

Onogaki, T. (1962). 2-Isopropylcyclopentanone from Japanese mint oil in Hokkaido. *Nippon Kagaku Zasshi*, **83**, 206–208.

Osaki, K. (1992). Flavor of fresh leaves of *Mentha* ssp., *Koryo*, **(176)**, 77–86.

Pandey, A.K. and Chowdhury, A.R. (2000). GC–MS studies of Japanese mint (*Mentha arvensis* L.) oil. *J. Med. Arom. Plant Sci*., **22**, 468–469.

Patra, N.K., Khanuja, S.P.S., Shasang, A.K., Singh, H.P., Singh, V.R., Tanveer, H., Kalra, A., Singh, H.B., Mengi, N., Tyagi, N.K., Naqvi, A., and Kumar, S. (2000). Genetic improvement of cultivated species of Cymbopogon and *Mentha* for yield, quality and adaptation. *J. Med. Arom. Plant Sci*., **22**, 263–277.

Peiris, B.D., Balasubramaniam, S., Jayawardene, A.L., and Herath, H.M.W. (1982). Effect of plant maturity on oil composition of two mint varieties recently introduced to Sri Lanka. *J. Natl. Sci. Counc. Sri Lanka*, **10(1)**, 143–147.

Peyron, L. (1961). Precursors of certain sulphur compounds encountered with essential oils. *Bull. Soc. France Physiol. Vegatal*., **7**, 46–47.

Pichitakul, N. and Sthapitanonda, K. (1977). The constituents of oil from different mint varieties. *J. Nat. Res. Council Thai*., **9(2)**, 1–13.

Platin, S., Özer, E.O., Akman, U., and Hortaçsu, O. (1994). Equilibrium distributions of key components of spearmint oil in sub/supercritical carbon dioxide. *J. Amer. Oil Chem. Soc*., **71**, 833–837.

Porsch, F. and Farnow, H. (1962). New discoveries about the constituents of peppermint oils by gas chromatography and infrared spectroscopy. *Dragoco Rept*., **(7)**, 163–178.

Prasad, J. (1997). Mentha cultivation, its problems and prospects in Uttar Pradesh. *Indian Perfum*., **41(2)**, 57–61.

Qiu, N-Y., Chen, Y-S., Wu, Q., Liu, J-H., and Sheng, L-S. (1990). Fast determination of peppermint oil by GC.FTIR. *Zhongguo Yaoke Daxue Xuebao* (*J. Chin. Pharmaceut. Univ*.), **21(4)**, 205–207.

Rajeswara Rao, B.R., Singh, S.P., and Prakasa Rao, E.V.S. (1984). Effect of row spacings and nitrogen application on biomass yield, essential oil concentration and essential oil yield of bergamot oil (*Mentha citrata* Ehrh.). *Indian Perfum*., **28,** 150–152.

Rajeswara Rao, B.R., Bhattacharya, A.K., Mallavarapu, G.R., and Ramesh, S. (1999). Volatile constituents of different paths of cornmint (*Mentha arvensis* L.). *Flav. Fragr, J*., **14**, 262–264.

Rajeswara Rao, B.R., Kaul, P.N., Mallavarapu, G.R., and Ramesh, S. (2000). Comparative composition of whole herb, flowers, leaves and stem oils of cornmint (*Mentha arvensis* L. f. *piperascens* Malinv. ex Holmes). *J. Essent. Oil Res*., **12**, 357–359.

Rajeswara Rao, B.R. (2002). Biomass yield, essential oil yield and essential oil composition of rose-scented geranium (*Pelargonium* species) as influenced by row spacings and intercropping with cornmint (*Mentha arvensis* L. f. *piperascens* Malinv. ex Holmes). *Indust. Crops Prod*., **16**, 133–144.

Ravid, U., Putievsky, E., and Katzir, I. (1994a). Enantiomeric distribution of piperitone in essential oils of some *Mentha* ssp. *Calamintha incana* (Sm.) Heldr. and *Artemisis judaica* L., *Flav. Fragr. J*., **9**, 85–87.

Ravid, U., Putievsky, E., and Katzir, I. (1994b). Chiral GC analysis of menthone and isomenthone with high enantiomeric purities in laboratory-made and commercial essential oils. *Flav. Fragr. J*., **9**, 139–142.

Ravid, U., Putievsky, E., and Katzir, I. (1994c). Chiral GC analysis of (1R)-(+)-pulegone with high enantiomeric purity in essential oils of some Lamiaceae aromatic plants. *Flav. Fragr. J.*, **9**, 205–207.

Ravid, U., Putievsky, E., and Katzir, I. (1994d). Chiral GC analysis of enantiomerically pure (R)-(−)-linalyl acetate in some Lamiaceae, Myrtle and Petitgrain essential oils. *Flav. Fragr. J.*, **9**, 275–276.

Ravid, U., Putievsky, E., Katzir, I., Ikan R., and Weinstein, V. (1992a). Determination of the enantiomeric composition of citronellol in essential oils by chiral GC analysis on a modified γ-cyclodextrin phase. *Flav. Fragr. J.*, **7**, 235–238.

Ravid, U., Putievsky, E., Katzir, I., Weinstein, V., and Ikan, R. (1992b). Chiral GC analyses of (S)-(+)- and (R)-(−)-carvone with high enantiomeric purity in caraway, dill and spearmint oils. *Flav. Fragr. J.*, **7**, 289–292.

Reitsema, R.H. (1958). A biogenetic arrangement of mint species. *J. Amer. Pharm. Assoc. Sci. Edn.*, **47**, 267–269.

Reverchon, E., Ambruosi, A., and Senatore, F. (1994). Isolation of peppermint oil using supercritical CO_2 extraction. *Flav. Fragr. J.*, **9**, 12–23.

Rohloff, J. (2002). Essential oil composition of Sakhalin mint from Norway detected by solid-phase microextraction and gas chromatography–mass spectrometry analysis. *J. Agric. Food Chem.*, **50**, 1543–1547.

Rojan, W., Hammerschmidt, F.J., Ziegler, E., and Hefendehl, F. (1977). The occurrence of viridiflorol in peppermint oil (*Mentha piperita* L.), *Dragoco Rept.*, **(10)**, 230–232.

Romito, L. (1990). Personal correspondence.

Rothbächer, H., Kraus, A., and Suteu, F. (1970). Esters and carbonyl components in Romanian peppermint oil, *Mentha piperita. Herba Hung.*, **9(1)**, 55–66.

Rothbächer, H., Suteu, F., and Kraus, A. (1967). Mono- and bicyclic terpene hydrocarbons in Romanian peppermint oil. *Planta Med.*, **15**, 434–438.

Sacco, T. and Nano, G.M. (1970). Contributo allo studio botanico e chimico del genre *Mentha* gruppo *arvensis*. *Allionia,* **16**, 59–64.

Sacco, T. and Gallino, M. (1976). Sulla composizione dell'olio essenziale di due forme ibride di *Mentha piperita* Huds. realizzate in U.R.S.S. e sperimentale in Piemonta. *Atti-Conv. Nax. Olii Essenz. Sui. Deriv. Agrum.*, 82–91.

Sacco, T., Maffei, M., and Chialva F. (1989). New hybrids from a seed population of *Mentha arvensis* var. *piperascens* Clone 7303. In S.C. Bhattacharyya, N. Sen, and L.K. Sethi, Eds., *Proceedings of the 11th International Congress of Essential Oils, Fragrances and Flavors*. Vol. 3, Oxford & IBBT Publ. Co., New Delhi, India, pp. 27–34.

Sacco, T., Nano, G.M., and Scannerini, S. (1969). On the cytotaxonomy of selected mints of the section Spicatae. III. Chromosome number and chemotaxonomical data of mints with prevalent carvone in the oil *Mentha longifolia* (L.). Huds. var. *crispa* Ten. and *Mentha longifolia* (L.) Huds. var. *candicans* Crantz. *Allionia*, **15**, 23–35.

Sakata, I. and Hashizume (1971). Constituents of Japanese peppermint oil. Part III. Isolation and identification of two new constituents from "Shubi." *Agric. Biol. Chem.*, **35**, 128–131.

Sakata, I. and Hashizume, T. (1972). Isolation and identification of (+)-β-methyl adipic acid from "Shubi." *Agric. Biol. Chem.*, **36**, 1237–1238.

Sakata, I., and Hashizume, T. (1973). Isolation and identification of 2,3-dimethyl-4-hydroxy-2-nonenioc acid lactone from "Shubi." *Agric. Biol. Chem.*, **37**, 2442–2442.

Sakata, I., Kasano, H., and Kawaza, K. (1982). Constituents of essential oils of two new cultivars of Japanese peppermint in Hokkaido, Hokkai 19 and Hokkai 20. *Nippon Nogei Kagaku Kaishi*, **56**, 787–794.

Sakata, I., and Koshimizu, K. (1980). Seasonal variations in menthyl glucoside, menthol, menthone and related monoterpenes in developing Japanese peppermint. *Nippon Nogeikagaku Kaishi*, **54**, 1037–1043.

Sakurai, K., Takahashi, K., and Yoshida, T. (1983a). Two new *m*-cresol derivatives and a new terpene alcohol from peppermint oil. *J. Agric. Food Chem.*, **47**, 1249–1256.

Sakurai, K., Takahashi, K., and Yoshida, T. (1983b). Pyridine derivatives in peppermint oil. *Agric. Biol. Chem*., **47**, 2307–2312.

Shahi, A.K., Chandra, S., Dutt, P., Kaul. B.L., Tava, A., and Avato, P. (1999). Essential oil composition of *Mentha xpiperita* L. from different environments of north India. *Flav. Fragr. J*., **14**, 5–8.

Sheldon, R.M., Walters, L.A., and Druell, W.E. (1972). The synthesis of flavor chemicals and oils from domestic raw materials. Paper #38, presented at 164th National Amer. Chem. Soc. Symp. on Indust. Prod. Ingred., Aug. 1972, New York.

Shi, M., Tang, M-H., and Hu, Z-Y. (1996). Quality evaluation of spearmint oil by gas chromatography. *Fenxi Kexue Xuebao*, **12(3)**, 180–184.

Shimizu, S. (1985). *Mentha piperita* L. and Japanese *Mentha arvensis* var. piperascens Malinv., *Aromatopia*, **4(4)**, 10–14.

Shimizu, S. (1988). Studies on the mints containing dextro- or levo-rotatory linalool as a dominant constituent in the essential oils. *Bull. Iida Women's Junior College* (*Japan*), **9**, 24–33.

Shimizu, S. and Ikeda, N. (1962). Studies on the essential oils of interspecific hybrids in the genus *Mentha*. Part VIII. The essential oils of *Mentha spicata* L. with 36 or 48 somatic chromosomes. *Agric. Biol. Chem*., **26**, 543–545.

Shimizu, S., Shibata, H., Karasawa, D., and Kozaki, T. (1990). Studies on terpene glycosides in *Mentha* plants. Part II. Carvyl—and dihydrocarvyl-β-D-glucosides in spearmint. *J. Essent. Oil Res*., **2**, 81–86.

Singh, N.P. and Nand, K. (1979). Response of *Mentha citrata* Ehrh. to dates of planting and row spacing. *Indian Perfum*., **23**, 50–52.

Singh, A., Shahi, A.K., and Das, B. (1979a). Effect of harvest management on the second cutting in *Mentha citrata* Ehrh., *Indian Perfum*., **23**, 216–217.

Singh, V.P., Bist, H.S., and Duhan, S.P.S. (1979b). Studies on the split application of nitrogen through soil and foliage on the herb and oil yield of *Mentha citrata* Ehrh., *Indian Perfum*., **23**, 100–102.

Singh, R.B., Goswami, A., Nigam, M.C., and Thakur, R.S. (1980). 1-vinylmenth-4(8)-ene, a terpenoid hydrocarbon from *Mentha citrata. Phytochemistry*, **19**, 2466.

Singh, A., Singh. D.V., and Thakur, R.S. (1989). Spearmint cultivation in India—past and present scenario. *PAFAI J*., **(Oct/Dec)**, 39–41.

Singh, A.K., Kare, M., Sharma, S., Naqvi, A.A., and Kumar, S. (2001). Instant prediction of the major components of essential oil of menthol mint (*Mentha arvensis)* on the basis of congealing point: a ready reference manual. *J. Med. Arom. Plant. Sci*., **23**, 372–378.

Singh, K., Rajeswara Rao, B.R., Singh, C.P., Kaul, P.N., Mallavarapu, G.R., and Ramesh, S. (2002a). Comparative performance of menthol mint (*Mentha arvensis* L. f. *piperascens* Malinv. and ex. Holmes) cultivars in semi-arid tropical climate. *J. Spices Arom. Crops*, **11**, 67–69.

Singh, K., Rajeswara Rao, B.R., Rajput, D.K., Bhattacharyya, A.K., Chauhan, H.S., Mallavarapu, G.R., and Ramesh, S. (2002b). Composition of essential oils of menthol mint (*Mentha arvensis* var. *piperascens*), citronella (*Cymbopogon winterianus*) and palmarosa (*Cymbopogon martini* var. *motia*) grown in the open and partial shade of poplar (*Populus deltoides)* trees. *J. Med. Arom. Plant Sci*., **24**, 710–712.

Smith, D.M., and Levi, L. (1961). Determination of geographical origins of peppermint oil by gas chromatographic analysis. *J. Agric. Food Chem*., **9**, 230–244.

Smith, D.M., Skakum, W., and Levi, L. (1963). Determination of the botanical and geographical origin of spearmint oils by gas chromatographic and ultraviolet analysis. *J. Agric. Food Chem*., **11**, 268–276.

Spencer, J.S. (1992). Distillation water composition from the field distillation of peppermint and spearmint. In H. Woidich and G. Buchbauer, Eds., *Proceedings of 12th International Congress of Flavors, Fragrances and Essential Oils*, Austrian Assoc. Flav. Fragr. Industry, Vienna, Austria, pp. 306–314.

Spencer, J.S., Dowd, E., and Faas, W. (1997). The genuineness of two mint essential oils. *Perfum. Flavor*, **22(3)**, 37–45.

Srinivas, S.R. (1986). Atlas of essential oils. Published by author, Bronx, NY, pp. 711–714 and 1084.

Stengele, M. (1994). Beitrage zur Rolle glykosidisch gebundener flüchtiger Komponenten in ätherisches Öle fürhrenden Pflanzen. Unterschungen an ausgenwählten Arten der Gattung *Mentha* L. Ph. D. thesis, University of Hamburg, Germany.

Surburg, H. and Köpsel (1989). Naturally occurring sesquiterpenes from Scotch spearmint oil (*Mentha cardiaca* Ger.). *Flav. Fragr. J.*, **4**, 143–147.

Takahashi, K., Someya, S., Muraki, S., and Yoshida, T. (1980). A new keto-alcohol, (−)-mintlactone and (+)-isomintlactone and minor components in peppermint oil. *Agric. Biol. Chem.*, **44**, 1535–1543.

Takahashi, K., Muraki, S., and Yoshida, T. (1981). Chemical composition of fragrant materials. Part V. Synthesis and distribution of (−)-mintsulfide, a novel sulfur-containing sesquiterpene. *Agric. Biol. Chem.*, **45**, 129–132.

Talwar, Y.P., Nigam, M.C., and Handa, K.L. (1963). Essential oil from *Mentha arvensis. Parfum. Kosmet.*, **44(4)**, 93–95.

Thacker, J. (1997). Personal correspondence.

Todd, W.P. and Murray, M.J. (1968). New essential oils from hybridization of *Mentha citrata* Ehrh. *Perfum. Essent. Oil Rec.*, **59**, 97–102.

Topalov, V. and Zheljazkov, V. (1991). Effect of harvesting stages on the yield of fresh material, essential oil and planting material from *Mentha piperita* Huds. and *Mentha arvensis* L., *Herba Hung.*, **30(1/2)**, 60–67.

Tsuneya, T., Ishihara, M., Takatori, H., Yoshida, F., Yamanishi, K., and Ikenishi, T. (1998). Acidic compounds in Scotch spearmint oil (*Mentha gracilis* Sole). *J. Essent. Oil Res.,* **10**, 507–516.

Tsuneya, T., Ishihara, M., Shiga, M., Kawashima, S., Satoh, H., Yoshida, F., and Yamagishi, K. (1993). Trace components in spearmint oil and their sensory evaluation. In R. Teranishi, R.G. Buttery, and H. Sugisawa, Eds., *Bioactive Volatile Compounds from Plants*. ACS Symp. Series No. 525, Amer. Chem. Soc., Washington, DC, USA, pp. 137–158.

Tucker, A.O., and Fairbrothers, D.E. (1990). The origin of *Mentha* ×*gracilis* (Lamiaceae) 1. Chromosome numbers, fertility and three morphological characters. *Econ. Bot.*, **44**, 182–213.

Tucker, A.O., Hendriks, H., Bos, R., and Fairbrothers, D.E. (1991). The original of *Mentha gracilis* (Laminaceae). II. Essential oils. *Econ. Bot.*, **45**, 200–215.

Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., and Webb, D.A. (1972). *Flora Europea. Vol. 3. Diapensiaceae to Myoporaceae*, Cambridge Univ. Press, Cambridge, UK, p. 184.

Umemoto, K. and Nagasawa, T. (1980). Studies on the wild mints of Tokai Districts. Part X. Constituents in essential oil of *Mentha spicata* L. var. *crispa* Benth. *Nagoya Gakuin Daigaku Ronshu, Jinbun, Shizen Kagakuhen*, **17(1)**, 139–153.

Varshney, S.C. (1986). Progress in essential oils in North India. *Indian Perfum.*, **30**, 425–428.

Velasco-Negueruela, A., Perez-Alonso, M.J., and Mata Rico, M. (1987). Aceites esenciales de Lamiaceas Ibericas con pulegone como componente fundamental. *Anal. Bromatol.*, **39**, 375–372.

Virmani, O.P., Nigam, M.C., and Datta, S.C. (1972). Gas chromatography of the essential oil of *Mentha citrata* raised in Lucknow. *Indian Perfum.*, **16(2)**, 1–2.

Vlahov, R. and Ognyanov, I. (1964). Oxygen containing terpene compounds in Bulgarian peppermint oil. *Compt. Rend. Acad. Bulg. Sci.*, **17**, 145–147.

Vlahov, R., Holub, M., Ognyanov, I., and Herout, V. (1967a). Terpenes. CLXXXIV. Sesquiterpene hydrocarbons from the essential oil of *Mentha piperita* of Bulgarian origin. *Coll. Czech. Chem. Communs.*, **32**, 808–821.

Vlahov, R., Holub, M., and Herout, V. (1967b). Terpenes CLXXXV. Structure of two hydrocarbons of the cadinene type isolated from *Mentha piperita* of Bulgarian origin. *Coll. Czech. Chem. Communs.*, **32**, 822–829.

von Schantz, M. and Norri, R. (1968). Über die Veränderungen der Olzusammensetzung der Blattinsertionen der ukrainischen Pfefferminze Während der Entwicklung. *Sci. Pharm.*, 36, 187–189.

Voronina, E.P., Dmitriev. L.B., and Greenberg, I.I. (1990). Composition of the essential oil of certain mint species and varieties introduced in Central Botanic Garden. *Izv. Timiryazevsk. S-kh. Akad.*, **(4)**, 166–170.

Wenninger, J.A., Yates, R.L., and Dolinsky, M. (1967). High resolution infrared spectra of some naturally occurring sesquiterpene hydrocarbons. *J. Assoc. Offic. Anal. Chem.*, **50**, 1313–1335.

Werkoff, P. and Hopp, R. (1986). Isolation and gas chromatographic separation of menthol and menthone enantiomers from natural peppermint oils. In E.J. Brunke, Ed., *Progress in Essential Oil Research,* Walter de Gruyter, Berlin, Germany, pp. 529–549.

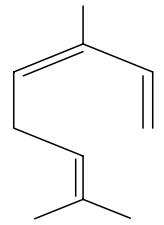
Wong, W. (1972). The changes that occur in peppermint during aging. *Ph*.D. Thesis, Rutgers University, New Brunswick, NJ.

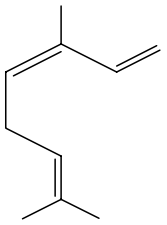
Wüst. M. and Mosandl, A. (1999). Important chiral monoterpenoid ethers in flavours and essential oils—enantioselective analysis and biogenesis. *Eur. Food Res. Technol*., **209**, 3–11.

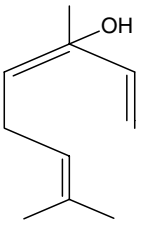
Zheljazkov, V., Yankov, B., and Topalov, V. (1996). Comparison of three methods of mint propagation and their effect on the yield of fresh material and essential oil. *J. Essent. Oil Res*., **8**, 35–45.

Zhu, L-F., Li, Y-H., Li, B-L., Lu, B-Y., and Xia, N-H. (1993). *Aromatic Plants and Essential Constituents*. South China Institute of Botany, Chinese Academy of Sciences, Hai Feng Publish. Co. distributed by Peace Book Co. Ltd., Hong Kong, p. 97.

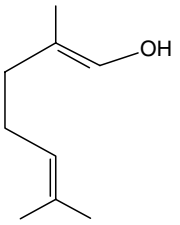
Appendix
Structures of Some Important Constituents of Mint Oils


(Z)-β-ocimene

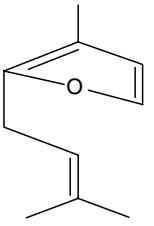

(E)-β-ocimene



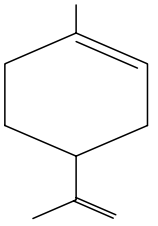
linalool

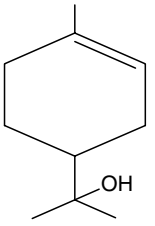


geraniol

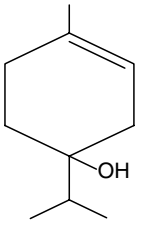


rosefuran

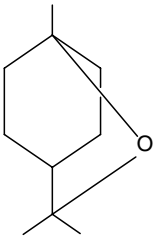

imonene



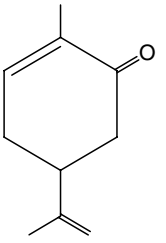
α-terpineol



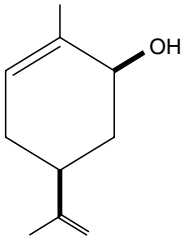
terpinen-4-ol



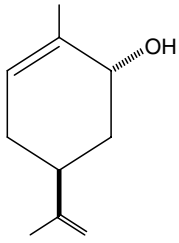
1,8-cineole



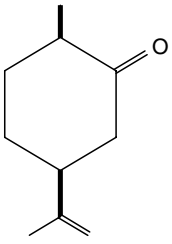
carvone



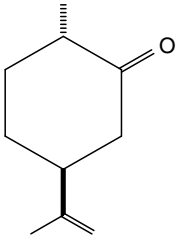
cis-carveol



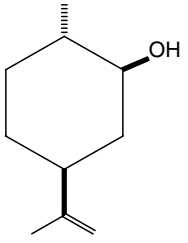
trans-carveol



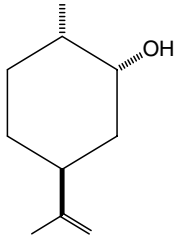
cis-dihydrocarvone



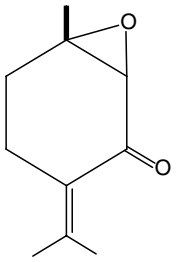
trans-dihydrocarvone



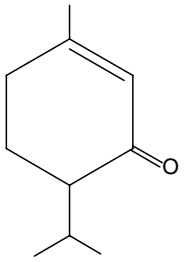
dihydrocarveol



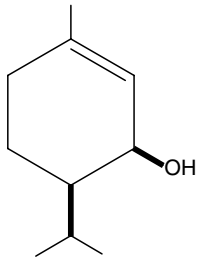
neodihydrocarveol



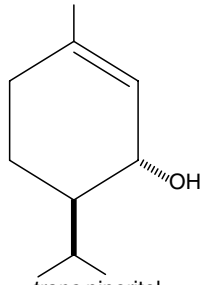
piperitenone oxide



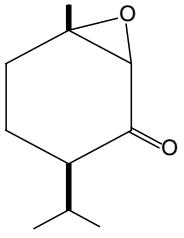
piperitone



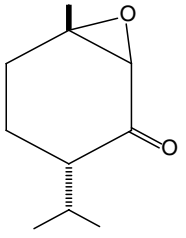
cis-piperitol



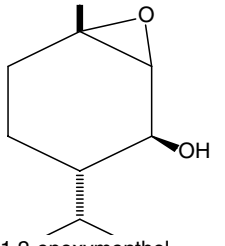
trans-piperitol



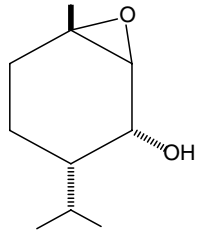
cis-piperitone oxide



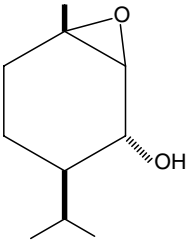
trans-piperitone oxide



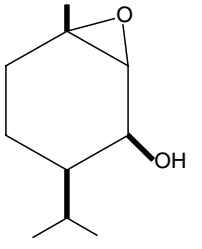
1,2-epoxymenthol



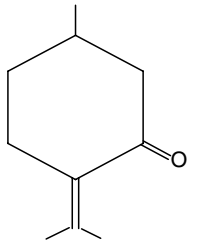
1,2-epoxyneomenthol



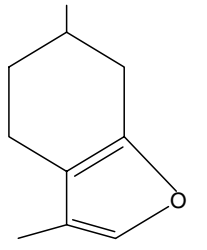
1,2-epoxyisomenthol



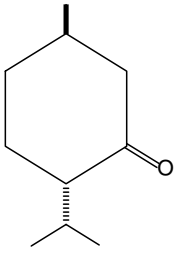
1,2-epoxyneoisomenthol



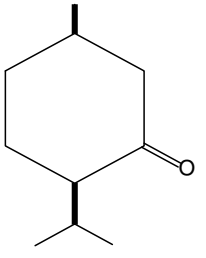
pulegone



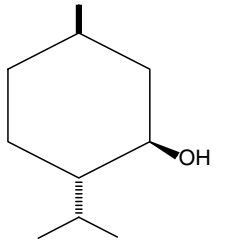
menthofuran



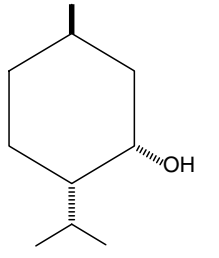
menthone



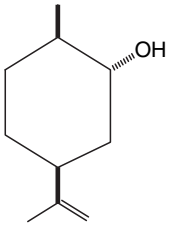
isomenthone



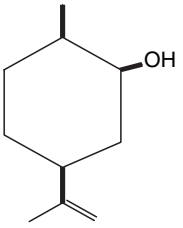
menthol



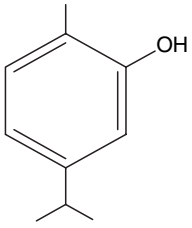
neomenthol



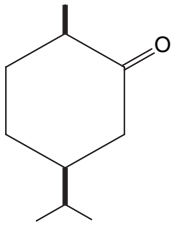
isodihydrocarveol



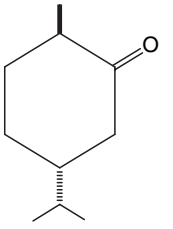
neoisodihydrocarveol



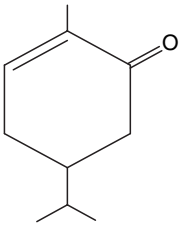
carvacrol



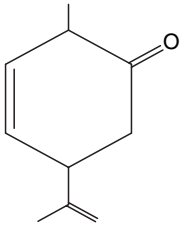
cis-carvomenthone



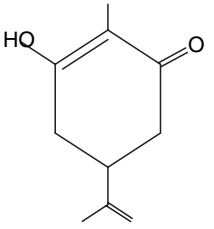
trans-carvomenthone



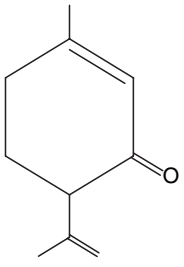
carvotanacetone



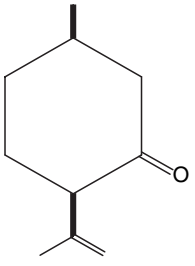
spicatone



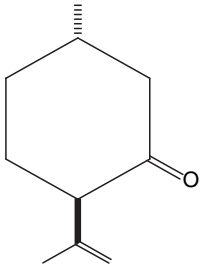
6-hydroxycarvone



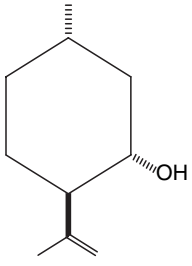
isopiperitenone



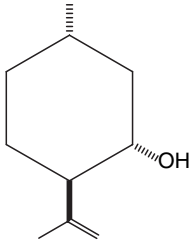
cis-isopulegone



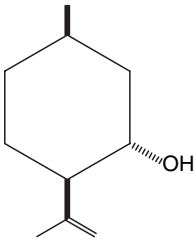
trans-isopulegone



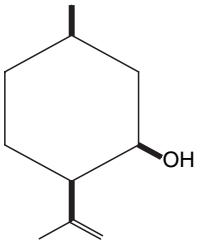
isopulegol



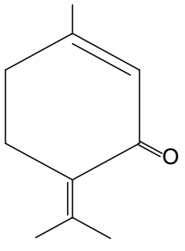
neoisopulegol



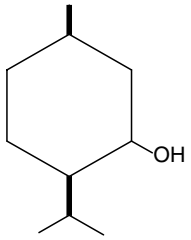
iso(iso)pulegol



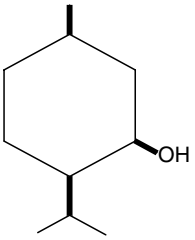
neoiso(iso)pulegol



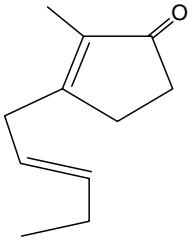
piperitenone



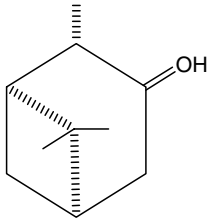
isomenthol



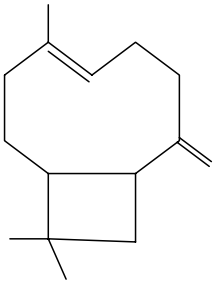
neoisomenthol



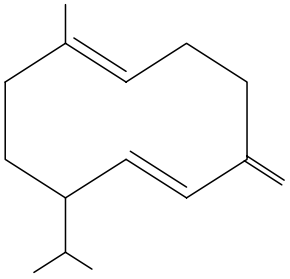
(Z)-jasmone



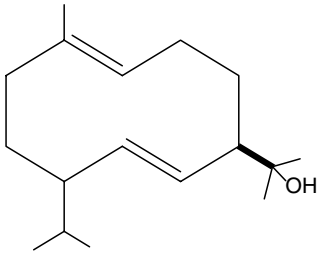
isopinocamphone



β-caryophyllene



germacrene D



elemol

8 Oil Composition of Other *Mentha* Species and Hybrids

Brian M. Lawrence

CONTENTS

8.1 INTRODUCTION

In the previous chapter, the composition of commercially important mints was discussed. However, as can be seen from Chapter 1, there are 18 species, numerous subspecies and varieties, and 11 hybrids and varieties known. As a result, this chapter is devoted to the composition of the *Mentha* species, subspecies, and varieties that are of little or no commercial value. It proceeds in the same order as that found in the first chapter.

8.1.1 *Mentha aquatica* l. var. *aquatica*

Examination of the previously published data on the oil of *M. aquatica* reveals that menthofuran has been found as a constituent of this oil in all studies. However, the amount of menthofuran has been found to vary considerably as have the other components with which it is associated. A selection of the oil compositions can be seen in Table 8.1.

8.1.2 *Mentha aquatica* l. var. *citrata* (Ehrh.) Fresen

The oil composition of *M. aquatica* var. *citrata* appears to be quite stable. All oils have been found to be rich in linalool and linalyl acetate, as shown in Table 8.1.

8.2 *Mentha arvensis* L.

A review of the published literature shows that a wide variation in the oil composition of *M. arvensis* has been found. For example, oils rich in acyclic nonterpenoid compounds, linalool, *trans*-sabinene hydrate, and terpinen-4-ol, α-terpinyl acetate, 3-substituted *p*-menthanes, β-pinene and *cis*- and *trans*-piperitone oxide, and β-caryophyllene. A selection of oil studies that have been reported on the chemotypes listed earlier can be seen in Table 8.1.

8.3 *Mentha australis* R. BR.

A survey of the literature reveals that no studies have been reported on the composition of *M. australis* oil.

8.4 *Mentha canadensis* L.

The oils of *M. canadensis* are chemically diverse; this has been proven as oils that are rich in pulegone and related 3-substitutes *p*-menthanes, *cis*- and *trans*-isopulegone, menthol and related 3-substituted *p*-menthanes, menthofuran, 1,2-epoxy-*p*-menthanes, carvone, 3- and 6-substituted *p*-menthanes, acyclic monoterpenes, 1,8-cineole and (Z)- and (E)-β-ocimene, and limonene have been found. A selection of oil studies that have been reported on the chemotypes listed earlier is shown in Table 8.1.

8.5 *Mentha cervina* L.

Examination of the published data on the oils of *M. cervina* reveals that some variation has been found to exist with the main constituents of these oils, as shown in Table 8.1.

8.6 *Mentha dahurica* FISCH. ex BENTH.

The oils of *M. dahurica* have not been the subject of much study thus far. Oils in which the main constituents have varied can be seen in Table 8.1.

TABLE 8.1
The Main Components Found in the Oils of *Mentha* Species and Hybrids

Components			References
8.1.1	*Mentha aquatica* var. *aquatica*		
	1a	Menthofuran (70.0–89.0%)	Sticher and Flück (1968)
	1b	Menthofuran (33.0%) Viridiflorol (20.6%)	Lawrence (1978)
	1c	Menthofuran (8.1%) 1,8-Cineole (23.5%)	Ibid.
	1d	Menthofuran (8.1%) Elemol (23.1%) Viridiflorol (16.1%)	Umemoto (1994)
	1e	Menthofuran (4.8%) β-Caryophyllene (17.4%) Germacrene D (20.9%) Viridiflorol (13.0%)	Ibid.
	1f	Menthofuran (11.4–32.8%) β-Caryophyllene (9.6–20.2%) Caryophyllene oxide (2.4–11.7%)	Baser et al. (1999)
	1g	Menthofuran (11.1–34.2%) β-Caryophyllene (5.9–10.2%) Elemol (11.6–24.4%)	Ibid.
	1h	Menthofuran (7.6–8.4%) β-Caryophyllene (15.0–19.1%) Caryophyllene oxide (6.1–14.5%) Viridiflorol (15.7–26.7%)	Ibid.
	1i	Menthofuran (28.8%) Terpinen-4-ol (12.8%)	Ibid.
8.1.2	*Mentha aquatica* var. *citrata*		
	1a	Linalool (55.2%) Linalyl acetate (34.0%)	Lawrence (1978)
	1b	Linalyl acetate (60.9%) Linalool (23.8%)	Malizia et al. (1996)
8.2	*Mentha arvensis*		
	1a	3-Octanol (54.0–80.0%)	Sacco and Shimizu (1965)
	1b	3-Octyl acetate (41.0%) 3-Octanol (35.0%)	Ibid.
	1c	3-Octanone (35.3%) Geranyl acetate (16.6%)	Malingré (1971)
	1d	3-Octanone (39.6–71.6%) (Z)-β-ocimene (3.5–10.8%)	Lawrence (1978)
	1e	3-Octanone (52.6%) 3-Octanol (12.7%)	Ibid.
	1f	3-Octanone (52.6%) *trans*-Sabinene hydrate (21.0%)	Ibid.
	2a	Linalool (55.1%)	Tucker et al. (1991)
	2b	Linalool (39.8–45.3%) 3-Octanone (22.9–27.5%)	Lawrence (1978)
	3	Geraniol (44.0%) Limonene (18.0%)	Malingré (1971)
	4	*trans*-Sabinene hydrate (44.5%) Terpinen-4-ol (16.5%)	Tucker et al. (1991)

continued

TABLE 8.1 (continued)
The Main Components Found in the Oils of *Mentha* Species and Hybrids

Components			References
	5	α-Terpinyl acetate (78.7%)	Ibid.
	6	Pulegone (54.6%)	Ibid.
	7	β-Pinene (25.4%) *trans*-Piperitone oxide (14.3%) *cis*-Piperitone oxide (9.9%)	Lawrence (1978)
	8	β-Caryophyllene (21.4%) 1,8-Cineole (19.1%)	Ibid.
8.4	*Mentha canadensis*		
	1a	Pulegone (81.5%)	von Rudloff and Hefendehl (1966)
	1b	Isomenthone (41.5–47.0%) Pulegone (24.6–39.1%) Menthone (8.0–21.0%)	Lawrence (1978)
	2a	*trans*-Isopulegone (33.8–60.3%) *cis*-Isopulegone (18.2–27.1%) Pulegone (1.5–13.7%)	Ibid.
	2b	*cis*-Isopulegone (41.4–78.0%) Pulegone (4.6–7.0%) *trans*-Isopulegone (0.7–2.6%)	Lawrence (1978)
	3a	Menthol (64.7–82.6%)	Ikeda et al. (1971)
	3b	Menthol (43.0–54.6%) Menthone (25.0–32.7%)	Ibid.
	3c	Menthone (71.5–76.8%) Menthol (27–9.6%)	Ibid.
	3d	Pulegone (32.8–49.4%) Menthone (30.6–43.3%) Menthol (3.0–16.0%)	Ibid.
	3e	Menthone (42.8–48.5%) Piperitone (20.2–29.7%)	Ibid.
	3f	Menthol (47.6%) Piperitone (23.1%) Neomenthol (11.4%)	Ibid.
	3g	Piperitone (30.9–50.2%) Menthone (23.5–38.4%)	Umemoto (1993)
	3h	Neomenthol (58.1%) Pulegone (15.0%) Menthone (13.9%)	Lawrence (1978)
	4a	Pulegone (16.6–40.6%) Menthofuran (13.7–28.6%) Menthone (10.6–22.6%)	Nagasawa et al. (1976a,b)
	4b	Menthofuran (38.1%) Menthol (18.9%)	Sacco et al. (1989)
	5a	*trans*-Piperitone oxide (28.5%) Piperitenone oxide (21.8%)	Umemoto and Tsuneya (1988)
	5b	Piperitenone oxide (32.1–43.3%) Linalool (1.1–11.3%)	Li et al. (1996)
	6	Carvone (63.1–68.2%)	Maffei (1988)
	7	Menthone (39.4%) Dihydrocarvone (4.9–20.7%)	Chou and Zhou (1993)

TABLE 8.1 (continued)
The Main Components Found in the Oils of *Mentha* Species and Hybrids

Components			References
	8a	Linalool (63.6–85.4%)	Lawrence (1978)
	8b	Linalool (34.8–67.1%)	Ibid.
		(E)-β-ocimene (9.2–19.9%)	
	9a	1,8-Cineole (24.8–47.1%)	Ibid.
		(Z)-β-ocimene (4.9–20.7%)	
	9b	(Z)-β-ocimene (31.1–38.0%)	Ibid.
		1,8-Cineole (20.1–28.0%)	
		(E)-β-ocimene (4.4–16.3%)	
	10	Limonene (41.7%)	Chou and Zhou (1993)
		β-Pinene (14.9%)	
8.5	*Mentha cervina*		
	1a	Pulegone (39.0%)	De Pascual Teresa et al. (1983)
		Piperitenone (34.0%)	
	1b	Isomenthone (41.5%)	Lawrence (1978)
		Piperitone (19.2%)	
		Piperitenone (18.6%)	
	1c	Pulegone (60.8%)	Velasco-Negueruela et al. (1987)
		Menthone (11.1%)	
	1d	Pulegone (31.7%)	Ibid.
		Menthyl acetate (19.2%)	
		Menthone (10.4%)	
8.6	*Mentha dahurica*		
	1	α-Terpineol (30.0%)	Lawrence (1978)
		Linalool (18.7%)	
	2	Piperitone (69.9%)	Chou and Zhou (1993)
8.7	*Mentha diemenica*		
		Menthone (32.2–32.4%)	Brophy et al. (1996)
		Pulegone (24.9–43.6%)	
		Neomenthyl acetate (0–18.3%)	
8.8	*Mentha gattefossei*		
		Pulegone (68.4%)	Fujita and Fujita (1967)
		Menthone (10.4%)	
8.9	*Mentha grandiflora*		
		Piperitenone oxide (36.2%)	Brophy et al. (1997)
		trans-Piperitone oxide (21.4%)	
		Pulegone (19.1%)	
8.10	*Mentha japonica*		
	1a	Menthone (50.8%)	Fujita and Fujita (1970)
		Isomenthone (18.6%)	
		Pulegone (12.6%)	
	1b	Pulegone (50.0%)	Lawrence (1978)
		Menthone (27.9%)	
	2	Menthol (50.3%)	Fujita et al. (1977)
		Pulegone (22.0%)	
		Menthone (11.8%)	
8.12.1	*Mentha longifolia ssp. longifolia*		
	1a	*cis*-Piperitone oxide (4.0–13.6%)	Lawrence (1978)
		trans-Piperitone oxide (50.8–70.8%)	

continued

TABLE 8.1 (continued)
The Main Components Found in the Oils of *Mentha* Species and Hybrids

Components			References
	1b	*cis*-Piperitone oxide (20.7%) *trans*-Piperitone oxide (14.3%) Piperitenone oxide (11.1%)	Ibid.
	1c	Piperitenone oxide (77.4%)	Maffei (1988)
	1d	*cis*-Piperitone oxide (15.4%) Piperitone (13.9%) Piperitenone (13.8%)	Fleisher and Fleisher (1991)
	1e	*trans*-Piperitone oxide (30.9%) 1,8-Cineole (17.8%)	Gavalas et al. (1998)
	1f	*cis*-Piperitone oxide (72.9%)	Baser et al. (1999)
	2a	Carvone (41.0–77.0%)	Lawrence (1978)
	2b	Carvone (42.2–55.3%) *cis*-Dihydrocarvone (11.0–13.4%)	Ibid.
	2c	Dihydrocarveol (37.8%) Dihydrocarvone (32.0%)	Umemoto and Nagasawa (1980)
	2d	Carvone (56.2–66.3%) *trans*-Dihydrocarvone (0.9–32.6%)	Kokkini et al. (1995)
	2e	Carvone (21.5%) β-Caryophyllene (22.0%)	Baser et al. (1999)
	2f	*trans*-Dihydrocarvone (30.6%) *cis*-Dihydrocarvone (15.9%)	Matovic and Lavadinovic (1999)
	3a	Piperitone (51.2%) Piperitenone (33.8%)	Calvarano and Codignola (1976)
	3b	Menthone (37.7–66.5%) Isomenthone (10.6–13.7%)	Kapelev and Akimov (1980)
	3c	Menthone (26.5–45.6%) Isomenthone (6.8–10.4%) Pulegone (19.1–11.0%)	Ibid.
	3d	Pulegone (34.8–52.4%) Menthone (12.1–25.8%)	Ibid.
	3e	Menthone (41.4–47.2%) Piperitone (35.7–35.9%)	Ibid.
	3f	Piperitone (57.8–76.1%)	Ibid.
	3g	Menthone (43.5–75.5%) Pulegone (4.0–20.7%)	Vidal et al. (1985)
	3h	Isomenthone (42.3%) Menthone (11.9%)	Mimica-Dukic et al. (1991)
	3i	Isomenthone (78.1%)	Baser et al. (1999)
	4a	Menthyl acetate (16.4%) Menthol (10.l4%)	Chopra et al. (1964)
	4b	Menthone (41.7–66.5%) Menthol (9.2–18.1%)	Kapelev and Akimov (1980)
	4c	Menthone (22.3%) Isomenthone (25.9%) Menthol (13.7%)	Ibid.
	4d	Piperitone (34.7%) Menthol (19.8%)	Ibid.

TABLE 8.1 (continued)
The Main Components Found in the Oils of *Mentha* Species and Hybrids

Components			References
	4e	Piperitone (31.2–31.6%)	Ibid.
		Menthone (12.1–26.6%)	
		Menthol (11.8–12.1%)	
	4f	Menthol (24.7–46.5%)	Ibid.
		Menthone (11.6–25.7%)	
	4g	Menthol (31.9%)	Fadel and Aesia (1994)
		Piperitone oxide (13.8%)	
	4h	Piperitone (28.3%)	Dolya et al. (1999)
		Menthone (16.2%)	
		Menthol (15.5%)	
		α-Terpineol (10.2%)	
	5a	Linalool (90%)	Shimizu and Ikeda (1962)
	5b	Linalool (57.7–62.2%)	Baser et al. (1999)
		Linalyl acetate (16.1–23.7%)	
	5c	Linalool (27.8–50.3%)	Ibid.
		1,8-Cineole (15.8–19.9%)	
	6a	*trans*-Dihydrocarvone (18.3%)	Mimica-Dukic et al. (1991)
		Isomenthone (11.5%)	
	6b	*cis*-Piperitol (44.4–52.8%)	Baser et al. (1999)
		cis-Dihydrocarvone (16.7–19.1%)	
	7a	β-Caryophyllene (26.3%)	Lawrence (1978)
		Germacrene D (17.7%)	
	7b	3-Octanol (41.1%)	Ibid.
	7c	Piperitol (44.8–45.0%)	Kapil and Sinha (1979)
		Geranyl acetate (16.5–20.5%)	
	7d	1,8-Cineole (26.4–34.5%)	Fleisher and Fleisher (1998)
		Menthone (10.0–16.7%)	
	7e	Isomenthone (17.4%)	Baser et al. (1999)
		Linalool (12.2%)	
	7f	α-Terpinyl acetate (42.1%)	Ibid.
	7g	Terpinen-4-ol (39.8%)	Ibid.
		trans-Sabinene hydrate (14.8%)	
8.12.2	*Mentha longifolia* ssp. *typhoides*		
	1a	Piperitenone (40.6%)	Lawrence (1978)
		trans-Piperitone oxide (25.4%)	
		Piperitenone (17.3%)	
	1b	*trans*-Piperitone oxide (79.6%)	Ibid.
	1c	*trans*-Piperitone oxide (61.7%)	Baser et al. (1999)
		Piperitenone oxide (22.8%)	
	1d	*trans*-Piperitone oxide (58.8%)	Ibid.
		cis-Piperitone oxide (16.0%)	
	1e	*cis*-Piperitone oxide (62.0%)	Ibid.
	1f	Menthone (30.5%)	Ibid.
		trans-Piperitone oxide (23.6%)	
	1g	Caryophyllene oxide (11.1–28.5%)	Ibid.
		trans-Piperitone oxide (12.9–13.2%)	
		β-Caryophyllene (11.6–12.4%)	

continued

TABLE 8.1 (continued)
The Main Components Found in the Oils of *Mentha* Species and Hybrids

Components			References
	2a	Piperitone (62.3%)	Özgüven et al. (1995)
	2b	Pulegone (42.1%)	Ibid.
	3a	*trans*-Sabinene hydrate (71.0%)	Baser et al. (1999)
	3b	*trans*-Sabinene hydrate (25.6%) Carvone (25.0%)	Ibid.
	3c	*trans*-Sabinene hydrate (51.2%) Terpinen-4-ol (14.8%)	Ibid.
	4a	Linalool (86.7%)	Ibid.
	4b	Linalool (63.0–79.4%) Linalyl acetate (0–18.5%)	Ibid.
	4c	Linalool (27.1%) 1,8-Cineole (18.1%)	Ibid.
	5a	1,8-Cineole (58.3%)	Özgüven et al. (1995)
	5b	1,8-Cineole (47.6%) Pulegone (25.8%)	Ibid.
	6	Carvone (39.4–58.3%)	Baser et al. (1999)
8.12.3	*Mentha longifolia* ssp. *caucasica*		
	1	Linalool (60.0–90.0%)	Bogonina et al. (1964)
	2	*trans*-Sabinene hydrate + Terpinen-4-ol (58.0–71.0%)	Chobanu (1977)
8.12.4	*Mentha longifolia* ssp. *noèana*		
	1	Carvone (39.8–45.9%) Dihydrocarvone (11.0–17.7%)	Kokkini (1983)
	2	*trans*-Piperitone oxide (57.3–85.9%) 1,8-Cineole (2.4–13.4%)	Karousou et al. (1998)
8.12.5	*Mentha longifolia* ssp. *royleana*		
	1	*trans*-Piperitone oxide (55.6%) Piperitenone oxide (21.7%)	Lawrence (1978)
8.12.6	*Mentha longifolia* ssp. *hymalaiensis*		
	1a	Piperitenone oxide (58.9%) *cis*-Piperitone oxide (25.7%)	Mathela et al. (1989)
	1b	*cis*-Piperitone oxide (23.2%) *trans*-Piperitone oxide (21.1%) Piperitenone oxide (18.6%)	Kharkwal et al. (1994)
8.12.7	*Mentha longifolia* ssp. *schimperi*		
	1	Piperitone (42.7%) 1,2-Epoxyneomenthyl acetate (15.4%) *cis*-Piperitone oxide (13.9%) Piperitenone oxide (13.3%)	Lawrence (1978)
	2	Piperitenone (21.6%) 1,8-Cineole (11.6%)	Soliman et al. (1997)
8.12.8	*Mentha longifolia* ssp. *wissii*		
	1	*cis*-Piperitone oxide (29.7%) *trans*-Piperitone oxide (15.8%) 1,8-Cineole (11.7%) Piperitone (10.4%)	Lawrence (1978)

TABLE 8.1 (continued)
The Main Components Found in the Oils of *Mentha* Species and Hybrids

Components			References
8.12.9	*Mentha longifolia* var. *asiatica*		
	2a	Carvone (65.7%)	Lawrence (1978)
	2b	Piperitone (55.7–67.6%)	Jaimand and Rezaee (2002)
		Carvone (0–16.2%)	
		1,8-Cineole (11.4–11.6%)	
	3a	Piperitenone oxide (52.2%)	Sharipova et al. (1983)
	3b	*trans*-Piperitone oxide (64.5%)	Baser et al. (1997)
		Piperitenone oxide (12.4%)	
	4	Rosefuran oxide (63.2%)	Karasawa et al. (1995)
		Rosefuran (11.6%)	
8.13	*Mentha pulegium* var. *pulegium*		
	1a	Pulegone (50.5%)	Fujita and Fujita (1967)
		Menthone (26.4%)	
	1b	Pulegone (52.0–63.5%)	Sticher and Flück (1968)
		Isomenthone (18.9–42.1%)	
		Neomenthol (2.8–19.4%)	
	1c	Pulegone (90.7%)	Kokkini et al. (2002)
	1d	Pulegone (31.2–44.0%)	Lawrence (1978)
		Neoisomenthol (10.7–14.8%)	
		Neoisomenthyl acetate (6.4–15.3%)	
		Menthone (0.6–15.8%)	
	1e	Menthone (25.1–59.1%)	Baser et al. (1999)
		Pulegone (26.6–57.3%)	
	1f	Isomenthone (41.7–52.0%)	Ibid.
		Pulegone (32.1–43.8%)	
	1g	Menthone (36.4%)	Ibid.
		Pulegone (25.2%)	
		Neomenthol (19.0%)	
	2	Piperitone (83.7–97.2%)	Kokkini et al. (2002)
8.15	*Mentha requienii*		
		Pulegone (79.2%)	Mucciarelli and Sacco (1999)
		Menthone (13.7%)	
8.16	*Mentha satureoides*		
		Pulegone (40.0%)	Jones and Berry-Smith (1926)
		Menthol (12.0%)	
8.17.1	*Mentha spicata* ssp. *spicata*		
	1a	Carvone (67.3–80.0%)	Sticher and Flück (1968)
	1b	Carvone (40.1%)	Nagasawa et al. (1976a,b)
		Neodihydrocarveol (16.5%)	
		Dihydrocarvyl acetate (10.8%)	
		Dihydrocarveol (10.1%)	
	1c	*cis*-Dihydrocarvone (21.6%)	Lawrence (1978)
		trans-Dihydrocarvone (21.2%)	
	1d	Dihydrocarvyl acetate (24.8%)	Ibid.
		Neoisodihydrocarvyl acetate (20.9%)	
		Carvone (20.4%)	
	1e	Carvone (35.2%)	Kokkini (1983)
		Dihydrocarvone (21.5%)	
		Dihydrocarvyl acetate (12.3%)	

continued

TABLE 8.1 (continued)
The Main Components Found in the Oils of *Mentha* Species and Hybrids

Components			References
	1f	Carvone (68.0%) 1,8-Cineole (16.0%)	Koyalta et al. (1993)
	1g	Dihydrocarveol (34.8%) Carvone (33.4%) *cis*-Dihydrocarvone (11.4%)	Sivropulou et al. (1995)
	1h	Dihydrocarveol (37.8%) *cis*-Dihydrocarvone (32.0%)	Umemoto and Nagasawa (1980)
	1i	Carvone (22.4%) Linalool (11.3%)	Hadjiakhoondi et al. (2000)
	2a	Menthone (43.8–57.1%) Pulegone (0.1–33.2%) Piperitone (0.5–10.5%)	Murray et al. (1972)
	2b	Menthone (57.8%) Isomenthone (18.2%)	Clark (1994)
	2c	Isomenthone (42.6%) Menthone (33.1%)	Lawrence (1978)
	2d	Isomenthone (41.3%) Neoisomenthyl acetate (10.7%) Neoisomenthol (10.3%)	Ibid.
	2e	Menthone (51.8%) Piperitone (10.7%)	Tucker et al. (1991)
	2f	Piperitone (40.8–48.2%) Piperitenone (16.2–32.4%)	Lawrence (1978)
	3a	*trans*-Piperitone oxide (60.8%)	Ibid.
	3b	Piperitenone oxide (72.4%)	Ibid.
	3c	*trans*-Piperitone oxide (42.6%) 1,2-Epoxyneomenthyl acetate (15.3%)	Ibid.
	3d	*trans*-Piperitone oxide (70.8%) Piperitenone (18.4%)	Kokkini et al. (1997)
	3e	*trans*-Piperitone oxide (40.2–42.8%) *cis*-Piperitone oxide (23.6–31.8%)	Ibid.
	4	Linalool (75.6–86.3%)	Gora and Kalemba (1979)
	5a	*trans*-Sabinene hydrate (85.3%)	Karasawa and Shimizu (1978)
	5b	*trans*-Sabinene hydrate (45.2%) Terpinen-4-ol (16.6%)	Kuwahara et al. (1979)
	6a	Linalool (30.0%) Carvone (20.0%)	Olszewski and Pluta (1974)
	6b	1,8-Cineole (26.3%) Carvacrol (17.8%)	Stoeva and Iliev (1997)
	6c	Carvone (36.0%) Menthone (21.0%)	Lawrence (1978)
	6d	1,8-Cineole (18.8%) Linalool (17.4%) Carvone (41.0%)	Baser et al. (1999)
	6e	*trans*-Sabinene hydrate (21.9%) Carvone (18.6%) Terpinen-4-ol (17.6%)	Ibid.

TABLE 8.1 (continued)
The Main Components Found in the Oils of *Mentha* Species and Hybrids

Components			References
8.17.2	*Mentha spicata* var. *crispata*		
	1	Carvone (45.6–68.4%)	Lawrence (1978)
	2	Piperitenone oxide (27.7%)	Ibid.
		cis-Piperitone oxide (17.7%)	
8.17.3	*Mentha spicata* ssp. *condensata*		
	1	Carvone (57.7%)	Lawrence (1978)
	2	Piperitenone oxide (68.0%)	Kokkini and Papageorgiou (1982)
8.18.1	*Mentha suaveolens* ssp. *suaveolens*		
	1a	Piperitenone oxide (87.3%)	Shimizu et al. (1960)
	1b	*trans*-Piperitone oxide (45.8%)	Lawrence (1978)
		Piperitenone oxide (28.7%)	
	1c	*trans*-Piperitone oxide (50.8%)	Ibid.
		1,2-Epoxyneomenthyl acetate (26.4%)	
		Piperitenone oxide (11.6%)	
	1d	1,2-Epoxyneomenthyl acetate (66.5%)	Ibid.
		trans-Piperitone oxide (15.0%)	
	1e	Piperitenone oxide (23.0%)	Umemoto (1996)
		trans-Piperitone oxide (22.3%)	
	1f	Piperitenone oxide (27.2%)	Ibid.
		cis-Piperitone oxide (15.3%)	
		trans-Piperitone oxide (13.8%)	
	1g	1,2-Epoxyneoisomenthyl acetate (27.8%)	Umemoto (1997)
		trans-Piperitone oxide (25.6%)	
		1,2-Epoxyisomenthyl acetate (10.2%)	
	1h	1,2-Epoxyneomenthyl acetate (22.5%)	Umemoto (1998)
		trans-Piperitone oxide (17.7%)	
		1,2-Epoxymenthyl acetate (10.1%)	
	2a	*cis*-Dihydrocarvone (43.2%)	Hendriks (1974)
		Neodihydrocarvyl acetate (17.0%)	
		Neodihydrocarveol (16.0%)	
	2b	*cis*-Dihydrocarvone (38.4%)	Lawrence (1978)
		trans-Dihydrocarvone (10.4%)	
	2c	Carvone (48.5%)	Ibid.
		cis-Carvyl acetate (22.5%)	
	2d	Carvone (62.3%)	Galambosi et al. (1998)
	3a	Menthol (48.3%)	Velasco-Negueruela et al. (1996)
		Pulegone (20.3%)	
	3b	Pulegone (50.0%)	Oumzil et al. (2002)
	4	Neoiso(iso)pulegol (52.3%)	Handa et al. (1964)
		Isopiperitenone (13.4%)	
	5a	Carvone (43.0%)	De laTorre and Torres (1977)
		1,8-Cineole (30.1%)	
	5b	*cis*-Piperitol (57.6%)	Perez Raya et al. (1990)
		Piperitenone oxide (10.4%)	
	5c	*trans*-Piperitone oxide (40.5%)	Avato et al. (1995)
		Hydroxy-*p*-menth-3-one (23.9%)	
		Piperitenone oxide (12.7%)	
	5d	*p*-Mentha-2,4,8(6)-triene-2,3-diol (14.5%)	Pino et al. (1999)
		Germacrene D (12.4%)	

continued

TABLE 8.1 (continued)
The Main Components Found in the Oils of *Mentha* Species and Hybrids

Components			References
8.18.2	*Mentha suaveolens* ssp. *insularis*		
	1	Pulegone (35.5%)	Lawrence (1978)
		Piperitone (13.7%)	
		Piperitenone (10.0%)	
	2	Carvone (62.0%)	Marongiu et al. (2001)
8.18.3	*Mentha suaveolens* ssp. *timija*		
	1	Pulegone (85–88%)	Sfiras (1952)
	2	*trans*-Piperitone oxide (41.5–49.1%)	Lawrence (1978)
		cis-Piperitone oxide (12.0–14.1%)	
		Piperitenone oxide (3.9–13.5%)	
8.20	*Mentha dalmatica*		
	1	Piperitone oxide (19.6%)	Tucker (1975)
	2	Carvone (58.0–70.1%)	Lawrence (1978)
8.21	*Mentha dumetorum*		
	1a	Menthofuran (25.1%)	Murray and Lincoln (1972)
		Pulegone (17.3%)	
		Menthol (11.1%)	
	1b	Menthyl acetate (33.3%)	Lawrence (1978)
		Menthofuran (27.3%)	
		Pulegone (10.4%)	
	2a	Piperitenone oxide (20.76%)	Ibid.
		cis-Piperitone oxide (12.9%)	
	2b	Pulegone (19.0%)	Ibid.
		β-Caryophyllene (11.8%)	
		cis-Piperitone oxide (10.7%)	
	2c	*trans*-Piperitone oxide (43.6%)	Baser et al. (1999)
		cis-Piperitone oxide (26.0%)	
	3	Carvone (51.0%)	Lawrence (1978)
	4	*trans*-Sabinene hydrate (29.4%)	Baser et al. (1999)
		Menthone (13.5%)	
		Isomenthone (12.5%)	
8.22	*Mentha gracilis*		
	1	3-Octanol (38.0%)	Lawrence (1978)
		3-Octanone (18.3%)	
	2	Pulegone (74.0–78.1%)	Nagasawa et al. (1975a,b)
	3a	Menthone (70.0–75.0%)	Ikeda et al. (1963)
	3b	Pulegone (0.3–58.7%)	Nagasawa et al. (1975a,b)
		Menthone (18.4–35.4%)	
		Neomenthol (1.5–31.4%)	
	3c	Neomenthol (9.8–44.6%)	Umemoto and Nagasawa (1978)
		Pulegone (1.8–42.6%)	
		Menthone (28.1–42.4%)	
	4a	Menthol (67.0–68.0%)	Ikeda et al. (1963)
	4b	Pulegone (30.0%)	Nagasawa and Umemoto (1976)
		Menthone (17.3%)	
		Piperitenone (14.5%)	
		Piperitone (13.2%)	

TABLE 8.1 (continued)
The Main Components Found in the Oils of *Mentha* Species and Hybrids

Components			References
	4c	Pulegone (24.0%) Menthol (23.0%) Menthyl acetate (12.5%)	Fujita et al. (1978)
	5a	Pulegone (21.5%) Piperitone oxide (17.9%) Piperitenone oxide (16.1%) 1,2-Epoxymenthyl acetate (10.6%)	Nagasawa et al. (1974)
	5b	Piperitenone oxide (31.2%) Pulegone (28.0%) *trans*-Piperitone oxide (13.1%)	Lawrence (1978)
	5c	Piperitenone oxide (35.7%) *trans*-Piperitone oxide (12.5%)	Tucker et al. (1991)
	6a	Carvone (46.4–78.4%)	Ibid.
	6b	Carvone (35.5%) 3-Octanol (22.0%)	Galambosi et al. (1998)
	6c	Carvone (23.3–46.4%) Linalool (37.2–39.9%)	Tucker et al. (1991)
	7a	Linalool (43.3–63.0%)	Lawrence (1978)
	7b	Linalool (48.0%) 3-dodecanone (13.0%) 3-Octanol (10.3%)	Ibid.
	7c	Linalool (57.0%) Menthyl acetate (10.0%)	Sacco and Nano (1968)
	8a	Carvone (20.4%) Dihydrocarvyl acetate (19.5%) Dihydrocarveol (14.5%) Menthyl acetate (13.8%)	Lawrence (1978)
	8b	Carvone (39.5%) Menthol (21.5%)	Tucker et al. (1991)
8.23	*Mentha maximilianea*		
	1a	Menthofuran (37.4%) β-Caryophyllene (11.7%)	Lawrence (1978)
	1b	Menthol (18.5%) 1,8-Cineole (10.2%)	Ibid.
	1c	Menthyl acetate (39.5%)	Ibid.
8.24	*Mentha piperita*		
	1a	Menthol (28.0–35.6%) Menthyl acetate (10.6–20.1%)	Lawrence (1978)
	1b	Pulegone (51.0%)	Ibid.
	1c	Pulegone (30.5%) Menthone (12.1%)	Kokkini (1983)
	1d	Menthofuran (23.5%)	Ibid.
	1e	Menthone (42.3%) Neomenthol (10.4%) 1,8-Cineole (10.2%)	Lawrence (1993)
	1f	Menthone (38.3%) Menthol (18.3%)	Özgüven et al. (1995)
	2	Carvone (61.0–68.0%) 1,8-Cineole (6.2–21.5%)	Ibid.

continued

TABLE 8.1 (continued)
The Main Components Found in the Oils of *Mentha* Species and Hybrids

Components			References
8.25	*Mentha rotundifolia*		
	1a	*cis*-Dihydrocarvone (68.9%)	Lawrence (1978)
		trans-Dihydrocarvone (12.2%)	
	1b	Carvone (60.0%)	Ibid.
		cis-Dihydrocarvone (10.8%)	
	2	Menthyl acetate (51.4%)	Kokkini (1983)
	3a	Piperitone oxide (74.7%)	Van Os and Hendriks (1975)
	3b	Piperitenone oxide (74.2–77.4%)	Kokkini (1983)
8.26	*Mentha smithiana*		
	1a	Carvone (22.1%–27.4%)	Lawrence (1978)
	1b	Carvone (38.4%)	Ibid.
		Dihydrocarvyl acetate (16.8%)	
	2	β-Pinene (17.1%)	Ibid.
		1,8-Cineole (10.9%)	
		β-Caryophyllene (10.4%)	
8.27	*Mentha verticillata*		
	1	3-Octanol (37.8%)	Lawrence (1978)
		3-Octanone (13.1%)	
	2a	Menthofuran (27.7%)	Ibid.
		1,8-Cineole (12.5%)	
	2b	Menthofuran (16.9%)	Ibid.
		Geranyl acetate (14.1%)	
		β-Caryophyllene (10.9%)	
	3	1,8-Cineole (21.8%)	Ibid.
		β-Caryophyllene (20.3%)	
	4	Terpinen-4-ol (19.7%)	Ibid.
		trans-Sabinene hydrate (11.4%)	
	5	α-Terpinyl acetate (74.6%)	Ibid.
	6	Decyl acetate (20.6%)	Ibid.
		trans-Piperitone oxide (12.2%)	
	7	Menthol (53.3%)	Maffei (1990)
		Menthyl acetate (17.4%)	
8.28.1	*Mentha villosa* var. *villosa*		
	1a	Carvone (68.6%)	Lawrence (1978)
	1b	Carvone (44.2%)	Ibid.
		Dihydrocarvyl acetate (18.0%)	
		cis-Dihydrocarvone (14.1%)	
	2a	Piperitone oxide (65.8%)	Kokkini (1983)
	2b	Piperitenone oxide (61.2%)	Ibid.
8.28.2	*Mentha villosa* var. *alopecuroides*		
	1a	Carvone (65.4–74.7%)	De Pooter and Schamp (1987)
	1b	Carvone (40.5%)	Lawrence (1978)
		cis-Dihydrocarvone (13.3%)	
8.29	*Mentha villoso-nervata*		
	1a	*trans*-Piperitone oxide (74.9–76.1%)	Karousou et al. (1998)
	1b	Piperitenone oxide (65.9%)	Kokkini (1983)
	1c	Germacrene D (21.4%)	Gavalas et al. (1998)
		Piperitenone oxide (19.8%)	
	2a	Carvone (69.6–80.1%)	Kokkini et al. (1995)

TABLE 8.1 (continued)
The Main Components Found in the Oils of *Mentha* Species and Hybrids

Components			References
	2b	Carvone (42.8%) Dihydrocarvone (15.9%)	Kokkini (1983)
	2c	*cis*-Dihydrocarvone (35.8%) *trans*-Dihydrocarvone (24.5%)	Baser et al. (1999)
	3	*trans*-Piperitone oxide (28.2%) *cis*-Dihyrocarvone (17.1%)	Ibid.
8.30	*Mentha longifolia var. asiatica*		
	2a	Carvone (65.7%)	Lawerence (1978)
	2b	Piperitone (55.7–67.6%) Carvone (0–16.2%) 1.8-Cineole (11.4–11.6%)	Jaimand & Rezaee, (2002)
	3a	Piperitenone oxide (52.2%)	Sharipova et al. (1983)
	3b	*trans*-Piperitone oxide (64.5%) Piperitenone oxide (12.4%)	Baser et al., (1999)
	4	Rosefuran oxide (63.2%) Rosefuran (11.6%)	Karasawa et al., (1995)
8.31	*Mentha ×piperita 'lavanduliodora'*		
	1	Linalool (49.7%) Linalyl acetate (23.9%)	Maffei et al. (1986)

8.7 *Mentha diemenica* SPRENG.

The oils of *M. diemenica* have been found to be rich in 3-substituted *p*-menthanes, as can be seen in Table 8.1.

8.8 *Mentha gattefossei* MAIRE

The oils of *M. gattefossei* appear to be stable in composition with oils rich in 3-substituted *p*-menthanes as shown in Table 8.1.

8.9 *Mentha grandiflora* BENTH.

To date only a single analysis of an oil of *M. grandiflora* has been reported, as can be seen in Table 8.1.

8.10 *Mentha japonica* (MIQ.) MAKINO

A review of the literature reveals that oils of *M. japonica* have always been found to be rich in 3-substituted *p*-menthanes, as shown in Table 8.1.

8.11 *Mentha laxiflora* BENTH.

No analyses on *M. laxiflora* oils have been reported in the literature thus far.

8.12 *Mentha longifolia* (L.) L. ssp. *longifolia*

Examination of the published literature on the oil composition of *M. longifolia* ssp. *longifolia* reveals that it can exist in a myriad of chemical forms, as can be seen from the main constituents found in these oils listed in Table 8.1. In addition, a summary of the various chemical types of the other *M. longifolia* subspecies analyzed thus far can also be seen in Table 8.1. Oils of the other *M. longifolia* subspecies not listed in the table have not been reported in the literature to date.

8.13 *Mentha pulegium* L.

The oils of *M. pulegium* have been found to be less chemically diverse than some other more commonly encountered *Mentha* species, although oils that are rich in pulegone and related 3-substituted *p*-menthanes and piperitone have been found as shown in Table 8.1.

8.14 *Mentha repens* (HOOK. f.) BRIQ.

The oil of *M. repens* has not been the subject of previous study thus far.

8.15 *Mentha requienii* BENTH.

A review of the literature reveals that the oils of *M. requienii* have all been found to be rich in 3-substituted *p*-menthanes, as can be seen in Table 8.1.

8.16 *Mentha satureoides* R. BR.

To date, only a single analysis has been reported for the oil of *M. satureoides*, as shown in Table 8.1.

8.17 *Mentha spicata* L.

Mentha spicata, which is found growing throughout the world, is one of the most polymorphic species found in the plant kingdom (Shimizu and Ikeda, 1962). In addition to existing in four cytotypic forms, a survey of the literature reveals that numerous chemical forms of this species have been found. A summary of the variations in oil composition of one variety and two subspecies of *M. spicata* from selected studies can be seen in Table 8.1.

8.18 *Mentha suaveolens* EHRH.

A survey of the published literature reveals that oils rich in 1,2-epoxy-*p*-menthanes, 6-substituted *p*-menthanes, 3-substituted *p*-menthanes, neoiso(iso)pulegol, and some miscellaneous compounds have been found. A selection of oil compositions of *M. suaveolens* ssp. *suaveolens* that demonstrate the variation encountered can be seen in Table 8.1. In addition to *M. suaveolens* ssp. *suaveolens*, the composition of other subspecies that have been the subject of analysis can also be found in the same table.

8.19 *Mentha* × *carinthiaca* HOST

A review of the literature reveals that the oil of *M. carinthiaca* has not been the subject of previous study.

8.20 *Mentha* × *dalmatica* TAUSCH

A limited number of studies have been performed on the oil of *M. dalmatica*. The oils have been found to possess some differences in oil composition, as can be seen from those listed in Table 8.1.

8.21 *Mentha* × *dumetorum* SCHULTES

A review of the literature reveals that the oil of *M. dumetorum* has been the subject of limited study. Nevertheless, a number of chemical forms have been found as can be seen in Table 8.1.

8.22 *Mentha* × *gracilis* SOLE

The oils of *M. gracilis* have been studied extensively. As a result, oils rich in acylic non-terpenoid compounds, 3-substituted *p*-menthanes, 1,2-epoxy-*p*-menthanes, 6-substituted *p*-menthanes, linalool, and some mixed constituents have been found, as shown in Table 8.1.

8.23 *Mentha* × *maximilianea* F.W. SCHULTZ

Only one study has been conducted on oils of *M. maximilianea* as shown in Table 8.1.

8.24 *Mentha* × *piperita* L.

Although *M. piperita* is the source of commercial peppermint oil, a few studies have been performed on oils produced from plants collected from their natural environment. A selection of these studies can be seen in Table 8.1.

8.25 *Mentha* × *rotundifolia* (L.) HUDS.

There has been considerable confusion in the literature with respect to the taxonomic origin of oils noted as originating from *M. rotundifolia*. It would appear that most of the early published literature on the oil composition of *M. rotundifolia* was actually misidentified and should have been reported as *M. suaveolens*. As a result, only oils whose taxonomic origin was irrevocably authenticated are included in this review. A selection of the studies reported can be seen in Table 8.1.

8.26 *Mentha* × *smithiana* R. GRAHAM

The oils of *M. smithiana* have only been the subject of a single analysis, although some compositional differences were characterized as shown in Table 8.1.

8.27 *Mentha* × *verticillata* L.

Although oils of *M. verticillata* have not been analyzed by many groups, it can be seen in Table 8.1 that from the results of these limited studies, a wide range in oil composition has been found.

8.27.1 *Mentha* × *villosa* Huds. var. *villosa*

Only two studies on the composition of oils of *M. villosa* var. *villosa* have been reported revealing that oils rich in 6-substituted *p*-menthanes and 1,2-epoxy-*p*-menthanes have been found, as shown in Table 8.1.

8.27.2 *Mentha* × *villosa* var. *alopecuroides* (Hull) Briq.

The limited studies on oils of *M. villosa* var. *alopecuroides* reveal that they are found to be rich in 6-substituted *p*-menthanes only, as can be seen in Table 8.1.

8.28 *Mentha* × *villoso-nervata* Opiz.

Although there have been limited studies published on the oils of *M. villoso-nervata*, it can be seen in Table 8.1 that oils rich in 1,2-epoxy-*p*-menthanes, 6-substituted *p*-menthanes, and mixed constituents have been found.

Mentha ×*piperita* "lavanduliodora" is not included in the earlier list of species and hybrids, although the composition of its oils has been the subject of previous study. (see Table 8.1)

REFERENCES

Avato, P., Sgarra, G. and Casadoro, G. (1995). Chemical composition of the essential oils of *Mentha* species cultivated in Italy. *Sci. Pharm*., 63, 223–230.

Baser, K.H.C., Nuriddinov, Kh.R., Nigmatullaev, A.M. and Aripov, Kh.N. (1997). Essential oil of *Mentha asiatica* Boriss. from Uzbekistan. *J. Essent. Oil Res*., 9, 453–454.

Baser, K.H.C., Kürkcüoglu, M., Tarimicilar, G. and Kaynak, G. (1999). Essential oils of *Mentha* species from northern Turkey. *J. Essent. Oil Res*., 11, 579–588.

Bogonina, Z.S., Gogol, O.N. and Koubrak, M.N. (1964). La Menthe de la flore del L'URSS comme materiel de depart pour I-obtention de sources hautement productives de linalool. In: *Compete rende du Troisiemme Congrés International des huiles essentielles*, May, Plodiv, Bulgaria, pp. 183–185.

Brophy, J.J., Goldsack, R.J., Lawrence, B.M. and Forster, P.I. (1996). Essential oil of *Mentha diemenica* (Lamiaceae). *J. Essent. Oil Res*., 9, 179–181.

Brophy, J.J., Goldsack, R.J., Forster, P.I. and Fookes, C.J.R. (1997). Leaf essential oil of *Mentha grandiflora* Benth. (Lamiaceae). *J. Essent. Oil Res*., 9, 459–461.

Calvarano, I. and Codignola, A. (1976). Chemical research on *Mentha longifolia* var. *mollissima* gathered from Stara Di Demonte Valley in the Caneo region. *Essenze Deriv. Agrum*., 44, 236–240.

Chobanu, V.I. (1977). Variability of the composition of terpenoids from *Mentha longifolia* L. ssp. *caucasica* Briq. in ontogenesis. *Aktual. Vopr. Fiziol. Biokhim. Rast. Mold., Mater. Resp. Konf. Fiziol. Biokhim* 2nd edn. Moraru, K., Ed., pp. 269–271.

Chopra, M.M., Vashist, V.N. and Handa, K.L. (1964). The essential oils of the species of genus *Mentha*. *Perfum. Essent. Oil Rec*., 55, 323–329.

Chou, G.-X. and Zhou, R.-H. (1993). The chemical components of essential oil in *Mentha dahurica* Fisch. ex. Benth. *Zhiwa Ziyan Yu Huajing*, 2, 55–57.

Clark, G.S. (1994). An aromatic profile. Menthone. *Perfum. Flavor*., 19, 41–45.

De la Torre, C.P. and Torres, O.A. (1977). Essential oil of *Mentha rotundifolia. Arch. Bioquim. Quim. Farm*., 20, 85–88.

De Pascual Teresa, J., Hernández, A., Calballero, M.C. and González, M.S. (1983). The essential oil of *Mentha cervina*. Proceedings of 9th International Congress of Essential Oils, March, 1983, Singapore. *Tech. Pap. Book* 3, 194–197.

De Pooter, H.L. and Schamp, N.M. (1987). The essential oil of *Mentha* × *villosa nm. alopecuroides. Flav. Fragr. J*., 2, 163–165.

Dolya, V.S., Mozul. V.I., Prikhodko, O.V., Kalyuzhna, T.V., Malykhin, V.V., Karpenko, V.V., Dolya, O.V. and Dolya, E.V. (1999). Oils of species of *Mentha* L. and *Thymus* L., *Farm. Zh. (Kiev)*, (5), 88–91.

Fadel, H.H.M. and Aeisa, M. (1994). Comparative studies on the essential oils of some *Mentha* species. *Bull. NRC Egypt*, 19, 135–441.

Fleisher, A. and Fleisher, Z. (1991). Aromatic plants of the Holy Land and the Sinai. Part IV. The essential oils from *Mentha longifolia* growing in Sinai and Israel. *J. Essent. Oil Res*., 3, 57–58.

Fleisher, A. and Fleisher, Z. (1998). Aromatic plants of the Holy Land and the Sinai. Part XIII. Volatile extract of *Mentha longifolia* growing in Israel. *J. Essent. Oil Res*., 10, 647–648.

Fujita, Y. and Fujita, S.I. (1967). Essential oil of *Mentha pulegium* and *M. gattefossei* viewed from the standpoint of comparative biochemistry. *Nippon Kagaku Zasshi*, 88, 767–769.

Fujita, S.-I. and Fujita, Y. (1970). Studies on the essential oils of the genus *Mentha*. Part VI. On the components of the essential oils of *Mentha japonica* Makino. *Agric. Biol. Chem*., 34, 1511–1513.

Fujita, S.-I., Nakano, T. and Fujita, Y. (1977). Studies on the essential oils of the genus *Mentha*. Part IX. A biochemical study of the essential oils of *Mentha japonica* Makino. *Nippon Nogei Kagaku Kaishi*, 51, 405–408.

Fujita, S.-I., Taka, K. and Fujita, Y. (1978). Studies on the essential oils of the genus *Mentha*. Part IX. On the components of the essential oils of *Mentha gentilis* Linn. var. *Ianata* (Rydb.), Fujita et Fujita (comb. Nov.). *Nippon Nogei Kagaku KIaishi*, 52, 277–280.

Galambosi, B., Aflatuni, A. and Sorvari, K. (1998). Effect of cultivation techniques on mint oils in northern Finland. *Perfum. Flavor.*, 23(5), 27–31.

Gavalas, N., Bosabalidis, A.M. and Kokkini, S. (1998). Comparative study of leaf anatomy and essential oils of the hybrid *Mentha* × *villosa-nervata* and its parental species *M. longifolia* and *M. spicata. Israel J. Plant Sci*., 46, 27–33.

Gora, J. and Kalemba, D. (1979). Chemical composition of essential oil from *Mentha spicata* L. *Herba Polonica*, 25, 269–275.

Hadjiakhoondi, A., Aghel, N., Zamanizadeh-Nadgar, N. and Vatandoost, H. (2000). Chemical and biological study of *Mentha spicata* L. essential oil from Iran. *DARU*, 8(1/2), 19–21.

Handa, K.L., Smith, D.M., Nigam, I.C. and Levi, L. (1964). Essential oils and their constituents. XXXIII. Chemotaxonomy of the genus *Mentha. J. Pharm. Sci*., 53, 1407–1409.

Hendriks, H. (1974). The essential oil of a new chemotype of *Mentha suaveolens* and a hybrid with *Mentha longifolia*. Ph.D. Thesis, State University, Groningen, Netherlands.

Ikeda, N., Shimizu, S. and Udo, S. (1963). Studies on *Mentha gentilis* L. *Japan. J. Breed*, 13, 31–41.

Ikeda, N., Shimizu, S., Karasawa, D., Origasa, T. and Ono, S. (1971). *Mentha arvensis* L. var. *piperascens* Mal. which grows wild in the northeastern part of Japan, III. Studies on the essential oils. *Sci. Rep. Fac. Agric. Okayama. Univ*., (37), 1–8.

Jaimand, K. and Rezaee, M.B. (2002). Chemical constituents of essential oils from *Mentha longifolia* (L.) Hudson var. *asiatica* (Boriss.) Rech. f. from Iran. *J. Essent. Oil Res*, 14, 107–108.

Jones, T.G.H. and Berry-Smith, F. (1926). The essential oils of Australian *Menthas*. I. *Mentha satureiodes. Proc. R. Soc. Queensland*, 37, 89–91.

Kapelev, I.G. and Akimov, Yu. A. (1980). Intraspecific chemical variability of *Mentha longifolia. Rastit. Resur*., 16(3), 436–441.

Kapil, V.B. and Sinha, G.K. (1979). Essential oil of *Mentha sylvestris* var. *incana* of Kumaon Hills (India). In: *Proceedings VIIth International Congress of Essential Oils*, Oct. 1977, Kyoto, Japan, pp. 190–192.

Karasawa, D. Shimizu, S. (1978). *Mentha candicans*, a new chemical strain of selection Spicatae, containing *trans*-sabinene hydrate as the principal component of essential oil. *Agric. Biol. Chem*. 42, 433–437.

Karasawa, D., Shatar, S., Erdenechimeg, A., Okamoto, Y., Tateba, H. and Shimizu, S. (1995). A study on Mongolian mints. A new chemotype from *Mentha asiatica* Borris. and constituents of *M. arvensis* L. and *M. piperita* L. *J. Essent. Oil Res*., 7, 255–260.

Karousou, R., Lanaras, T. and Kokkini, S. (1998). Piperitone oxide-rich essential oils from *Mentha longifolia* subsp. *petiolata* and *M.* ×*villoso-nervata* grown wild on the islands of Crete (S. Greece). *J. Essent. Oil Res.*, 10, 375–379.

Kharkwal, H., Shah, G.C., Mathela, C.S. and Laurent, R. (1994). Variation in the terpenoid composition of *Mentha longifolia* subsp. *himalaiensis. Indian Perfum.*, 38, 29–32.

Kokkini, S. (1983). Taxonomic studies in the genus *Mentha* in Greece. Ph.D. Thesis, University Thessaloniki, Greece.

Kokkini, S. and Papageorgiou, V.P. (1982). Morphological, cytological and chemical investigations of *Mentha spicata* L. in Greece. In: Margaris, N., Koedam, A. and Vokou, D., Eds., *Aromatic plants: basic and applied aspects*. Martinus Nijhoff, The Hague, Netherlands, pp. 131–140.

Kokkini, S., Karousou, R. and Lanaras, T. (1995). Essential oils of spearmint (carvone-rich) plants from the island of Crete (Greece), *Biochem. Syst. Ecol.*, 23, 425–430.

Kokkini, S., Karousou, R. and Lanaras, T. (1997). Essential oils with 1,2-epoxy-*p*-menthane derivatives from *Mentha spicata* plants growing across the island of Crete. *Bot. Acta*, 110, 184–189.

Kokkini, S., Hanlidou, E., Karousou, R. and Lanaras, T. (2002). Variations of pulegone content in pennyroyal (*Mentha pulegium* L.) plants growing wild in Greece. *J. Essent. Oil Res.*, 14, 224–227.

Koyalta, D., Sanaokko, A., Miralles, J. and Bassene, E. (1993). Etude des huiles essentilles de trois especes de *Menthe* acclimates au Senegal. *Rivista Ital. EPPOS*, (Numero Speciale), 544–547.

Kuwahara, Y., Kishino, K., Kayama, K. and Ito, K. (1979). A study on *Mentha candicans*. In: *Proceedings 7th International congress of Essential Oils*, Kyoto, Japan, Japan Flavor Fragrance Manufacturers Assoc., Tokyo, Japan, pp. 237–239.

Lawrence, B.M. (1978). A study of the monoterpene interrelationships in the genus *Mentha* with special reference to the origin of pulegone and menthofuran. Ph.D. Thesis, State University, Groningen, Netherlands.

Lawrence, B.M. (1993). Composition of the oil of wild Canadian *Mentha Peperita*. Unpublished data.

Li, Z.-Q., Li, Q.-C., Luo, L., Li, C., Huang, R. and Gao, T.-R. (1996). Chemical studies of *Mentha canadensis* from Yunnan province. *Yunnan Zhiwa Yanjiu*, 18, 115–122.

Maffei, M. (1988). Environmental factors affecting the oil composition of some *Mentha* species grown in northwest Italy. *Flav. Fragr. J.*, 3, 79–84.

Maffei, M. (1990). *Mentha* ×*verticillata*: a potential new source of menthol. *J. Essent. Oil Res.*, 2, 243–245.

Maffei, M., Gallino, M. and Sacco, T. (1986). Glandular trichomes and essential oils of developing leaves in *Mentha viridis lavanduliodora. Planta Med.*, 52, 187–193.

Malingré, Th.M. (1971). Chemotaxonomic study of *Mentha arvensis* L. *Pharm Weekbl.*, 106, 165–171.

Malizia, R.A., Molli, J.S., Cardell, D.A. and Retamar, J.A. (1996). Essential oil of *Mentha citrata* grown in Argentina. Variation in the composition and yield at full- and post-flowering. *J. Essent. Oil Res.*, 8, 347–349.

Marongiu, B., Porcedda, S., Della Porta, G. and Reverchon, E. (2001). Extraction and isolation of *Salvia desolenana* and *Mentha spicata* subsp. *insularis* essential oils by supercritical CO_2. *Flav. Fragr. J.*, 16, 384–388.

Mathela, C.S., Shah, G.C., Melkani, A.B. and Pant, A.K. (1989). Terpenoids of *Mentha longifolia* subsp. *himalaiensis. Fitorterapia*, 60, 349–350.

Matovic, M.M. and Lavadinovic, V. (1999). Essential oil composition of *Mentha longifolia* (L.) Huds. from the mountain Zlatar in Yugoslavia. *J. Essent. Oil Plants*, 2(2), 78–81.

Mimica-Dukic, N., Gasic, O., Kite, G., Fellow, L. and Jancic, R. (1991). A study of the essential oil of *Mentha longifolia* growing in Yugoslavia. *Planta Med.*, 57, A83-A84.

Mucciarelli, M. and Sacco, T. (1999). Glandular trichomes and essential oils of *Mentha requienii* Benth. *J. Essent. Oil Res.*, 11, 759–764.

Murray, M.J. and Lincoln, D.E. (1972). Oil composition of *Mentha aquatica–M. longifolia* F. hybrids and *M. dumetorium. Euphytica*, 21, 337–343.

Murray, M.J., Faas, W. and Marble, P. (1972). Effects of plant maturity on oil composition of several spearmint species grown in Indiana and Michigan. *Crop Sci.*, 12, 723–728.

Nagasawa, T. and Umemoto, K. (1976). Studies on chemical constituents of wild mints. Part IX. Essential oil of *Mentha gentilis* L. containing piperitone as a major component. *Nippon Nogei Kagagu Kaishi*, 50, 381–383.

Nagasawa, T., Umemoto, K., Tsuneya, T. and Shiga, M. (1974). Studies on wild mints of Tokai districts. Part 1. On the identification of d-1,2-epoxymenthol isolated from the essential oil of *Mentha gentilis* L. *Nippon Nogei Kagaku Kaishi*, 48, 39–42.

Nagasawa, T., Umemoto, K., Tsuneya, T. and Shiga, M. (1975a). Studies on the wild mints of Tokai districts. Part IV. New terpenic alcohols in essential oil of *Mentha gentilis* L. containing (+)-pulegone as a main component. *Nippon Nogei Kagaku Kaishi*, 49, 217–221.

Nagasawa. T., Umemoto, K., Tsuneya T. and Shiga, M. (1975b). Studies on the wild mints of Tokai districts. Part V. Essential oil containing (+)-neomenthol as a major constituent (note). *Nippon Nogei Kagaku Kaishi*, 49, 491–493.

Nagasawa, T., Umemoto, K., Tsuneya, T. and Shiga, M. (1976a). Studies on essential oil *Mentha spicata* L. var. *crispa* Benth. Part IV. Essential oil of *Mentha spicata* L. var. *crispa* Benth. grown in winter season. *Nippon Nogei Kagaku Kaishi*, 50, 287–289.

Nagasawa, T., Umemoto, K., Tsuneya, T. and Shiga, M. (1976b). Studies on the wild mints of Tokai districts. Part VII. Essential oils of wild mints containing (+)-menthofuran as a major constituent. *Nippon Nogei Kagaku Kaishi*, 50, 291–293.

Olszewski, Z. and Pluta, J. (1974). Gas chromatographic study of *Mentha crispa. Farmacia Pol*., 30, 143–148.

Oumzil, H., Ghoulami, S., Rhajaoui, M., Ildrissi, A., Fkih-Tetouani, S., Faid, M. and Benjouad, A. (2002). Antibacterial and antifungal activity of essential oils of *Mentha suaveolens. Phytother. Res*., 16, 727–731.

Özgüven, M., Kirici, S. and Mengel, C. (1995). Wild *Mentha* species growing in southern Turkey and their essential oils. Paper presented at 43rd Annual Congress on Medicinal Plant Research, Secondary Products-Physiologically Active Compounds, Halle-Wittenberg, Germany, pp. 1–8.

Perez-Raya, M.D., Utrilla, M.P., Navarro, M.C. and Jimenez, J. (1990). CNS activity of *Mentha rotundifolia* and *Mentha longifolia* essential oil in mice and rats. *Phytother. Res*., 4, 232–234.

Pino, J.A., Rosado, A. and Fuentes, V. (1999). Chemical composition of the leaf oil of *Mentha rotundifolia* (L.) Hudson from Cuba. *J. Essent. Oil Res*., 11, 241–242.

Sacco, T. and Nano, G.M. (1968). To the question of botany and chemical composition of *Mentha arvensis* essential oil. In: *Proceedings IVth Congrés International des huiles essentielles*. Vol. II. *Selection et culture des plantes a parfum*. Sept. 1968, Tblissi, USSR, pp. 164–165.

Sacco, T. and Shimizu, S. (1965). Botanical and chemical studies on a mint of the arvensis group (*Mentha arvensis* var. *praecox*) self-sown in Piedmont Italy. *Perfum. Essent. Oil Rec*., 56, 211–213.

Sacco, T., Maffei, M. and Chialva, F. (1989). New hybrids from a seed population of *Mentha arvensis* var. *piperascens* clone 7303. In: Bhattacharyya, S.C., Sen, N. and Sethi, L.K., Eds., *Proceedings 11th International Congress of Essential Oils, Fragrances and Flavours,* New Delhi, Oxford & IBBTT Publ. Co., Pvt. Ltd., New Delhi, India, Vol. 3, 27–34.

Sfiras, J. (1952). A few constituents of *Mentha timija. Recherches*, (2), 39–41.

Sharipova, F.S., El'chibekova, L.A., Nedel'ko, E.S. and Gusak, L.E. (1983). Wild mints of Kazakhstan II. Study of the chemical composition of *Mentha arvensis* L., *Mentha longifolia* (L.), *Mentha crispa* L. and *Mentha interupta* Boriss. essential oils. *Izv. Akad. Nauk Kaz. SSr*, *Ser Khim*., (5), 74–76.

Shimizu, S. and Ikeda, N. (1962). Studies on the essential oils of interspecific hybrids in the genus *Mentha*. Part VIII. The essential oils of *Mentha spicata* L. with 36 or 48 somatic chromosomes. *Agric. Biol. Chem*., 26, 543–545.

Shimizu, S., Ikeda, N. and Ueda, H. (1960). Studies on the essential oil of *Mentha rotundifolia*. Part. V. Isolation of (+)-neoiso-isopulegol, a new stereoisomer of isopulegol, and piperitenone from a variety of *Mentha rotundifolia. Bull. Agric. Chem. Soc. Japan*, 24, 324–325.

Sivropulou, A., Kokkini, S., Lanaras, T. and Arsenakis, M. (1995). Antimicrobial activity of mint essential oils. *J. Agric. Food Chem*., 43, 2384–2388.

Soliman, F.M., El-Sohly, M.A., Fathy, M.M. and El-Sakhawy, F.S. (1997). A comparative study of the essential oils from certain *Mentha* and *Salvia* species grown in Egypt. *Egypt. J. Pharm. Sci*., 38, 553–564.

Sticher, O. and Flück, H. (1968). Die Zusmmensetzung von genuinen, extrahierten und destillierten ätherischen Ölen einiger *Mentha*-Arten. *Pharm. Acta Helv*., 43, 411–446.

Stoeva, T. and Iliev, L. (1997). Influence of some phenylurea cytokinins on spearmint essential oil composition. *Bulg. J. Plant Physiol.*, 23, 66–71.

Tucker, A.O. (1975). Morphological, cytological and chemical evaluation of the *Mentha* ×*gentilis* L.S.L. hybrid complex. Ph.D. Thesis, Rutgers University, New Brunswick, NJ, USA.

Tucker, A.O., Hendriks, H., Bos, R. and Fairbrothers, D.E. (1991). The origin of *Mentha* ×*gracilis* (Lamiaceae). II. Essential oils. *Econ. Bot.*, 45, 200–215.

Umemoto, K. (1993). Studies on the wild mints of Tokai districts. Part IIX. Essential oil of *Mentha arvensis* containing piperitone as a main component. *Nagoya Gakuin Univ. Review (Nat. Sci.)*, 30, 13–20.

Umemoto, K. (1994). Studies on wild mints of the Tokai districts. Part IIXI. Essential oil of self-pollinated plants of *Mentha aquatica* containing sesquiterpene alcohols as major compounds. *Nagoya Gakuin Univ. Rev.*, 30, 17–27.

Umemoto, K. (1996). Studies on wild mints of the Tokai districts. Part XXV. Essential oil of self-pollinated plants of *Mentha rotundifolia* with piperitenone oxide. *Nagoya Gakuin Univ. Rev.*, 32(2), 33–46.

Umemoto, K. (1997). Studies on the wild mints of the Tokai districts. Part XXX. Stereochemistry of four 1,2-epoxymenthyl acetates isolated from S_1 oils of *Mentha rotundifolia. Nagoya Gakuin Univ. Rev.*, 34(1), 13–35.

Umemoto, K. (1998). Two new stereoisomers of 1,2-epoxymenthyl acetate from self-pollinated plant oils of *Mentha rotundifolia. Nat. Prod. Lett.*, 11, 161–165.

Umemoto, K. and Nagasawa, T. (1978). Studies on chemical constituents of wild mints. Part XIII. Essential oil of *Mentha gentilis* L. containing pulegone–menthone–neomenthol as major components. *Nippon Nogei Kakaku Kaishi*, 52, 191–193.

Umemoto, K. and Nagasawa, T. (1980). Studies on the wild mints of Tokai districts. Part X. Constituents in essential oil of *Mentha spicata* L. var. *crispa* Benth. *Nagoya Gakuin Daigaku Ronshu, Jinbun Shinzen Kagakuhen*, 17(1), 139–153.

Umemoto, K. and Tsuneya. T. (1988). Studies on chemical constituents of wild mints. Part XIV. *Mentha arvensis* containing piperitone oxide and piperitone oxide as major components. *Nippon Nogei Kagaku Kaishi*, 62, 1073–1076.

Umemoto, K., Arai, T., Nii, H. and Furukawa, K. (1994). Chemical constituents of wild mints. Part XX. Essential oil of self-pollinated plants of *Mentha aquatica* with sesquiterpene alcohols as major components. *Nippon Nogei Kagaku Kaishi*, 68, 1567–1570.

Van Os, F.H.L. and Hendriks, H. (1975). Investigation of a population of *Mentha* ×*niliaca* Juss ex. Jacq. found in natural conditions at Oudemolen (DR). *Acta Bot. Neerl.*, 24, 129–133.

Velasco-Negueruela, A., Perez-Alonso, M.J. and Mata Rico, M. (1987). Aceites esenciales de Lamiaceas Ibericas con pulegona como components fundamental. *Anal. Bromatol.*, 39, 357–372.

Velasco-Negueruela, A., Perez-Alonso, M.J., Esteban, J.L., Garcia Vallejo, M.C., Zygadlo, J.A., Guzman, C.A. and Ariza-Espinar, L. (1996). Essential oils of *Calamintha nepeta* (L.) Savi and *Mentha aff. suaveolens* Ehrh., grown in Cordoba, Argentina. *J. Essent. Oil Res.*, 8, 81–84.

Vidal, J.-P., Noleau, I., Bertholon, G., Lamy, J. and Richard, H. (1985). Constituents volatils des huiles essentielles de *Menthes sylvestres* de la Drôme. *Parfum. Cosmet. Arômes*, (64), 83–87.

von Rudloff, E. and Hefendehl, F.W. (1966). Gas liquid chromatography of terpenes. XV. The volatile oil of *Mentha arvensis* var. *glabrata* ray. *Can. J. Chem.*, 44, 2015–2022.

9 North American Mint Oil Industry: The Production and Quality Control of Mint and Its Commercially Important Isolates

Ross M. Sheldon

CONTENTS

9.1 INTRODUCTION

Beginning with their initial introduction in the early 1800s, peppermint oil (*Mentha piperita*), Scotch spearmint (*Mentha gracilis*), and Native spearmint (*Mentha spicata*) have become the primary mint oils of commerce within the North American mint oil industry. Used principally throughout the oral care, chewing gum, and confection industries, mint oils can be used directly or as key components in mint flavor formulations.

This chapter explores key considerations related to the production and quality control of mint oils and their related isolates.

9.2 PRODUCTION SUMMARY AND GENERAL TRENDS

Beginning in the mid- to late 1800s, the Midwest region of the United States became the agricultural center of the mint industry with Michigan, Indiana, and later Wisconsin as the primary growing areas. In 1913, Oregon's Willamette Valley began producing commercial quantities of peppermint oil and, at that point, the industry witnessed the start of a growing trend toward new district development (Landing, 1969). Madras, Oregon followed in 1956, Idaho in 1962, and then the La Grande (Oregon), Northern California, South Dakota, and Nevada districts reached full commercial production during the 1990s.

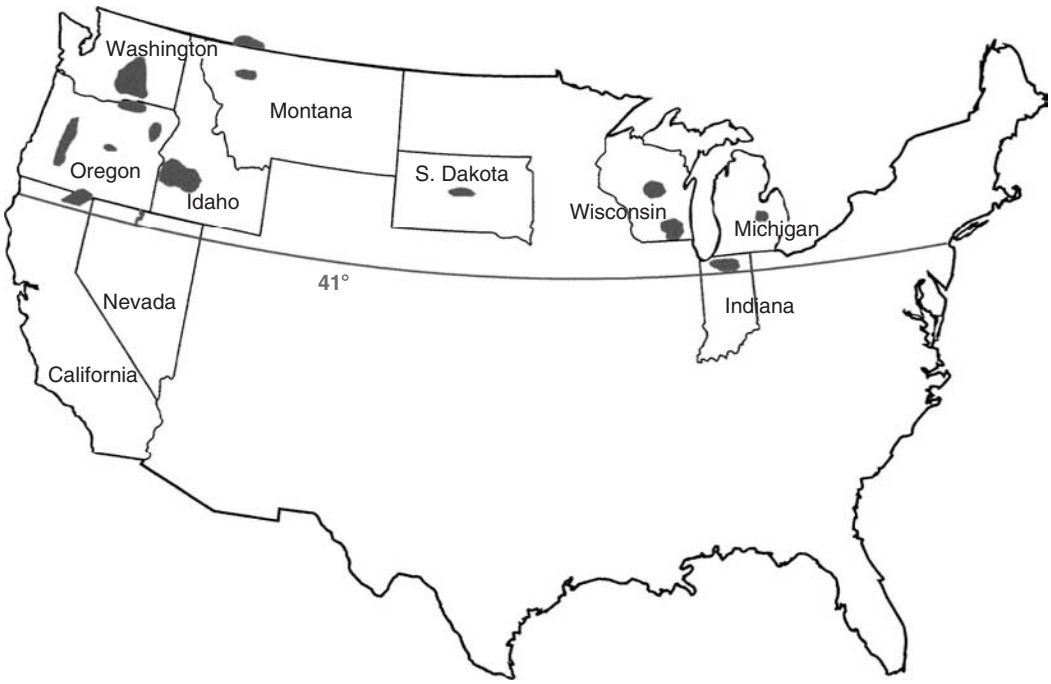


FIGURE 9.1 Mint oil growing districts in North America.

Figure 9.1 presents an overview of the key mint growing regions in North America. Importantly, these districts are located above the 41st Parallel where climatic conditions and other factors, notably the plant's day length or photoperiod, have favored the production of high-quality oil (Clark and Menary, 1979). Key considerations related to the general geography and climatic conditions have been presented in Table 9.1. Importantly, mint needs approximately 90 cm of water during the course of the growing season and, with the exception of parts of the Midwest, irrigation systems are in place to provide the necessary water. Figure 9.2 presents a summary of North American mint oil production for the period 1970 through 2004. Table 9.2 provides key details for each of the specified years in terms of volume (metric tonnes) and hectares under cultivation for each of the mint oils produced in the U.S.

For the specified period, the data underscore several important developments that have occurred within the mint oil industry. For peppermint oil, the production statistics speak of a relatively flat demand for the 20-year period from 1970 to 1990. Beginning in the 1990s, world demand experienced a marked increase, with today's global demand for *M. piperita* oil estimated at 4290 metric tonnes per year. While North America has been the primary source of supply, it is important to acknowledge the growing presence of other producing regions such as India. In 2004, total sales of true Indian *M. piperita* oil were estimated at approximately 1030 metric tonnes, an appreciable increase from several years ago when production totals were estimated at approximately 450 metric tonnes.

Considering circumstances specific to North America, recent years have witnessed two very important developments within the peppermint oil industry, namely, significant overproduction during the late 1990s, which led to major efforts throughout the agricultural community to *bring production in balance with demand*. Second, as production was reduced, the industry experienced a *dramatic shift in the production among key producing regions*.

TABLE 9.1
North American Mint Production. Geographic and Climatic Considerations

Region	General Characteristics	Climate
Midwest	Three distinct growing areas: Northern Indiana, Southern Wisconsin, and Central Michigan. Soil conditions in Northern Indiana are relatively unique. Classified as a muck soil, the organic content is high with some soils having organic contents as high as 60%. The rest of the region tends to be a sandy or silt loam, which is typical of the Midwest.	The summers are hot and humid with cooler temperatures during the evening. Although the winters are normally very cold, the snow cover is generally sufficient to protect the root stock. Precipitation occurs principally in the spring and winter along with frequent thunderstorms during the summer.
Washington	Primary growing areas include the Yakima Valley and the Columbia River Basin including the Boardman/Hermiston area (North Central Oregon). Soil conditions range from the heavy clay loam typical of the Yakima Valley to the sandy soils found in the Columbia River Basin.	The region has a semiarid climate with summer temperatures ranging from lows of 10°C to highs that frequently exceed 38°C. During the growing season, the high temperatures average in a range from 26°C to 32°C. Annual precipitation averages 15 to 25 cm with much of the total falling during the Spring.
Oregon	Primary growing areas include the Willamette Valley, Madras, and La Grande. Soil conditions vary widely throughout the region. In the Willamette Valley, the soil is largely river sediment that is dark and rich in organic material. Clay and sandy soils are also found in the valley. In Madras, the soil has a volcanic component with low levels of organic material. In the La Grande area, the soil ranges from heavy clay to sandy loam.	Protected by the Pacific Coast and Cascade mountain ranges, the Willamette Valley experiences warm, dry summers and mild, wet winters and springs. In the Madras and La Grande regions, the climate is best described as a high desert plains climate with hot summer days and cool nights. The winters are not extreme and the snowfall is generally sufficient to protect the fields.
Idaho	Falling along the Snake River Plain, the region includes areas in Eastern Oregon and Idaho on the central border between the two states. The soil type is generally a silt or clay loam and of volcanic origin with low levels of organic material.	The climate is best characterized as a high desert plains climate with hot summer days and cool nights. Like Washington, summer temperatures average in a range from 26°C to 32°C with frequent highs in excess of 38°C. The winters are generally mild with sufficient snow to protect the rootstock.

Addressing each point, efforts to reduce production began in earnest in the late 1990s when production and oil supply reached their peak. In 1995, the industry had more than 60,000 hectares of peppermint (Table 9.2) under cultivation and, within 6 years, this level had been reduced to 32,106 hectares, a 46.9% reduction. In terms of peppermint oil supply, the industry reached its peak in 1998, when the total North American supply, i.e., annual production plus carryover from the previous years, reached a total of 6067 metric tonnes, a quantity far in excess of the world demand. Market factors and a responsive agricultural community have brought production to a point where some reasonable balance has been achieved.

In recent years, the dramatic shift in the production among key producing regions has been a major development occurring within the peppermint oil industry. Figure 9.3 is a summary of North American peppermint oil production by region for the period 1994 through 2004 and the data offer a compelling example of the dramatic transitions that have occurred. Highlighting key points, attention should be drawn to the following:

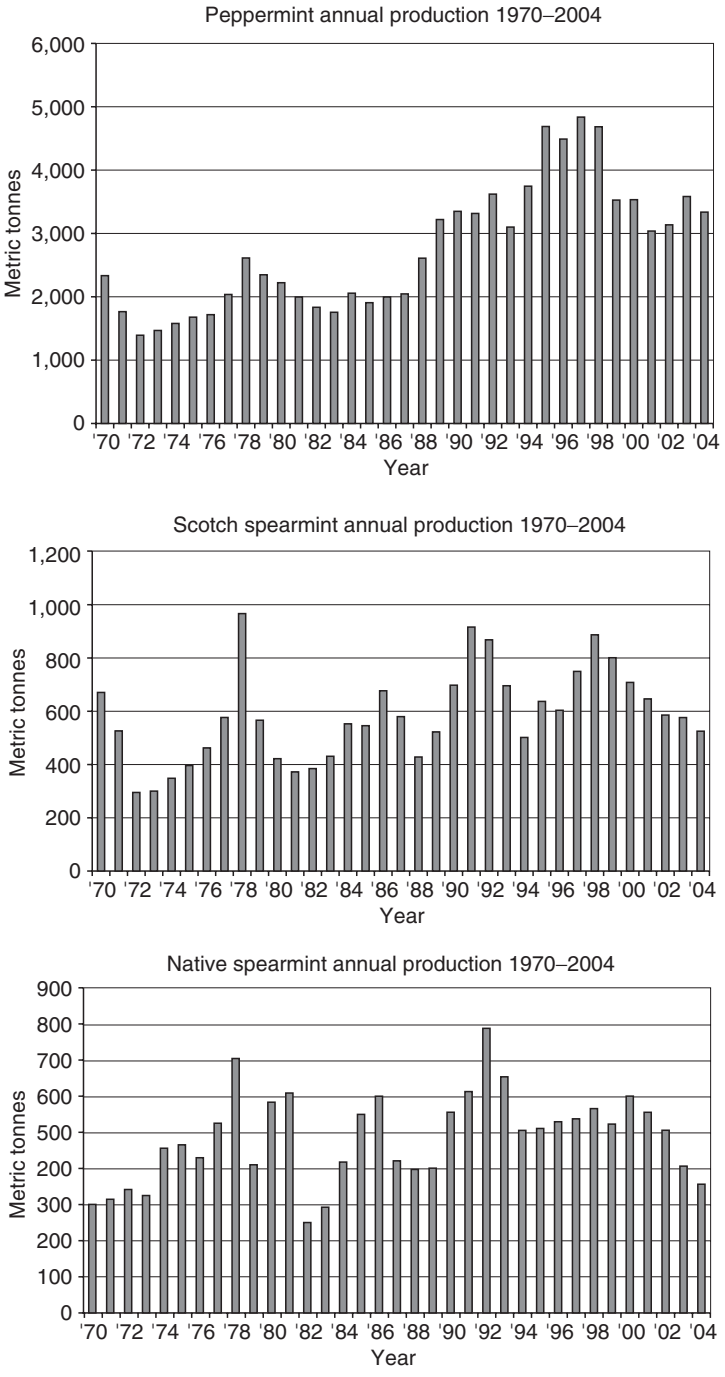


FIGURE 9.2 North American production summary. Peppermint oil (*M. piperita)*, Scotch spearmint (*M. gracilis*), Native spearmint (*M. spicata*).

TABLE 9.2
North American Production Statistics. Peppermint Oil (*M. piperita*), Scotch Spearmint Oil (*M. gracilis*), and Native Spearmint Oil (*M. spicata*)

	Peppermint		Scotch Spearmint		Native Spearmint	
Year	Hectares	Metric Tonnes	Hectares	Metric Tonnes	Hectares	Metric Tonnes
1970	32,174	2,330	9,490	670	3,951	302
1971	26,536	1,763	8,252	526	4,166	315
1972	25,015	1,394	6,390	294	3,963	342
1973	24,373	1,468	6,327	300	3,927	325
1974	26,012	1,574	6,956	347	4,548	458
1975	28,531	1,674	7,417	394	4,363	466
1976	30,399	1,714	8,418	460	4,216	429
1977	37,406	2,033	11,296	575	4,959	528
1978	43,201	2,605	13,706	965	7,009	707
1979	40,171	2,345	10,540	563	5,307	412
1980	37,001	2,220	7,617	419	6,023	585
1981	30,227	1,993	6,856	369	5,415	611
1982	26,968	1,827	6,681	383	2,841	252
1983	26,122	1,744	7,624	430	3,736	295
1984	29,820	2,047	8,410	550	4,439	416
1985	28,433	1,898	9,241	543	5,097	550
1986	27,998	1,985	9,821	674	4,546	599
1987	29,416	2,033	9,225	577	3,544	423
1988	38,385	2,600	8,590	426	3,622	399
1989	44,930	3,211	8,830	520	3,796	401
1990	45,038	3,342	10,655	694	5,056	555
1991	50,594	3,306	12,672	912	5,968	613
1992	50,188	3,608	13,456	865	6,249	788
1993	44,060	3,090	10,511	693	5,350	655
1994	49,430	3,728	8,298	498	4,270	505
1995	60,408	4,673	9,508	633	4,250	511
1996	57,914	4,478	8,899	599	3,917	529
1997	58,696	4,819	8,888	746	3,676	538
1998	49,853	4,669	9,714	882	3,527	566
1999	43,592	3,503	8,520	798	3,586	521
2000	37,037	3,514	7,554	704	4,004	598
2001	32,106	3,025	6,982	643	3,920	554
2002	32,311	3,120	6,713	582	3,577	501
2003	34,544	3,562	5,769	573	2,949	404
2004	33,688	3,318	4,835	478	2,671	346

- In 2004, peppermint oil production from Washington (Kennewick S/C and Yakima D/C[1]) and Idaho totaled 66.3% of the North American total, up 61.3% from that in 1994. During the referenced period, the Yakima (D/C) quality became a significant part of the overall product mix.

[1]Both the referenced Kennewick and Yakima product qualities are grown in Washington's Yakima Valley. The Yakima type is used to designate a Double Cut (D/C) quality. The Kennewick designation represents a Single Cut (S/C) quality. Because of the length of Washington's growing season, farmers can take two cuttings during the course of the season.

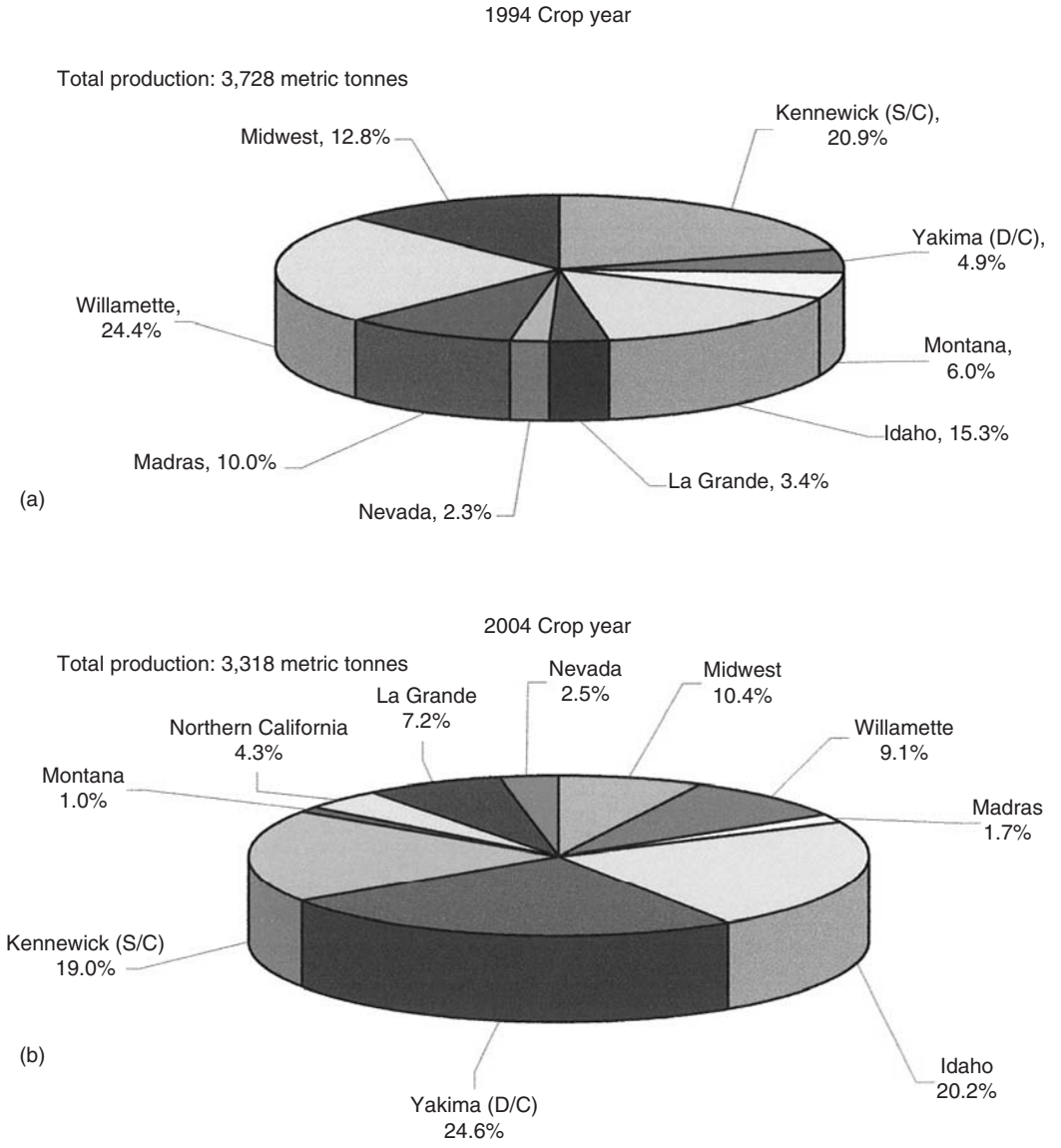


FIGURE 9.3 North American peppermint oil production by region in crop years 1994 and 2004.

- Peppermint oil production in the Willamette and Madras districts has experienced a marked decline from earlier levels. In 1994, the combined output totaled 34.4% of the North American supply. Today, the combined total is estimated at 10.8%.
- Peppermint oil production in the Midwest has also experienced a slight decline. The La Grande (Oregon), Northern California, and Nevada districts are relatively new and making important contributions to the industry.

Economics has been the fundamental factor driving the dramatic shift in district production. Although crop yields will vary from year to year, the most recent results from the 2004 crop year are typical of past results and trends. Table 9.3 is a summary of crude oil yields (kg/ha) at the farm level for the 2004 crop year. As the results indicate, there is a significant

TABLE 9.3
Peppermint Oil Yield Data by Region

2004 Crop Year	
Region	**Yield (kg/ha)**
Kennewick (S/C)	106
Yakima (D/C)	155
Montana	67
Idaho	97
La Grande	77
Nevada	101
Northern California	88
Madras	78
Willamette	103
Midwest	50

spread in the data with the higher yields translating directly as a reduced cost of production. Understanding that horticultural practice can also be a factor impacting yield, it should be noted that the marked yield differences shown in Table 9.3 are largely due to climatic conditions. In the case of peppermint, such concerns as *verticillium* wilt (a fungal disease) and insect pests will also have an adverse effect on product quality and yield.

With few exceptions, conditions within the spearmint oil market follow a close parallel to those within the peppermint oil industry. Both products are grown in many of the same regions by many of the same growers and the mint oils serve the same consumer product industries. Current world demand for Scotch spearmint oil (*M. gracilis*) and Native spearmint oil (*M. spicata*) is estimated at 1063 metric tonnes[2] and 836 metric tonnes[2], respectively. North American spearmint oil production represents approximately 67% of the world demand.

Figure 9.4 shows a profile of the North American spearmint oil production by region and Table 9.4, presents a summary of the support data. Importantly, the Farwest region is responsible for an estimated 68.8% of the current production in North America.

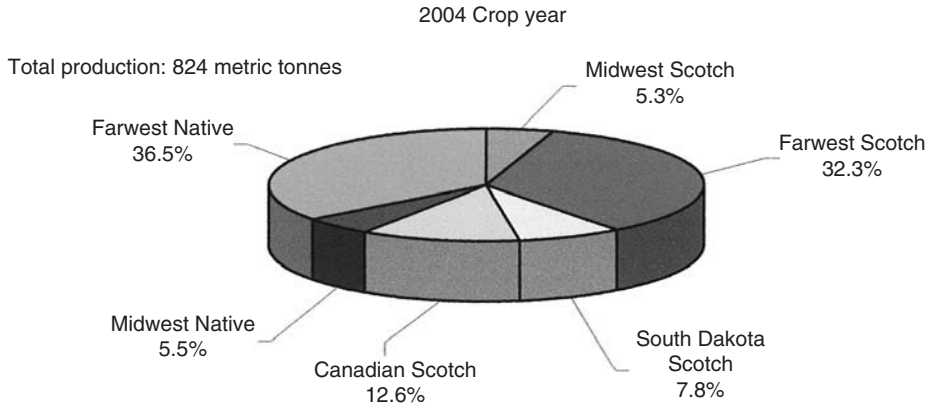


FIGURE 9.4 North American spearmint production by region.

[2]The world demand figure includes consideration of spearmint oil production from China and India.

TABLE 9.4
North American Spearmint Oil Production by Region

	2004 Crop Year	
Region	**Volume (MT)**	**%**
Midwest Scotch	44	5.3
Farwest Scotch	266	32.3
South Dakota Scotch	64	7.8
Canadian Scotch	104	12.6
Midwest Native	45	5.5
Farwest Native	301	36.5
Total (MT)	824	100.0

Like peppermint, the spearmint industry has also undergone some contraction as production has far outstripped demand. In 2003, the combined Scotch and Native production totaled an estimated 977 metric tonnes and this quantity was in addition to the carryover volume,[3] which was in excess of 1000 metric tonnes at the start of the 2003 crop year. Considering the 2004 combined spearmint oil production of 824 metric tonnes, there has been a 15.7% decrease from the previous year against an estimated 5% increase in sales for the same period. Again, serious efforts have been made to bring supply in balance with demand. Today, the spearmint oil carryover position is at its lowest level in many years.

Importantly, despite the excess supply, saleable volumes are regulated by a Federal marketing order, Spearmint Oil Marketing Order #985, which is unique to the spearmint oil industry. The marketing order was the result of proactive efforts by Farwest spearmint oil producers who, with the help of the USDA, prepared a draft for industry and government review. The USDA approved the order and it became a law on April 14, 1980. The marketing order applies to all spearmint oil grown in the Farwest region of the U.S which would include Washington, Oregon, Idaho, and the northern sections of Nevoda and Utah. In terms of benefits, end users have credited the marketing order for stabilizing the spearmint oil industry through a regulated supply at a stabilized price (Christensen, 1994). Notwithstanding the strength of the Farwest in terms of the overall spearmint oil supply, the balance of the production is extremely important to the market, with the volumes originating in the Midwest for Scotch and Native spearmint oils as well as Scotch spearmint oil production in Canada. In these cases, the production is not controlled by the marketing order and it is driven in direct response to market demand.

Finally, as noted, oversupply has been a key factor in recent years for both the peppermint and spearmint oil industries, and the agricultural community has taken some necessary steps to bring supply back in balance with demand. In this context, an understanding of competitive crop options at the grower level is essential as mint oil production is replaced with alternate crops. Specialty crops such as grass seed and ornamentals are very important for the Willamette Valley. For other areas, the full range of commodity crops such as corn, soy, wheat, and potatoes have been important alternate crops.

9.3 QUALITY CONTROL CONSIDERATIONS

Quality determinations need to be considered from a number of different standpoints, namely, compliance with established regulatory standards, ingredient authenticity, and the

[3]Carryover volume is a term used within the mint industry to represent unsold production from previous years.

organoleptic integrity of the essential oil or its isolate. In terms of regulatory standards, the documentation is widespread as both national and international specification requirements can be applicable given the different sets of conditions and circumstances.

All natural products, their isolates, and any chemical that has appeared in the scientific literature will have a Chemical Abstracts Service (CAS) number. In Europe, all substances that are used are listed in the European Inventory of Existing Chemical Substances (EINECS) and will have an EINECS number as an identification code. All substances that are traded anywhere in the world will have a unique Harmonization Tariff (HT) number, which is a six-digit identification code that identifies the material and becomes the basis for tariff assessments and the compilation of import and export trade statistics. Additionally, a four-digit country code is also added to the unique six-digit figure, thereby allowing each country to determine its own trade statistics. For a product to be approved for food use in the U.S., it must be Generally Recognized As Safe (GRAS) and it should have a Flavor Extract Manufacturer's Association (FEMA) number.

To determine if a natural oil or its isolate is a pure product, internationally accepted specifications have been developed. The main organizations that have broadly accepted specifications are the International Organization for Standardization (ISO), the Association Français de Normalisation (AFNOR [1996a–c]) providing French essential oil standards, United States Pharmacopoeia (USP), European Pharmacopoeia (EUP), Essential Oils Association of the USA (EOA), and the USA National Academy of Sciences Food Chemical Codex (FCC). Additionally, some individual countries have their own sets of specifications, for example, India (Indian Standardized Specifications), the United Kingdom (British Standard Specifications), etc. Significantly, these individual specifications are closely related to the aforementioned international standards. Typically, these specifications are a combination of physical and chemical measurements such as density (or specific gravity), refractive index, and optical rotation, together with an assay of the most important components that would be characteristic of the product.

The *density* of an oil or isolate is one of the criteria used to determine authenticity. Density is the weight per milliliter at a specific temperature (usually 20°C), whereas specific gravity is the weight of a known volume of an oil or isolate divided by the weight of the same volume of water at the same temperature, usually 20°C. If the density is measured at a temperature other than 20°C, then the density can be adjusted using the data reported by Nigam (1966).

The *refractive index* is another physical characteristic of essential oils or their isolates and is the ratio of the velocity of light in air to the velocity of light through a liquid sample at a specific temperature, usually 20°C. Again, if the measurements are taken at temperatures other than 20°C, the data can be adjusted according to the data reported by Nigam (1966).

All essential oils and their isolates that possess one or more chiral centers possess the ability to rotate the plane of polarized light, a property known as optical activity in which the measurement is known as *optical rotation*. Like density and refractive index, the optical rotation of an oil or isolate (for solids, a solution is used) is measured at a specific temperature, usually 20°C. As before, measurements made at temperatures other than 20°C can be adjusted (Nigam, 1966).

As noted earlier, the primary essential oils of commerce produced in the U.S. from the genus *Mentha* are peppermint, Scotch spearmint, and Native spearmint. Typical isolates recovered from these oils are shown in Table 9.5. For the sake of completeness, dementholized cornmint oil, *l*-Menthol, and *l*-Menthone (two isolates for cornmint oil) are included in this discussion because of their importance and close association with peppermint oil and its related applications. A summary of the CAS, EINECS, HT, and FEMA numbers for the oils and natural isolates is found in Table 9.6. Key physicochemical specifications for peppermint oil, dementholized cornmint oil, and spearmint oils, both Scotch and Native, have been

TABLE 9.5
Natural Isolates from Peppermint and Spearmint Oils

1,8-Cineole	Isobutyraldehyde
Dimethyl sulphide	Isovaleraldehyde
Ethyl 2-methylbutyrate	*l*-Limonene
2-Ethylfuran	Menthofuran
Germacrene-D	3-Octanol
cis-3-Hexenol	Viridiflorol

presented in Table 9.7, Table 9.8, and Table 9.9, respectively. For solids like *l*-Menthol, the melting point of 41°C to 42°C is as important a specification as is its miscibility in 70% aqueous ethanol.

An infrared spectrum of an oil or isolate can be a very useful proof of identity as it is a unique *fingerprint* of the material that is measured. Examples of infrared spectra for peppermint, dementholized cornmint, Scotch spearmint, and Native spearmint oils are shown in Figure 9.5.

Flash point is another specification and it is defined as the temperature at which the material in question will spontaneously burst into flames when exposed to an external

TABLE 9.6
Identity Numbers for Selected Mint Oils and Their Isolates

	Identity Numbers			
Material	**CAS**	**EINECS**	**HT**	**FEMA**
Essential oils				
Peppermint oil	8006-90-4	308-770-2	3301.25	2848
Spearmint oil (Scotch)	8008-79-5	283-656-2	3301.25	4221
Spearmint oil (Native)	8008-79-5	283-656-2	3301.25	3032
Cornmint oil	68917-18-0	290-058-5	3301.25	4219
Cornmint oil dementholized	68917-18-0	290-058-5	3301.25	4219
Isolates				
1,8-Cineole	470-82-6	207-431-5	2909.20	2465
Dimethyl sulphide	75-18-3	200-846-2	2930.90	2746
2-Ethylfuran	3208-16-0	221-714-0	2932.19	3673
Ethyl 2-methylbutyrate	7452-79-1	231-225-4	2915.90	2443
cis-3-Hexenol	928-96-1	213-192-8	2905.29	2563
l-Limonene	5989-54-8	227-815-6	2902.19	
Menthofuran	494-90-6	207-795-5	2932.19	3235
l-Menthol	2216-51-5	218-690-9	2906.11	2665
l-Menthone	89-80-5	201-941-1	2914.29	2667
3-Methylbutanal[a]	590-86-3	209-691-5	2912.19	2692
2-Methylpropanal[b]	78-84-2	201-149-6	2912.19	2220
3-Octanol	589-98-0	209-667-4	2905.16	3581
Viridiflorol	552-02-3	209-003-3		

Source: *Allured's Flavor and Fragrance Materials*, Allured Publishing Corp., Illinois, 2003.
[a]Also known as isovaleraldehyde.
[b]Also known as isobutyraldehyde.

TABLE 9.7
Physicochemical Specifications for Peppermint Oil

Specification	1	2	3	4	5	6	7
Density (20°C)	0.896–0.908[a]	0.896–0.908[a]	0.900–0.916	0.900–0.916	0.900–0.910	0.900–0.913	0.903–0.912
Refractive index (20°)	1.459–1.465	1.459–1.465	1.457–1.467	1.460–1.467	1.462–1.464	1.460–1.465	1.460–1.464
Optical rotation (20°)	−32° to −18°[a]	−32° to −18°[a]	−30° to −10°	−29° to −10°	−23° to −16°	−30° to −20°	−28° to −17°
Total alcohols (%) (as menthol)	50.0 min	50.0 min	32.8–65.0	47.6–70.6	47.6–61.4	58.2–79.8	55.4–68.1
Total esters (%) (as menthyl acetate)	5.0 min	5.0 min	2.8–10.0	4.9–6.7	4.9–12.0	3.8–9.9	4.9–6.7
Total ketones (%) (as menthone)			15.5–47.0	14.9–29.6	18.7–29.6	14.8–31.6	18.7–31.6

Source: 1. *US Pharmacopoeia*, United States Pharmacopoeia Convention Inc., Rockville, Maryland, 2004; 2. *Food Chemical Codex* National Academy Press, Washington, D.C., 1996; 3. Europaisches Arzneibuch, Pfefferminzol, *Mentha piperitae aestheroleum*. 4. Aurgabe Grundwerbe, Verlag österreich Gmbh, Berlin, 2002, 2641–2643., 4. International Organization for Standardization, ISO 856, Oil of peppermint (*Mentha piperita* L.) ISO, Geneva, 1981, 12pp. (French Oils); 5. International Organization for Standardization, ISO 856, Oil of peppermint (*Mentha piperita* L.) ISO, Geneva, 1981, 12pp. (Italian Oils); 6. International Organization for Standardization, ISO 856, Oil of peppermint (*Mentha piperita* L.) ISO, Geneva, 1981, 12pp. (English Oils); 7. International Organization for Standardization, ISO 856, Oil of peppermint (*Mentha piperita* L.) ISO, Geneva, 1981, 12pp. (USA Oils).

[a]At 25°C.

TABLE 9.8
Physicochemical Specifications for Dementholized Cornmint Oil

Specification	China	India
Density (20°C)	0.890–0.908	0.890–0.910
Refractive index (20°)	1.457–1.465	1.457–1.465
Optical rotation (20°)	−24° to −15°	−22° to −13°
Total alcohols (%) (as menthol)	40.0–60.0	40.0–60.0
Total esters (%) (as menthyl acetate)	3.0–9.0	3.0–9.0
Total ketones (%) (as menthone)	25.0–45.0	25.0–40.0

Source: International Organization for Standardization, ISO 9776, Oil of *Mentha arvensis* partially dementholized (*Mentha arvensis* var. *Piperascens* Malinv. and var. *glabrata* Holmes), ISO, Geneva, 1999, 6 pp.

flame during the course of the test procedure. This specification is extremely important from a handling, storage, packaging, labeling, and transportation standpoint. In the U.S., the Department of Transportation requires special handling and labeling instructions for materials whose flash point is below 60.5°C. Finally, additional specifications such as weight loss on evaporation, ash content, acid value, ester value, trace metal determinations, etc., have become important secondary considerations depending on the material and test circumstances. Secondary specifications are often considered only during an annual or biannual audit.

Originally, determinations of total alcohols, esters, or ketones were made using wet chemical methods. With the advent of gas chromatography (GC), these measurements have been replaced with specific component composition determinations by GC or, more commonly, a GC profile analysis.

Typical GC conditions would be as follows:

- Capillary column: 60 m × 0.25 mm (ID)
- Liquid phase: carbowax 10 (polar); DB-1 (nonpolar)
- GC conditions: carrier gas, helium at a flow rate of 1 mL/min
- Injection volume: 0.5 μL split 1:150 or an estimated 3 to 5 ng on-column injection
- Temperature program: 75°C to 220°C; initial hold for 8 min; 4°C per min; final hold for 20 min
- General parameters: injector temperature, 250°C; detector, 300°C; detection system, flame ionization detector (FID)

TABLE 9.9
Physicochemical Specifications for Spearmint Oil

Specification	1[a]	2[b]	3[a]
Density (20°C)	0.917–0.934[a]	0.921–0.932	0.917–0.934[a]
Refractive index (20°)	1.484–1.491	1.484–1.491	1.484–1.491
Optical rotation (20°)	−48° to −59°[a]	−48° to −59°[a]	−48° to −59°[a]
Total ketones (%) (as carvone)	55.0 min	60.0 min	55.0 min

Source: 1. *Food Chemical Codex* National Academy Press, Washington, D.C., 1996; 2. ISO International Organization for Standardization, Oil of Spearmint—Part 1: Native type (*Mentha spicata* L.), ISO 3033-1, Geneva, 2005, 6 pp. 3. Fragrance Materials Association of the United States 1991.

[a]Applicable to both Native and Scotch spearmint.

[b]Data applicable to Native spearmint.

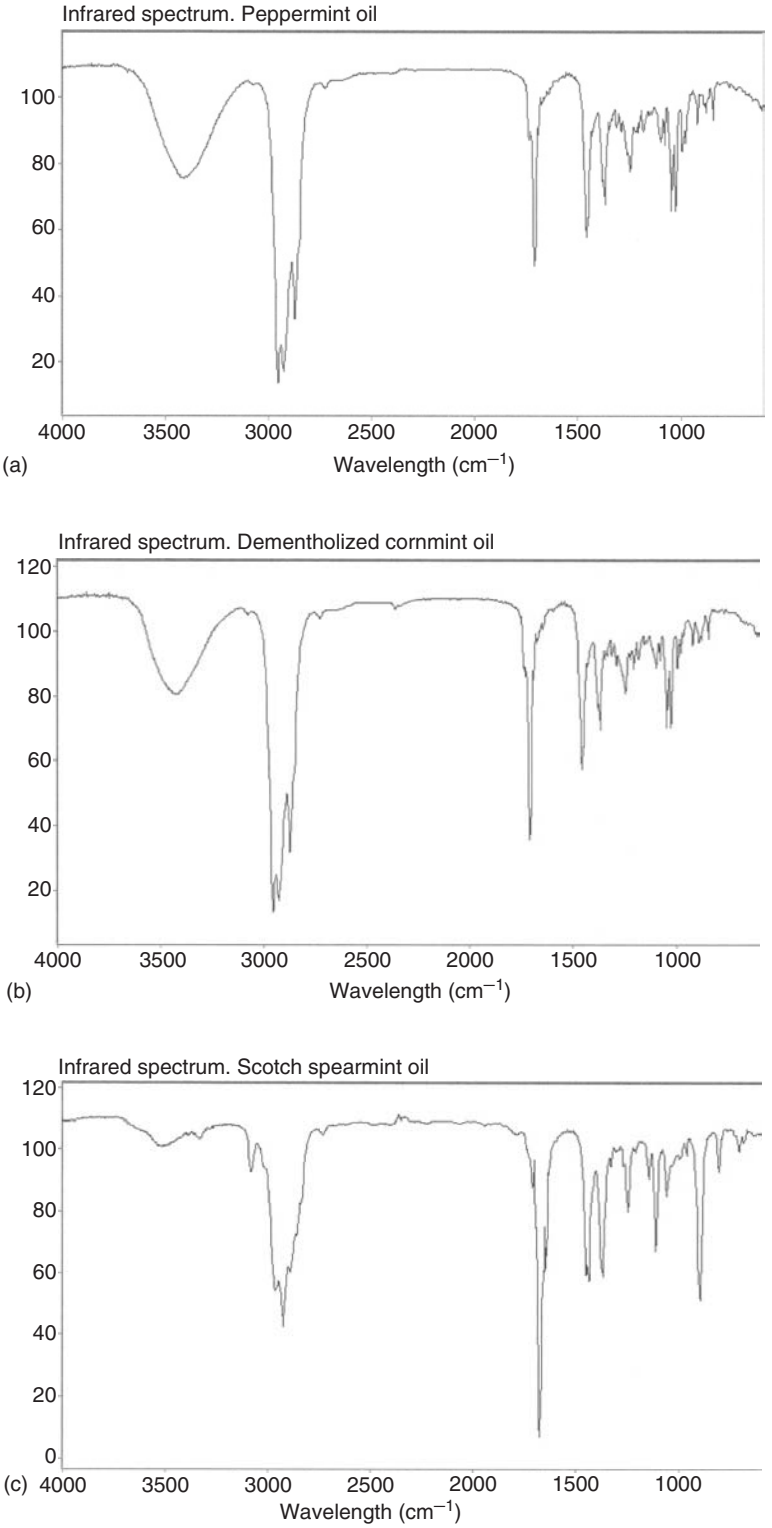


FIGURE 9.5 Infrared spectra for peppermint, dementholized cornmint, Scotch spearmint, and Native spearmint oils.

continued

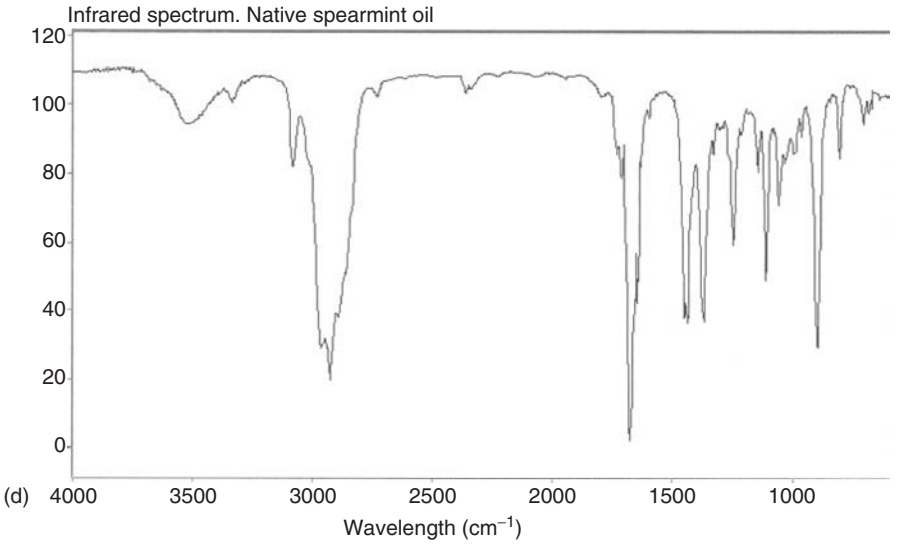


FIGURE 9.5 (continued)

Examples of GC profiles using both polar and nonpolar columns for peppermint, dementholized cornmint, Scotch spearmint, and Native spearmint oils are presented in Figure 9.6 and Figure 9.7, respectively.

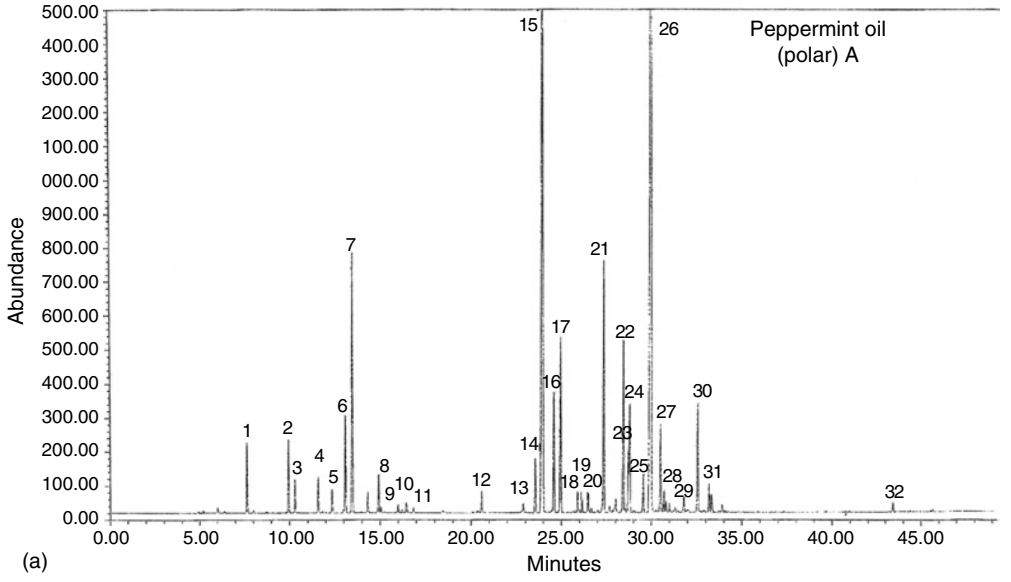


FIGURE 9.6 Polar GC profiles of peppermint, dementholized cornmint, Scotch spearmint, and Native spearmint oils. (a) 1. α-Pinene, 2. β-pinene, 3. sabinene, 4. myrcene, 5. α-terpinene, 6. limonene, 7. 1,8-cineole, 8. (Z)-β-ocimene, 9. γ-terpinene, 10. (E)-β-ocimene, 11. *p*-cymene, 12. 3-octanol, 13. 1-octen-3-ol, 14. trans-sabinene hydrate, 15. menthone, 16. menthofuran, 17. isomenthone, 18. β-bourbonene, 19. neomenthyl acetate, 20. linalool, 21. menthyl acetate, 22. neomenthol, 23. terpinen-4-ol, 24. β-caryophyllene, 25. neoisomenthol, 26. menthol, 27. pulegone, 28. (E)-β-farnesene, 29. α-terpineol, 30. germacrene D, 31. piperitone, 32. viridiflorol.

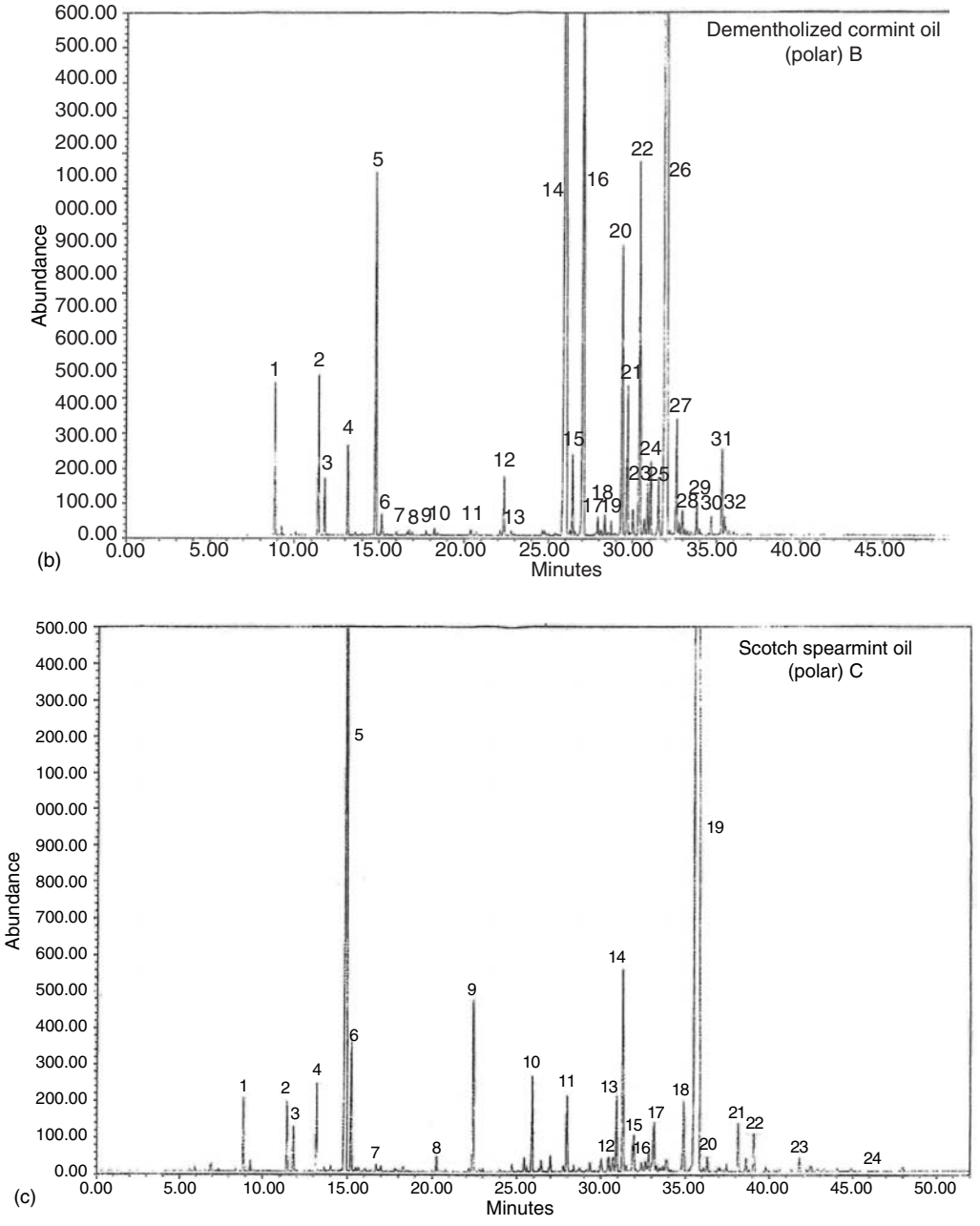


FIGURE 9.6 (continued) (b) 1. α-Pinene, 2. β-pinene, 3. sabinene, 4. myrcene, 5. linalool, 6. 1,8-cineole, 7. (Z)-β-ocimene, 8. γ-terpinene, 9. (E)-β-ocimene, 10. terpinolene, 11. 3-octyl acetate, 12. 3-octanol, 13. 1-octen-3-ol, 14. menthone, 15. (Z)-3-hexenyl isovalerate, 16. isomenthone, 17. β-bourbonene, 18. neomenthyl acetate, 19. linalool, 20. menthyl acetate, 21. isopulegol, 22. neomenthol, 23. terpinen-4-ol, 24. β-caryophyllene, 25. neoisomenthol, 26. menthol, 27. pulegone, 28. (E)-β-farnesene, 29. α-terpineol, 30. germacrene D, 31. piperitone, 32. carvone. (c) 1. α-Pinene, 2. β-pinene, 3. sabinene, 4. myrcene, 5. limonene, 6. 1,8-cineole, 7. γ-terpinene, 8. 3-octyl acetate, 9. 3-octanol, 10. menthone, 11. β-bourbonene, 12. terpinen-4-ol, 13. β-caryophyllene, 14. cis-dihydrocarvone, 15. menthol, 16. (E)-β-farnesene, 17. dihydrocarvyl acetate, 18. germacrene D, 19. carvone, 20. piperitone, 21. cis-carvyl acetate, 22. cis-carveol, 23. (Z)-jasmone, 24. viridiflorol.

continued

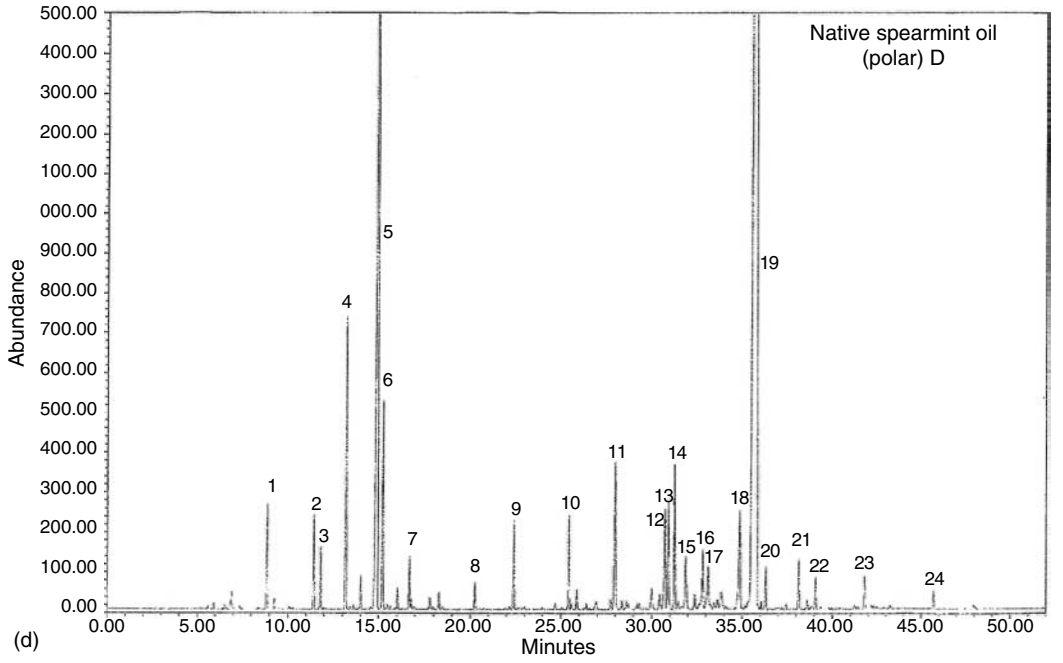


FIGURE 9.6 (continued) (d) 1. α-Pinene, 2. β-pinene, 3. sabinene, 4. myrcene, 5. limonene, 6. 1,8-cineole, 7. γ-terpinene, 8. 3-octyl acetate, 9. 3-octanol, 10. trans-sabinene hydrate, 11. β-bourbonene, 12. terpinen-4-ol, 13. β-caryophyllene, 14. cis-dihydrocarvone, 15. menthol, 16. (E)-β-farnesene, 17. α-terpineol, 18. germacrene D, 19. carvone, 20. piperitone, 21. cis-carvyl acetate, 22. cis-carveol, 23. (Z)-jasmone, 24. viridiflorol.

9.4 REGULATORY CONSIDERATIONS

From a regulatory standpoint, the specifications defining peppermint oil state that the oil must be obtained from *M. piperita* by physical means, usually steam distillation, which precludes the use of related whole oils and by-products from such products as cornmint oil (*M. canadensis*, sometimes erroneously known as *M. arvensis*). Noting market practice in food applications, regulatory standards covering the use of mint oil blends containing *M. piperita* and *M. canadensis* would be to classify the finished products as a natural peppermint flavor.

Table 9.7 and Table 9.8 present specifications for both peppermint and dementholized cornmint oils. As is evident from the data, the oils share many similarities, while at the same time they are quite different. Chemically, the differences are largely quantitative resulting in substantial differences in quality as measured by the odor and flavor characteristics of the oil.

For peppermint oil, CAS 8006-90-4, specification guidelines are available through monographs published by the USP, FCC, and ISO regulatory committees. Interestingly, all the specifications define peppermint oil as *M. piperita* L. (Family Labiatae) which, by definition, precludes *M. canadensis* or products containing *M. canadensis* from peppermint oil labeling under USP, FCC, or ISO standards of identity. Additionally, a study of the specifications indicates some important variances in the data. For example, the USP and FCC specifications for peppermint oil are closely related whereas the ISO specification allows for a significantly wider range for *l*-Menthol. Under the USP and FCC guidelines, total menthols (four isomers) must be present at the level of not less than 50%. The ISO specification recommends a range from 32% minimum to 49% maximum. This provision is important

because it permits the inclusion of the Asian grades of *M. piperita* oil, particularly those produced in India in which the levels of *l*-Menthol are generally lower than those of North American peppermint oil.

For spearmint oil, CAS 8008-79-5, specification guidelines are available through FCC and ISO monographs. Both the Scotch and Native spearmint oil grades are covered under the same FCC specification while the ISO specification covers only the Native spearmint oil quality. The specifications differ slightly with respect to the content of *l*-Carvone, the primary component of spearmint oil. In the case of the ISO specification, the *l*-Carvone level of the oil must fall within a 60% to 70% range. The FCC guidelines permit a minimum *l*-Carvone level of 55%. Importantly, the lower level permits the inclusion of the Native spearmint oil produced in India, which tends to have lower levels of *l*-Carvone, generally in the 55% to 60% range.

Beyond the FCC, ISO, and USP regulatory guidelines, other compliance factors become equally important to ensure that all peppermint and spearmint oils used in commercial applications are pure and safe for human consumption. The regulation of agrochemicals is one such example with the Environmental Protection Agency (EPA) in the U.S. actively mandating guidelines covering the use of agrochemicals on all agricultural crops. Table 9.10 presents a summary of the regulated materials specific for mint. Moreover, this list of approved materials is the subject of a constant review with the list and all subsequent updates recorded in the U.S. Code of Federal Regulations (CFR).

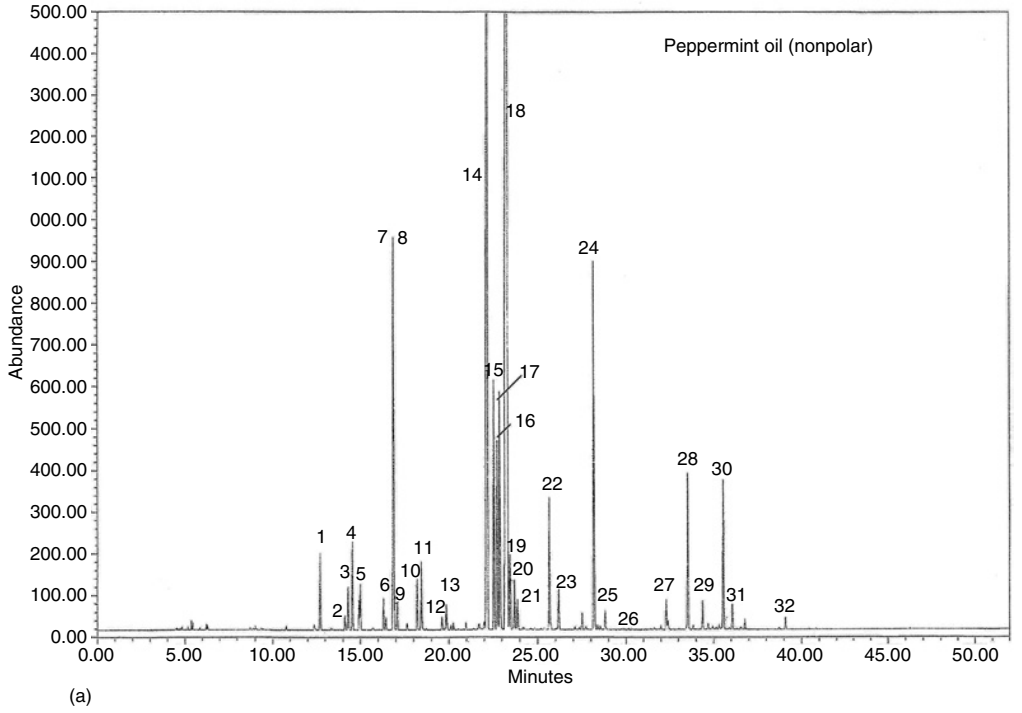


FIGURE 9.7 Nonpolar GC profiles of peppermint, dementholized cornmint, Scotch spearmint, and Native spearmint oils. (a) 1. α-Pinene, 2. 1-octen-3-ol, 3. sabinene, 4. β-pinene, 5. 3-octyl acetate, 6. α-terpinene, 7. limonene, 8. 1,8-cineole, 9. (Z)-β-ocimene, 10. γ-terpinene, 11. trans-sabinene hydrate, 12. terpinolene, 13. 3-octyl acetate, 14. menthone, 15. menthofuran, 16. isomenthone, 17. neomenthol, 18. menthol, 19. terpinen-4-ol, 20. cis-dihydrocarvone, 21. neodihydrocarveol, 22. pulegone, 23. trans-carvyl acetate, 24. menthyl acetate, 25. cis-carvyl acetate, 26. (Z)-jasmone, 27. β-bourbonene, 28. β-caryophyllene, 29. (E)-β-farnesene, 30. germacrene D, 31. spathulenol, 32. viridiflorol.

continued

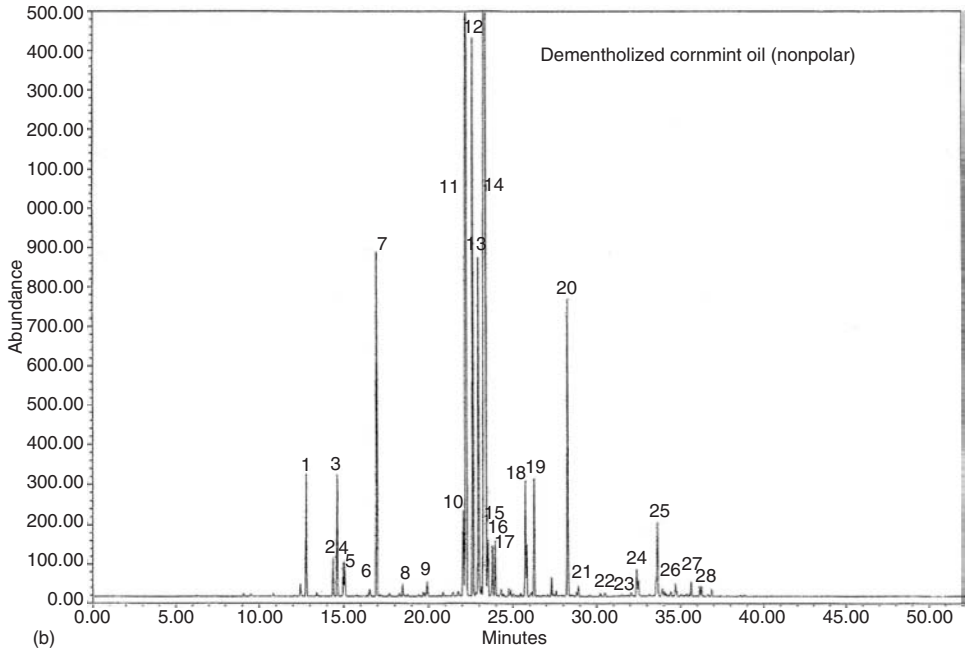


FIGURE 9.7 (continued) (b) 1. α-Pinene, 2. sabinene, 3. β-pinene, 4. myrcene, 5. 3-octanol, 6. α-terpinene, 7. limonene + 1,8-cineole, 8. γ-terpinene, 9. 3-octyl acetate, 10. isopulegol, 11. menthone, 12. isomenthone, 13. neomenthol, 14. menthol, 15. terpinen-4-ol, 16. cis-dihydrocarvone, 17. neodihydrocarveol, 18. pulegone, 19. piperitone, 20. menthyl acetate, 21. trans-carvyl acetate, 22. cis-carvyl acetate. 23. (Z)-jasmone, 24. β-bourbonene, 25. β-caryophyllene, 26. (E)-β-farnesene, 27. germacrene D, 28. spathulenol.

Referring to the materials outlined in Table 9.10, the detection limits and their unique chemistry require the use of specialized analytical procedures to ensure an accurate and complete analysis. To this end, special gas chromatographic detectors such as electron capture, flame photometric, and thermal ionic specific detectors are used. Additionally, high pressure liquid chromatography finds wide application in the analysis of these agrochemicals.

The published studies of Habenicht (1996) present an important example covering the application of this work. In studies targeting an analysis of organochlorine pesticides, Habenicht systematically evaluated the best-known methods for residue analysis and then analyzed a number of essential oils including peppermint and cornmint for possible contamination. In this study, both peppermint and cornmint oils produced in Asia, Europe, and North America during the period 1985 through 1995 were shown to contain trace levels of several organochlorine pesticides. Without knowing the origin of the oils and knowing that North American mint growers have not used organochlorine pesticides since the early 1970s, full comment on the data is difficult. Understanding that organochlorine pesticides can have a long half-life in the soil, residual contamination from the soil could be a subject worthy of further study. Importantly, as the Habenicht study would suggest, agrochemical analyses are valuable methodologies in order to guarantee ingredient purity and safety.

9.4.1 Organoleptic Integrity

By their very nature, published specifications are focused on physical data with the associated measurements targeted on the chemical constants and composition of a specific mint oil.

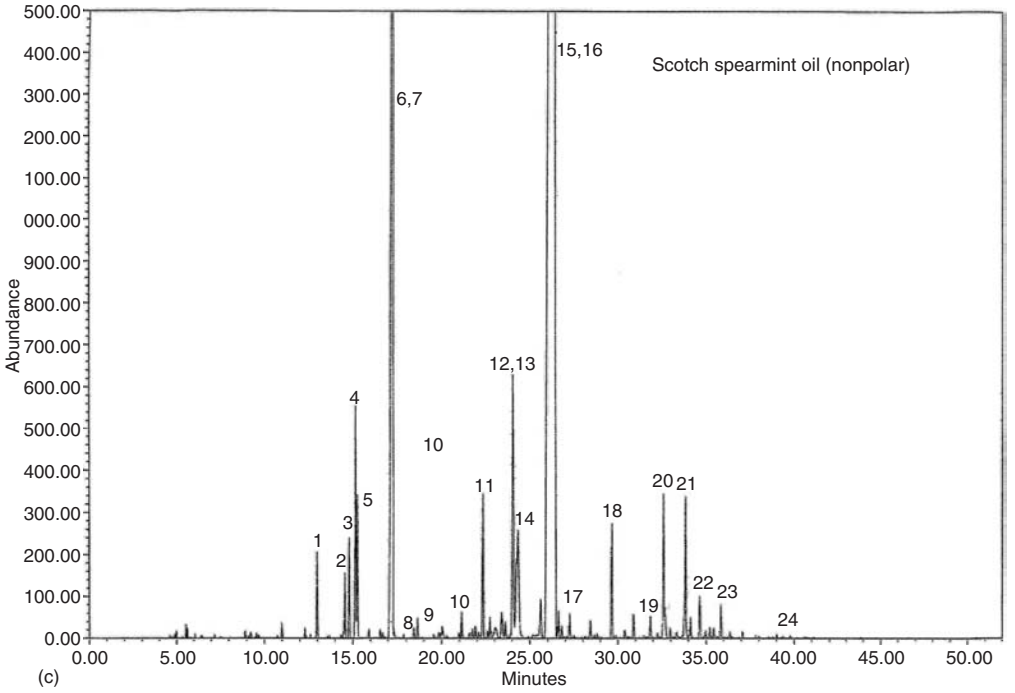


FIGURE 9.7 (continued) (c) 1. α-Pinene, 2. sabinene, 3. β-pinene, 4. 3-octanol, 5. myrcene, 6. limonene, 7. 1,8-cineole, 8. γ-terpinene, 9. trans-sabinene hydrate, 10. linalool, 11. menthone, 12. terpinen-4-ol, 13. cis-dihydrocarvone, 14. trans-dihydrocarvone, 15. carvone, 16. trans-carveol, 17. piperitone, 18. cis-carvyl acetate, 19. (Z)-jasmone, 20. β-bourbonene, 21. β-caryophyllene, 22 (E)-β-farnesene, 23. germacrene D, 24. viridiflorol.

continued

Understanding that peppermint and spearmint oils are used principally because of their flavor and odor, organoleptic measurements need to be a central consideration of all quality determinations.

Peppermint and spearmint oils are extremely complex and each oil is known to contain several hundred different constituents. Significantly, the flavor and odor characteristics of an oil are a function of a unique qualitative or quantitative balance of these various constituents. While the physical data are clearly controlled by those constituents present at the highest concentration, it is important to realize that trace constituents can often determine the ultimate character and quality of the oil. In this context, organoleptic significance becomes a consideration whenever an ingredient is present at levels that are higher than its detection threshold. Using dimethyl sulfide as an example, this material was found to have a detection threshold of 3 to 10 ppb and that it can be present in mint oils at levels of up to 100 ppm. Despite the very low concentration of dimethyl sulfide, the compound is organoleptically significant, because when it is present at 100 ppm, it is approximately 1000 times higher than its detection threshold. Each growing district or region will have its own distinctive character and profile and the organoleptic differences between the various peppermint oil types will be quantitative in nature. This same analogy will apply to spearmint oil as well. Sensory testing methodologies for flavor and odor need to be a central part of every quality control program. Figure 9.8 is an example of how a standard descriptive analysis methodology can be used to profile a typical peppermint and Scotch spearmint oil.

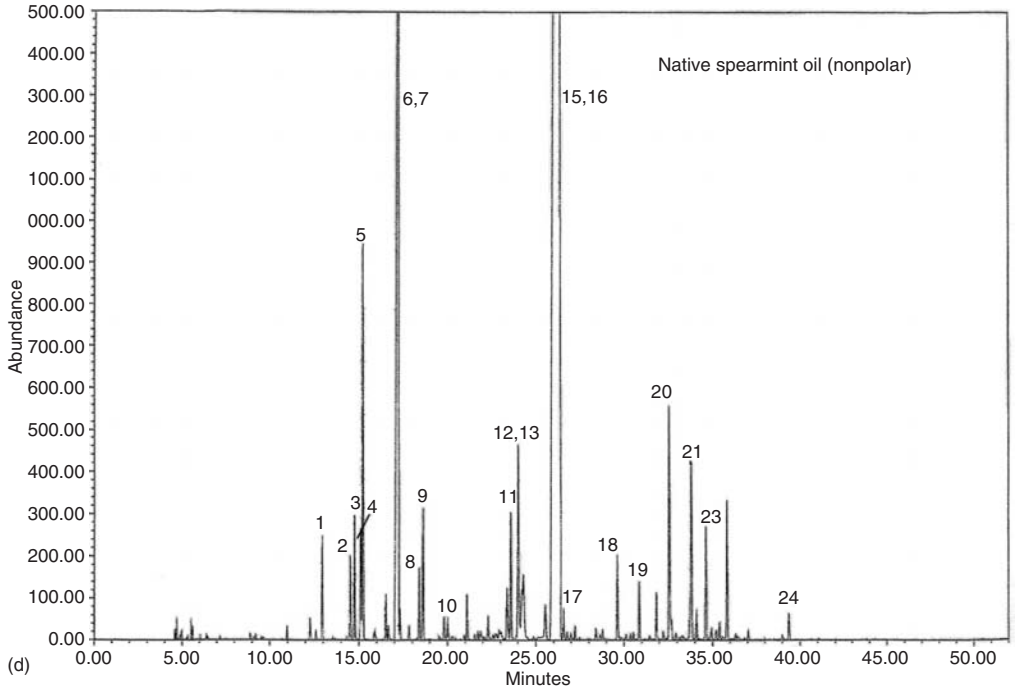


FIGURE 9.7 (continued) (d) 1. α-Pinene, 2. sabinene, 3. β-pinene, 4. 3-octanol, 5. myrcene, 6. limonene, 7. 1,8-cineole, 8. γ-terpinene, 9. trans-sabinene hydrate, 10. 3-octyl acetate, 11. menthol, 12. terpinen-4-ol, 13. cis-dihydrocarvone, 14. trans-dihydrocarvone, 15. carvone, 16. trans-carveol, 17. piperitone, 18. cis-carvyl acetate, 19. (Z)-jasmone, 20. β-bourbonene, 21. β-caryophyllene, 22. (E)-β-farnesene, 23. germacrene D, 24. viridiflorol.

Mint oil composition is clearly a function of many factors with the metabolic or biosynthetic pathways as the key determinants in controlling the formation and ultimate level of the various components in the oil. In addition, however, it is important to acknowledge the role of many other factors that have a direct impact on the manner in which these components are formed. For example, extrinsic conditions such as climate, environment, horticultural practice, etc. and intrinsic conditions such as the cultivar and maturity of the plant at the time of harvest are extremely important. As a final consideration, the recovery of the oil from the mint herbage by steam distillation becomes an important matter capable of impacting the overall quality and composition of the oil. In this regard, it is important to acknowledge the presence of mint oil components that are formed during the distillation process.

9.4.2 Peppermint Oil Authenticity

Ingredient authenticity is a critical consideration in terms of labeling compliance and, in light of market practice, this is especially important for peppermint oil. As noted, ingredient statements that specify peppermint oil, by definition, must be formulated with *M. piperita* and not include cornmint or related by-products such as dementholized cornmint oil. Importantly, through an analysis of production and export statistics from peppermint oil exporting countries, it becomes immediately clear that, in recent years, export volumes of

TABLE 9.10
Approved Agrochemicals for Peppermint and Spearmint Cultivation

Chemical Name	Manufacturer	EPA Max Tolerance Level (ppm)
Assure II (Quizalofop)	DuPont	2.00
Basagran (Bentazon)	BASF	4.00
Buctril (Bromoxynil)	Rhone-Poulenc	0.10
Chateau (Flumioxazin)	Valent	0.04
Command (Clomazone)	FMC	0.05
Devrinol (Napropamide)	Zeneca	0.10
Goal (Oxyfluorfen)	Rohm & Haas	0.25
Gramoxone (Paraquat)	ICI	3.00
Karmex (Diuron)	DuPont	2.00
Poast (Sethoxydim)	BASF	30.00
Sinbar (Terbacil)	DuPont	2.00
Select (Clethodim)	Valent	0.50
Stinger (Clopyralid)	DowElanco	3.00
Spartan (Sulfentrazon)	FMC	0.30
Tough (Pyridate)	Novartis	0.20
Treflan (Trifluralin)	Dow AgroSciences	2.00
Vine-der (2,4 D-B)	Platte	0.20
Comite (Propargite)	Uniroyal	50.00
Confirm (Tebufenozide)	Rohm & Haas	10.00
Dyfonate (Fonofos)	Zeneca	0.10
Kelthane (Dicofol)	Rohm & Haas	25.00
Lannate (Methomyl)	DuPont	2.00
Lorsban (Chlorpyrofos)	DowElanco	8.00
Malathion 8 (Malathion)	Wilbur-Ellis	8.00
MSR (Oxydemeton-M)	Gowan	12.50
Onager (Hexythiozox)	Gowan	2.00
Orthene (Acephate)	Valent	15.00
Tedion (Tetradifon)	FMC	100.00
Bravo (Chlorothalonil)	ISK Biotech	2.00
Quadris (Azoxystrobin)	Syngenta	30.00
Rally (Myclobutanil)	Rohm & Haas	3.00
Vydate (Oxymyl)	DuPont	10.00
Tilt (Propiconizole)	Novartis	0.30

M. piperita oil far exceed the reported production volumes. Given this circumstance, end users need a careful monitoring of their source of supply.

Beyond supplier audits, analytical measurements have become an important means to validate authenticity. Table 9.11 presents an important summary covering established component averages that have been developed over an extended period of time (Spencer et al., 1997). For purposes of comparison, Table 9.12 presents typical levels for those same key peppermint components found in dementholized cornmint oil of both Indian and Chinese origins. As can be seen from the data, Isomenthone and Isopulegol have become useful markers to distinguish the presence of cornmint oil and its by-products in mint flavor formulations. With reference to the data, elevated levels of these components become an especially good indicator of peppermint oil adulteration.

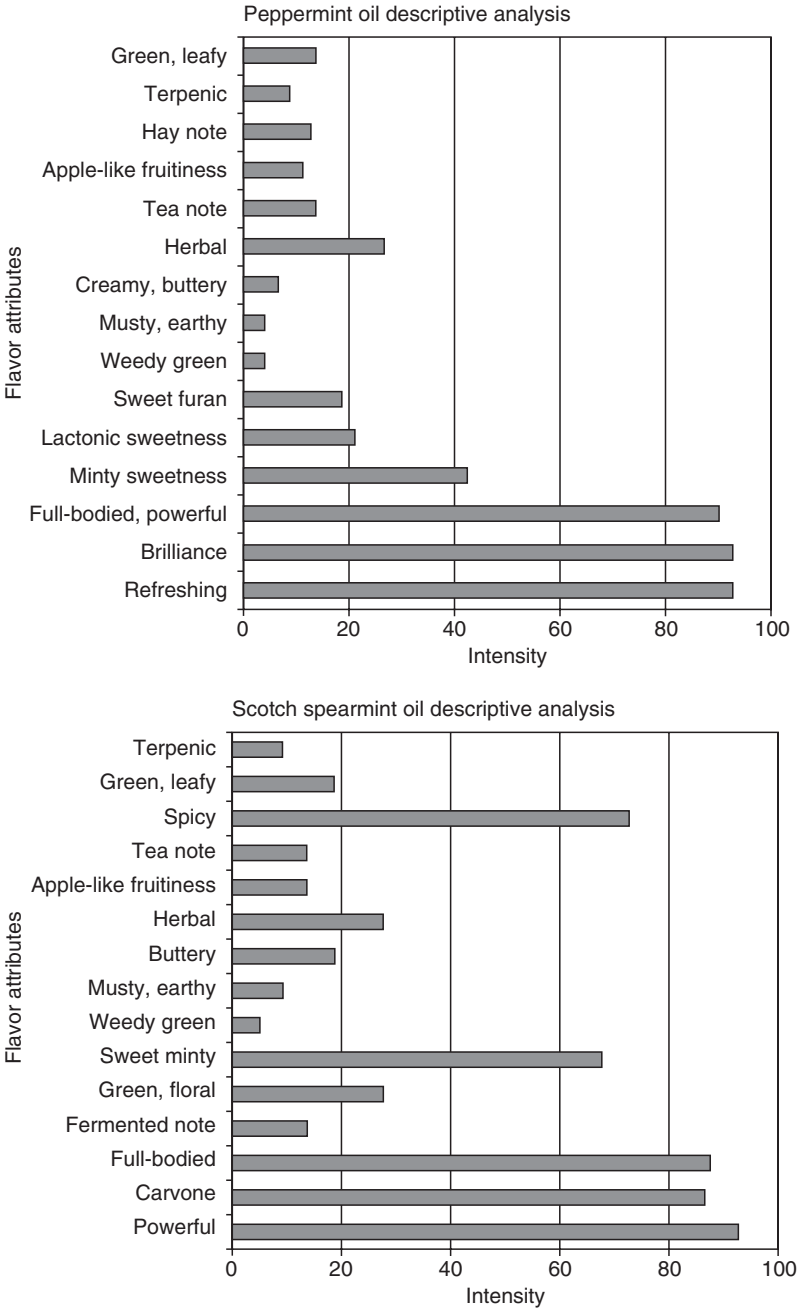


FIGURE 9.8 Descriptive analysis, peppermint, and Scotch spearmint oils.

9.5 IMPROVEMENT OF OIL QUALITY OR COMPONENT ISOLATION

Blackwell et al. (1984) published a patent detailing a procedure to reduce the menthofuran content by a Diels–Alder reaction with maleic anhydride. The maleic anhydride forms a crystalline adduct with menthofuran, which is removed by filtration. The removal of harsh notes from either peppermint or spearmint oils through a treatment of the oil by an oxidizing

TABLE 9.11
Average Composition (%) of Key Peppermint Oil Components over a Period of Five Crop Years

	Crop Year Results (%)					
Component	**1 (263)[a]**	**2 (243)[a]**	**3 (279)[a]**	**4 (330)[a]**	**5 (237)[a]**	**Mean**
Limonene	1.342	1.426	1.508	1.527	1.455	1.452
1,8-Cineole	5.337	4.835	4.970	4.890	4.918	4.990
trans-Sabinene hydrate	1.010	0.954	0.891	0.969	1.054	0.976
Isomenthone	2.570	2.734	2.739	2.728	2.527	2.660
Isopulegol	0.055	0.080	0.068	0.070	0.076	0.070
Neoiso(iso)pulegol	0.019	0.036	0.028	0.031	0.031	0.029
Piperitone	0.336	0.524	0.519	0.457	0.449	0.457

[a]Number of samples.

agent such as hydrogen peroxide, ozone, or mixtures thereof and in combination with acids such as hydrochloric acid, sulphuric acid, or phosphoric acid was patented by Hussein (1987). The reduction of pulegone in peppermint oil by a stereospecific method of reduction by hydrogenation of the pulegone *in situ* at a neutral pH with a saturated solution of sodium sulphite in glacial acetic acid was patented by Spencer (1989).

Peppermint and spearmint oils can be used directly as the recovered oil from the steam distilled mint herbage or they can be further distilled or rectified (fractional distillation) in order to refine the character and adjust the overall profile. In practice, rectification schemes normally involve the removal of varying amounts of the volatile and high boiling fractions and then collecting a heart-cut or center fraction as the rectified oil. Through this process, significant volumes of by-products containing some very interesting ingredients are made available. The by-product streams are further processed and become a source of natural flavor chemicals for use by flavorists in new flavor formulations. Examples of these flavor chemicals were mentioned earlier and presented in Table 9.5.

9.6 MINT OIL STORAGE CONDITIONS

Mint oils should be packaged in suitable glass, galvanized aluminum, plastic, stainless steel, or epoxy-lined drums. As mint oils are recovered from the mint herbage by steam distillation,

TABLE 9.12
Average Composition (%) of Key Peppermint Oil Components in Indian and Chinese Cornmint Oils

Component	**Indian Cornmint Oil**	**Chinese Cornmint Oil**
Limonene	2.200	2.875
1,8-Cineole	0.500	0.440
trans-Sabinene hydrate	0.006	0.022
Isomenthone	10.270	9.200
Isopulegol	2.020	1.218
Neoiso(iso)pulegol	1.800	1.030
Piperitone	1.080	1.406

there can be a tendency to have a small amount of water dissolved in the oil, which can later separate or cause a cloudiness to develop in the product. Therefore, before storage, the oils should be examined for moisture and any residual water removed from the bottom of the drum. The drums should be filled to within 95% of their capacity to reduce the headspace above the oil while allowing space for the likelihood of temperature-induced expansion. Prior to sealing, it is preferable to purge the headspace of the drum with nitrogen or another inert gas to reduce the presence of oxygen. It is recommended that the drums be stored in a cool and protected location.

REFERENCES

Allured's Flavor and Fragrance Materials (2003). Allured Publishing Corp., Carol Stream, IL.

Association Français de Normalization (1996a). AFNOR Specification, Huile essentielle de Menthe poivrée. NF T 75–210. In: *Huiles Essentielles*, Vol. 2, AFNOR, Paris, France, pp. 93–95.

Association Français de Normalisation (1996b). AFNOR Specifications, Huile essentielle de Menthe crêpue (*Mentha spicata Linnaeus*). NF T 74–245. In: *Huiles Essentielles*, Vol. 2, AFNOR, Paris, France, pp. 269–273.

Association Français de Normalisation (1996c). AFNOR specification, Huile essentielle de *Mentha arvensis* patiellement dementholée. NF T 75–306. In: *Huiles Essentielles*, Vol. 2, AFNOR, Paris, pp. 385–391.

Blackwell, B., Netherwood, S., and Piccolo, D.J. (1984). Process for flavoring chewing gum with stabilized peppermint oil. US Patent 4,456,621, June 26. 7 pp.

Christensen, R. (1994). The establishment and functionality of the Farwest Spearmint Marketing Order. In: Verlet, N., Ed., *4èmes Recontres Techniques et Economiques. Plantes Aromatiques et Médicinales*. Office National Interprofessionnel des Plates à Parfum Aromatiques et Médicinales, Nyons, France, pp. 180–197.

Clark, R.J. and Menary, R.C. (1979). Effects of photoperiod on the yield and composition of peppermint oil. *J. Am. Soc. Hortic. Sci.*, 104, 699–702.

Europäisches Arzneibach (2002). Pfefferminzöl, *Mentha piperitae aetheroleum*. 4. Aurgabe Grundwerbe 2002, B and 2 Monographier A–Z, Verlag Österreich GmbH, Berlin, pp. 2641–2643.

Food Chemical Codex, 4th Edition (1996). National Academy Press, Washington, D.C.

Habenicht, M. (1996). Rückstandsuntersuchung auf Organochlorpestizide in ätherischen Ölen. PhD thesis. Freign Universität, Berlin, 210 pp.

Hussein, M.M. (1987). Essential oils treated to remove harsh notes there from. US Patent 4,708,880, Nov. 24, 5 pp.

International Organization for Standardization (1981). ISO 856, Oil of peppermint (*Mentha piperita* L.) ISO, Geneva, 12 pp.

International Organization for Standardization (1999). ISO 9776, Oil of *Mentha arvensis* partially demontholized (*Mentha arvensis piperascens* Malinv. and var. *glabrata* Holmes). ISO, Geneva, 6 pp.

International Organization for Standardization (2005). Oil of Spearmint—Part 1: Native type (*Mentha spicata* L.), ISO, Geneva, 6 pp.

Landing, J.E. (1969). *American Essences. A History of the Peppermint and Spearmint Industry in the United States*, Kalamazoo Public Museum, Kalamazoo, MI.

Nigam, I.S. (1966). Analysis of volatile oils. In: *Pharmaceutical Chemistry* Vol. I, Chatten, L.G., Ed., Marcel Dekker, New York, pp. 439–479.

Spencer, J.S. (1989). Stable peppermint oil having reduced pulegone content and method of producing the same. US Patent 4,861,616, Aug. 29. 7 pp.

Spencer, J.S., Dowd, E., and Faas, W. (1997). The genuineness of two mint essential oils. *Perfum. Flavor*, 22 (3): 37–45.

US Pharmacopoeia, 26th Edition (2004). United States Pharmacopoeia Convention Inc., Rockville, Maryland.

10 Natural and Synthetic Menthol

Rudolph Hopp and Brian M. Lawrence

CONTENTS

10.1 INTRODUCTION

Natural menthol is obtained by freeze crystallization from crude cornmint oil. Although the oil has been produced in a number of different countries, the major cornmint oil producers are India and China. In general, 48% to 50% of the menthol present in cornmint oil can be

isolated by freeze crystallization. For example, from 100 kg of oil (in which menthol content ranges from 70% to 77%), 33.6 to 38.5 kg of menthol can be isolated using normal production procedures.

10.2 NATURAL MENTHOL

L-Menthol [(*1R*, *3R*, *4S*)-(−)-menthol] is an extremely important flavor and, to a lesser extent, fragrance item of commerce because of its well-known, unique cooling characteristics. Both natural and synthetic versions of this enantiomer can be obtained. The former is obtained from cornmint oil, and there are a number of ways for its synthetic production. The physicochemical properties of (−)-menthol are as follows:

Mpt.: 41°C−43°C
$[\alpha]_D^{20}$: −45° to −51° (10% solution in ethanol)
Assay: 99.3%
Heavy metals: <10 ppm Pb, <4 ppm As
Solubility: slightly soluble in H_2O (ca. 0.04%),
completely soluble in 90% aq. ethanol, propylene glycol, diethyl ether, chloroform, and liquid paraffin,
1:20 in 50% aq. ethanol,
1:2 in diethyl phthalate

A typical flow scheme for the isolation of natural menthol from crude cornmint oil is as follows:

1. The oil is collected at receiving stations located within cornmint oil production regions. Oil producers are paid according to the menthol content of their oil.
2. The crude oil is bulked in 200-kg mild steel drums and sent or sold to a crystallizing factory or an independent menthol powder producer (India only).
3. The crude oil is filtered to remove water and foreign material and bulked into large tanks.
4. The bulked oil is vacuum distilled by some crystallizers to remove the unpleasant top notes, which are bulked and sold to flavor and fragrance companies for the isolation of *cis*-3-hexenol [(Z)-3-hexenol)] or fractionally distilled by the menthol producer for the production of value-added products.
5. The oil containing 68% to 78% menthol is put into slightly tapered galvanized steel containers, which are, in turn, placed upright in a deep freezer. The temperature is maintained at −40°C or lower. This process, which is known as fast crystallization, yields an intermediate product known as menthol powder.
6. Once the block of menthol powder has been formed, the container is removed from the freezer and inverted on a drip tray to allow the residual dementholized mint oil (DMO) to drip.
7. After the oil has dripped away from the block of powder, it is broken up and centrifuged to remove the rest of the residual oil.
8. In India, a portion (up to 30%) of the total powder production is carried out on a small scale. This powder is sold to the crystallizers for incorporation with their own menthol powder or it is sold to China.
9. The crude menthol powder is melted and mixed with a small portion of DMO to produce a hot, enriched menthol solution in DMO.

10. In some of the crystallizer factories, this solution, which has a menthol content between 88% and 94%, is initially filtered to remove any foreign material that could be present in the purchased powder. It is then poured into a second galvanized steel container (60 to 80 kg capacity), which is, in turn, placed upright in a second freezer.
11. The enriched solution is slowly crystallized under a strict controlled cooling regimen in the freezer, in which the cold contact is applied at the bottom of the container. A temperature differential is maintained from the bottom to the top (which is less cool), thereby allowing large menthol crystal formations to take place from the bottom upward. To assist in crystallization, the enriched solution is seeded with pure menthol.
12. The cooling regimen in this slow crystallization stage is carefully controlled from room temperature to ca. –10°C over a 15 to 35-day period.
13. When the crystals have formed throughout the container, it is removed from the freezer and the block of large crystals is inverted on a drip tray to allow the remnants of DMO to be removed.
14. The large crystals are carefully broken up and centrifuged to remove any residual occluded DMO.
15. The menthol crystals are sieved or graded into large, medium, and small powder crystals. The menthol powder and small crystals can either be dried and packaged for sale or combined with virgin menthol powder to rework through the enriched menthol solution stage.
16. The medium and large menthol crystals are further dried to remove traces of residual DMO. This can be done by rack drying or with the aid of a belt dryer.
17. High-grade menthol crystals produced this way are packaged in polyethylene bags and packed into 25 or 50-kg fiber drums or in 25-kg corrugated cardboard boxes ready for sale.

A diagrammatic representation of the menthol isolation process can be seen in Figure 10.1 and Figure 10.2.

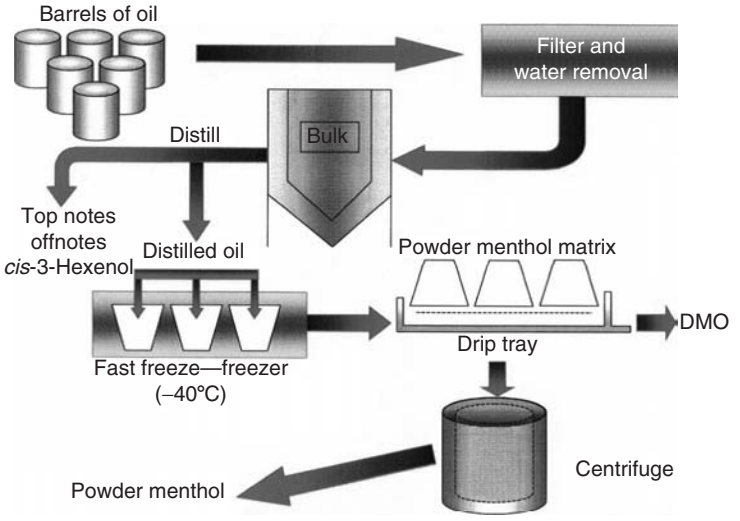


FIGURE 10.1 Natural menthol process Part 1.

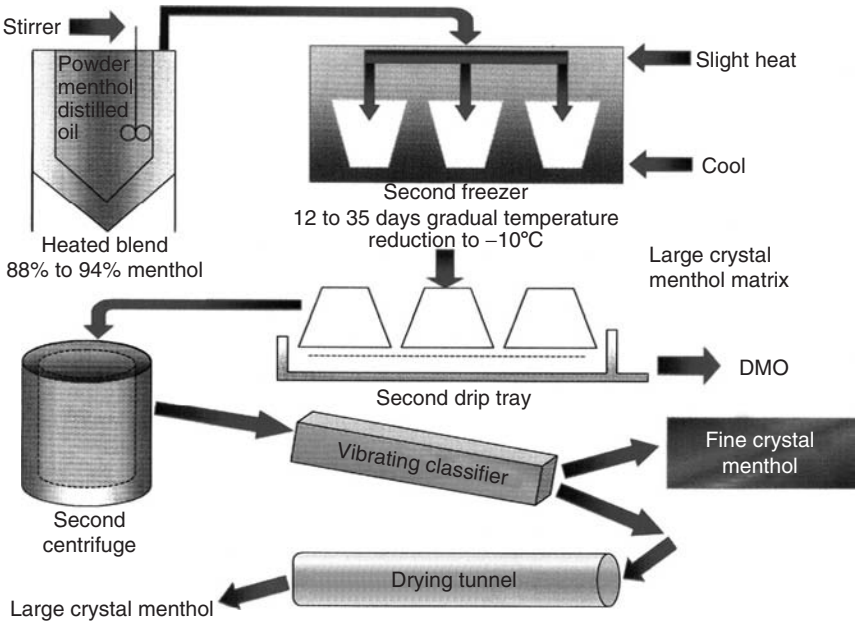


FIGURE 10.2 Natural menthol process Part II.

To understand the changing situation for worldwide menthol production, it is important to examine the history of cornmint oil cultivation and menthol production in Japan, Brazil, Paraguay, China, and India, the main countries of production.

10.2.1 Japan

Historically, the cornmint plant (along with tea) was introduced in Japan from China by Enzan, a Japanese priest, some 1750 years ago. The oldest record of cornmint oil being produced for its medicinal value was in 984. By 1870, a large acreage of cornmint was planted in Yamagata prefecture in the northern part of Japan's main island. Between 1870 and 1880, the technique of menthol freeze crystallization from crude cornmint oil was developed (Hiraizumi, 1959).

The first exports of menthol from Japan occurred in 1883. In the years between 1885 and 1895, cornmint cultivation spread to the Sapporo district and the Kitami province of Hokkaido (Japan's north island) and the southeastern part of Honshu (Japan's main island) in the Bizen and Bitchiu districts of Okayama and Hiroshima prefectures.

By 1909, although three harvests could be obtained from cornmint grown in the Bizen, Bitchiu, and other Honshu districts and only one harvest in the Kitami and Ischikari, Techio and Tokachi regions of Hokkaido, the cornmint oil production of the much larger Hokkaido region was about 60 tonnes, which represented over 90% of the total production (Gildemeister and Hoffmann, 1961). The authors who reported this also found that by 1912, 112 tonnes of oil was produced. The amount of oil and menthol produced between 1935 and 1957 can be seen in Table 10.1 (Guenther, 1949).

By the 1970s, crude cornmint oil production had decreased to less than 50 tonnes, although menthol production increased substantially because Takasago had started to produce synthetic menthol (see Table 10.2). More recent information on Japanese menthol production is not readily available, although it is believed that cornmint oil production has dropped to less

TABLE 10.1
Production Statistics for Crude Cornmint Oil and Menthol Export (Tonnes) from Japan (1935 to 1957)

Year	Crude Oil	Menthol
1935	500	309
1936	711	295
1037	807	375
1938	887	233
1939	645	269
1940	313	59
1941	172	131
1942	153	69
1943	150	24
1944	138	—
1945	84	—
1946	30	—
1947	30	—
1948	20	—
1949	20	—
1950	60	30
1951	200	40
1952	200	50
1953	110	50
1954	70	4
1955	260	10
1956	330	40
1957	360	90

than 10 tonnes. Natural menthol is still being produced in Japan for consumption within the country. It is produced from crude cornmint oil imported from both India and China. Synthetic production in Japan has become stabilized at about 1000 tonnes annually.

10.2.2 Brazil/Paraguay

After the great Tokyo earthquake of 1923, more than 200,000 Japanese immigrated to Brazil. It was these Japanese settlers who introduced cornmint cultivation and oil production starting in 1936. By 1943, oil production had grown to 80 tonnes. Before Japan entered the Second World War, it was the largest producer and exporter of menthol either from Japan itself or from material produced internally or from its controlled territories in China. Once the supply of menthol to the Western world had dried up, Brazil commenced production of crude cornmint oil and, more recently, Paraguay also commenced cornmint oil production. The production statistics for cornmint oil and menthol production in Brazil and Paraguay between the years of 1955 to 1975 and 1976 to 1999 can be seen in Table 10.3 and Table 10.4, respectively.

In Brazil, cultivation of cornmint uses the slash-and-burn practice, in which the virgin forest is cleared, the high-value timber trees are sold, a portion of the wood is saved as fuel, and the rest of the debris is burnt to clear the area. Although the area is cleaned of vegetation, the roots and some unburnt trunks are left throughout. In the partially cleaned

TABLE 10.2
Production Statistics for Crude Cornmint Oil and Menthol Export (Tonnes) from Japan (1958 to 1978)

Year	Crude Oil	Menthol
1958	200	60
1959	150	50
1960	130	40
1961	100	40
1962	100	40
1963	100	40
1964	100	40
1965	30	40
1966	80	40
1967	200	50
1968	180	50
1969	90	50
1970	60	60
1971	60	40
1972	50	70
1973	50	160*
1974	40	160*
1975	40	180*
1976	30	500*
1977	30	770*
1978	30	900*

*Includes synthetic menthol.

area, rooted cuttings (ca. 5 cm high) are planted. Because the land receives no fertilization from season to season, cornmint is productive as a source of oil for only 3 to 5 years at most on the same soil. After this time period, the soil is exhausted, so the land is sold to an arable crop farmer, and the cornmint grower clears another patch of virgin forest, and the cycle starts again.

In Brazil, cornmint was grown in the Ata Sorocaba zone (10%) in Saõ Paulo state and the Rio Ivai zone (90%) in Parana near the Paraguayan border. At one time, there were more than 1000 small distilleries in Parana (Sacco, 1967). These were found in the triangular region formed by the Rio Paraná and Rio Paranapanema junction along the railroad between Araguassú and Presidente Prudente, the most important production areas being Santo Anastácio and Presidente Prudente (Guenther, 1949).

According to Clark (1988), the price of menthol in the late 1960s was in such a depressed state that cornmint planting in Brazil had become unpopular. More recently, cornmint cultivation and oil production moved across the Brazilian border into Paraguay; however, because of the availability of Indian cornmint oil and the decreased prices for oil, production of oil in Paraguay has become very small. As can be seen from Table 10.4, the area under cultivation must have been extremely small as only 10 tonnes of oil was produced in 1999. Currently, menthol is being produced in Brazil and Paraguay, mainly from imported Indian cornmint oil. Although cornmint oil production has ceased in Brazil, a limited cultivation and oil production are still done in Paraguay.

TABLE 10.3
Production Statistics for Crude Cornmint Oil and Menthol Export (Tonnes) from Brazil and Paraguay (1955 to 1975)

	Oil		Menthol Export
Year	Paraguay	Brazil	Paraguay + Brazil
1955	—	300	170
1956	—	380	230
1957	—	500	290
1958	—	900	430
1959	—	1500	370
1960	—	600	350
1961	—	1800	620
1962	—	2000	930
1963	—	2000	1350
1964	—	2000	1010
1965	—	1700	720
1966	—	2100	870
1967	50	2900	1280
1968	50	3000	1400
1969	80	3000	1530
1970	100	3000	1380
1971	30	3500	1560
1972	150	4700	2230
1973	180	6300	2940
1974	260	3200	1680
1975	570	2800	860

10.2.3 China

Cornmint has been cultivated as a herbal crop for at least two centuries, as it is used in Chinese herbal medicine because of its cooling properties. It was cultivated in a small area in Jiangsu province. In 1923, the first large-scale production of cornmint oil and menthol was started in Shanghai. From the late 1920s until 1994, at least seven other large menthol crystallizing facilities that process cornmint oil were started (Watts, 1997). During this same time frame, cornmint cultivation spread into three provinces. As a result, cornmint cultivation and oil production can be found in Anhui province in the vicinity of Taihe, Linquan, and Bozhou; in Jiangsu province in the vicinity of Dongtai, Gaoyu, and Hiamen; and in Henan province in the vicinity of Shanqiu and Yongchen (Figure 10.3). These growing areas are within 200 km of the major provincial capitals of Heifei (Anhui), Nanjing (Jiangsu), and Zhengzhou (Henan) (Watts, 1997).

In these areas, all the cultivation is in the hands of smallholders. Menthol of Chinese origin is sold under a range of brand names such as White Cat, Penguin, Polar Bear, White Bear, Snow Peak, Glacier, AE, E&O, etc.; however, these brand names are meaningless as they are merely trademarks for menthol. Any of the crystallizers can produce any of these brand names as long as a fee is paid to the trademark holder for use of the name.

A summary of the crude cornmint oil and natural menthol produced in China between 1977 and 1999 can be seen in Table 10.5.

TABLE 10.4
Production Statistics for Crude Cornmint Oil and Menthol Export (Tonnes) from Brazil and Paraguay (1976 to 1999)

	Oil		Menthol Export
Year	Paraguay	Brazil	Paraguay + Brazil
1976	1000	1800	1300
1977	1500	1500	800
1978	2000	1000	800
1979	2000	500	800
1980	1500	400	700
1981	1500	300	700
1982	1500	100	600
1983	1500	100	600
1984	1000	100	500
1985	1600	50	500
1986	1100	50	500
1987	1100	40	500
1988	100	30	400
1989	500	35	400
1990	400	30	300
1991	200	25	200
1992	50	20	200
1993	50	10	200
1994	50	15	200
1995	25	—	400
1996	25	—	400
1997	25	—	200
1998	10	—	350
1999	10	—	300

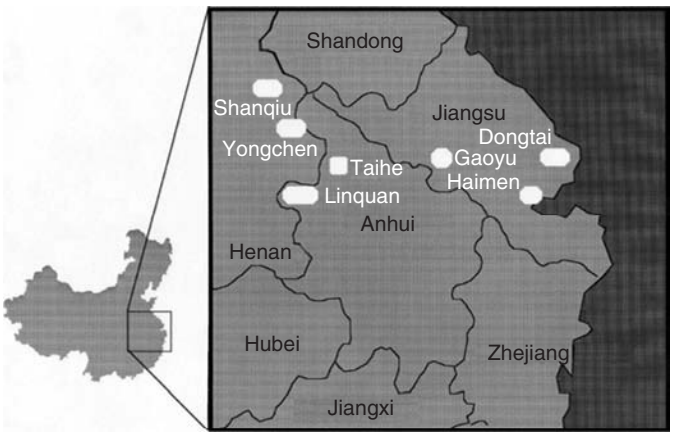


FIGURE 10.3 (See color plate following page 398.) Chinese cornmint growing areas.

TABLE 10.5
Production Statistics for Crude Cornmint Oil and Menthol (Tonnes) in China (1977 to 1999)

Year	Crude Oil	Menthol
1977	1000	540
1978	1200	650
1979	2500	1400
1980	4000	2200
1981	5000	2700
1982	6000	3300
1983	6500	3500
1984	5000	2700
1985	8000	4300
1986	7500	4100
1987	6500	3500
1988	5000	2700
1989	4500	2400
1990	5000	2200
1991	5500	3000
1992	6500	3500
1993	5500	3000
1994	4000	2200
1995	4500	2400
1996	2500	1400
1997	4500	2400
1998	4000	2000
1999	3500	1800
2000	2800	1500
2001	2500	1400
2002	2600	1450
2003	2300	1300
2004	2300	1300

10.2.4 India

Cornmint was introduced in 1955 from Japan by the Regional Research Laboratory (RRL), Jammu and Kashmir, with the assistance of UNESCO. Plantings were established in Jammu and the Tarai region of Uttar Pradesh after initial clonal multiplication at RRL. Over the next 20 or 50 years, this clone of cornmint was indiscriminately multiplied and divided by local farmers to a point at which there was a deterioration in oil yield and, more importantly, menthol content (Clark, 1998). As a result, there was an effort to hybridize, develop, and select new strains of cornmint that would yield more oil with a menthol content of about 70% under rural farming conditions. This program, which was carried out in the government supported RRLs and Central Institutes of Medicinal and Aromatic Plants, resulted in the release of a number of new strains of cornmint, such as MAS-1, HY-77, and Shivalik 88. The Shivalik strain became the most popular and from 1988 onward it has been widely grown as the main cornmint cultivar (Nijjar, 1990). More recently, Saksham and Kushal (two new cultivars) have been introduced on a limited basis (Vaze, 2004). It is expected that these high oil-yielding cultivars will start to replace the Shivalik cultivar over the next few years.

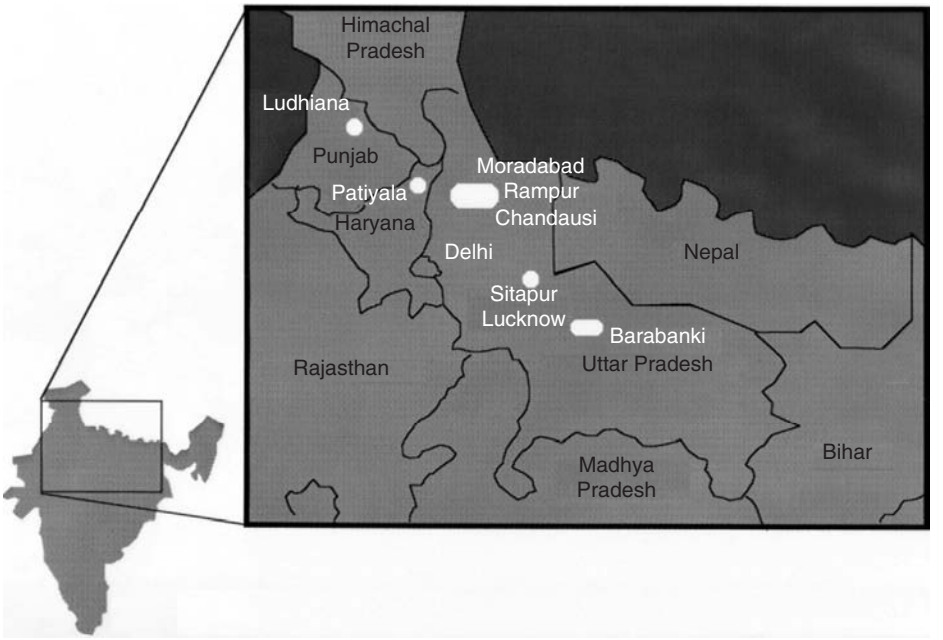


FIGURE 10.4 (See color plate following page 398.) Indian cornmint growing areas.

Cornmint oil production in India is in the hands of small farmers. It is estimated that there are more than 100,000 ha devoted to cornmint cultivation and oil production (Figure 10.4). The cultivation and oil production of cornmint result in the employment of more than 500,000 people during the crop and oil production cycle. The main areas of cornmint production are as follows:

1. Moradabad district (Uttar Pradesh) around towns of Sambhal, Amroha, Chandausi, Bahjoi, and Moradabad
2. Badaun and Bareilly districts (UP) around the towns of Badaun, Bareilly, Bisauli, and Gawar
3. Rampur district (UP) around the towns of Rampur, Bilaspur, Suar, Bazpur, Tanda, Bhojpur, and Shahabad
4. Barabanki district (UP) around the towns of Barabanki, Lucknow, Sitapur, and Masauli
5. Punjab state around Ludhiana and Jalandhar
6. Haryana state around Patiyala
7. Bihar state close to the UP border

It should be noted that the amount of cornmint oil produced in Punjab and Haryana is quite small in comparison with that obtained from Uttar Pradesh. From mid-1960 to 1970, the production of cornmint grew from 2 to 46 metric tonnes. A summary of the production of crude cornmint oil from 1970 to 1999 can be seen in Table 10.6.

In the late 1960s, the first menthol freeze crystallization program began in India. Once this first factory (Richardson Hindustan) showed that fine crystalline menthol could be successfully produced, numerous companies began their own crystallization factories. Some of these factories were developed with the help of Chinese and Japanese technologies whereas others developed their processes independent of outside assistance. As can be seen from Table 10.6, India has become the largest producer of natural menthol over the last few years.

TABLE 10.6
Production Statistics for Crude Cornmint Oil and Menthol Production (Tonnes) in India (1970 to 1999)

Year	Crude Oil	Menthol
1970	46	25
1971	100	55
1972	191	105
1973	174	94
1974	190	105
1975	250	92
1976	250	135
1977	250	135
1978	200	100
1979	250	135
1980	265	145
1981	300	160
1982	350	190
1983	400	200
1984	780	420
1985	2,000	1,100
1986	1,800	1,000
1987	2,200	1,200
1988	2,500	1,400
1989	2,600	1,400
1990	2,800	1,500
1991	3,000	1,600
1992	3,500	1,900
1993	3,800	2,100
1994	3,500	1,900
1995	5,500	3,000
1996	6,200	3,400
1997	6,500	3,500
1998	14,000	7,300
1999	11,000	6,500
2000	11,000	6,500
2001	13,000	7,800
2002	13,000	7,800
2003	15,000	9,000
2004	12,000	7,200
2005	15,000	7,800*

*Estimate.

In 1977, additional quantities of cornmint oil and menthol were produced in Taiwan (200 to 300 tonnes oil, 300 tonnes menthol), Thailand (30 tonnes oil, 30 tonnes menthol), and Argentina (100 tonnes oil, 40 tonnes menthol). Since then, Taiwan and Thailand have ceased producing cornmint oil and menthol, whereas approximately the same production levels are maintained in Argentina primarily to satisfy the internal menthol needs of L-menthol and DMO.

Natural menthol is also produced in Hong Kong, Singapore, Taiwan, and Vietnam. In the former three countries, it is produced from imported Indian cornmint oil. In Vietnam, the establishment of a cornmint oil and menthol industry is currently in its infancy.

10.3 SYNTHETIC MENTHOL

10.3.1 Historical Review

For more than 100 years, cornmint oil (*Mentha canadensis* oil) has been the main source of (−)-menthol, though numerous ways of partial or total synthesis had been developed during the course of the last century. In the past, until around 1970, synthetic (−)-menthol was produced only on a smaller scale by partial syntheses when menthol prices were high, because of shortfalls in the production of natural menthol. Preferred natural raw materials for these semisynthetic processes were optically active terpenoids, such as (−)-menthone or dementholized cornmint oil, respectively, and (−)-piperitone (ex *Eucalyptus dives* oil) and (+)-citronellal (ex citronella oil).

During the 1960s and 1970s, several multistep processes were developed, starting from readily available optically active terpenes, e.g., (+)-3-carene (ex Western U.S. or Indian turpentine), (−)-α-phellandrene (ex turpentine or *E. dives* oil), (−)-β-pinene from Eastern U.S. turpentine, and (+)-limonene from orange oil. None of these processes was successful in the long run, though the pinene and the carene route had been commercialized for some years. The drawbacks of these processes will be explained later.

A breakthrough in the production of synthetic (−)-menthol was achieved only when two manufacturers, Haarmann and Reimer (H&R) and Takasago International Co., made considerable investments to produce this aroma chemical on a large scale, using different technologies.

Haarmann and Reimer, using the thymol production of Bayer AG (Voges, 1991) as the starting material for (±)-menthol, applied the technique of enantioselective crystallization (see later) to resolve racemic menthyl benzoate to its optical antipodes (Fleischer et al., 1971). The first plant in Holzminden, Germany, went onstream in the year 1973, and the U.S. plant in Bushy Park near Charleston, South Carolina, has been in operation since 1978. Today, the overall capacity is 2000 metric tonnes a year.

Before 1984, Takasago used several of the already mentioned partial syntheses for (−)-menthol production. We are not sure if the enantioselective hydrolysis of racemic menthol esters by means of microorganisms, as claimed by a Takasago patent (Hattori et al., 1968), was also used on a commercial scale. Since 1984, Takasago has commercialized a modern process for manufacturing (−)-menthol by catalytic asymmetric synthesis (Akutagawa, 1992, 1999), converting myrcene to diethylgeranylamine, followed by the enantioselective isomerization to the enamine of (*R*)-(+) citronellal. (+)-Citronellal is a well-known raw material for the production of (−)-menthol via (−)-isopulegol. The Takasago facility at Iwata, Japan, has a capacity to manufacture about 2000 metric tonnes a year of (+)- or (−)-citronellal. Of this, 1500 tonnes was used for the production of (−)-menthol.

Thus, synthetic (−)-menthol production accounts for approximately 30% of the world market of about 12,000 metric tonnes [recommended reviews: Bauer et al. (1997); Bedoukian (1986); Hopp (1996); Leffingwell and Shackelford (1974); Traas (1982)].

10.3.2 The Stereoisomers of Menthol and Stereochemical Configuration

Owing to the three asymmetric carbon atoms in the molecule of *p*-menthan-3-ol, the four possible geometric isomers, menthol, neomenthol, isomenthol, and neoisomenthol, are existing as pairs of optical antipodes making a total of eight stereoisomers. The absolute

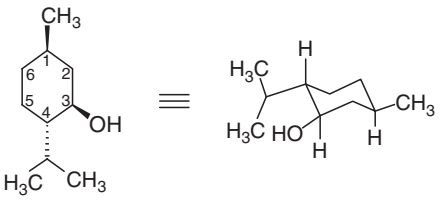


FIGURE 10.5 Stereoformulas of (−)-menthol.

configuration of (−)-menthol was determined as (1*R*, 3*R*, 4*S*)-menthol or, according to *Chemical Abstracts* nomenclature as [1*R*-(1α, 2β, 5α)]-5-methyl-2-(1-methylethyl)-cyclohexanol. Two preferred graphical formulas of (−)-menthol are shown in Figure 10.5. Formulas and (*RS*)-configurations of all menthol isomers can be seen in Figure 10.6.

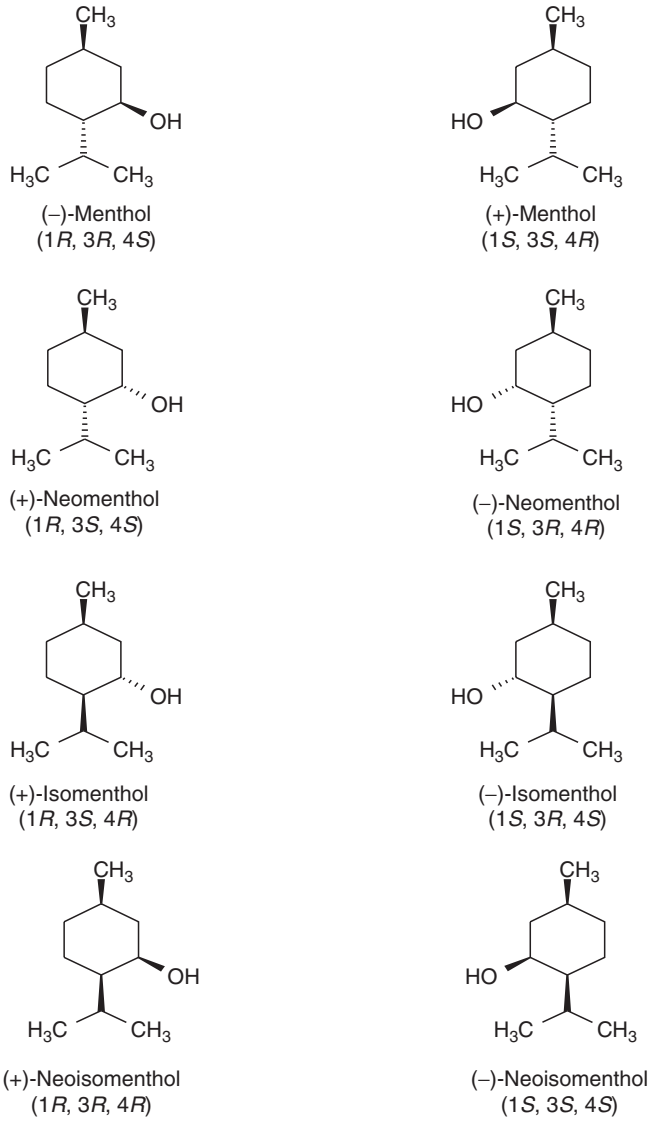


FIGURE 10.6 Stereoformulas and (*RS*)-configurations of the eight menthol isomers.

10.3.3 Sensory Properties and Physical Data

The unique sensory properties of (−)-menthol were confirmed by investigating the flavor profiles of the eight isomers at a concentration of 3 ppm in a 5% sugar solution (Emberger and Hopp, 1985). Each flavor attribute was evaluated by a panel of seven flavorists using a 5-point scale (1 very low, 5 very high). In Table 10.7 and Table 10.8 the flavor characteristics, scored higher than 1.0, and some physical constants of the (1*R*)- and (1*S*)-isomers, respectively, are summarized. (1*R*)-Menthols occur in peppermint oils, though (+)-neomenthol, (+)-isomenthol, and (+)-neoisomenthol are only minor or trace components.

(−)-Menthol obtained top values for freshness and cooling effect, whereas (+)-menthol received moderate ratings for "fresh" and "cool" but also showed some unpleasant notes like camphoraceous, musty, and bitter. The neomenthol isomers have no pronounced positive or negative flavor characteristics, but the isomenthol and neoisomenthol enantiomers obtained remarkably high scores of camphoraceous, musty, and earthy notes. Consequently, even traces of undesirable isomers may reduce the quality of synthetic (−)-menthol.

10.3.4 Partial Syntheses from Optically Active Raw Materials

10.3.4.1 General Aspects

At first sight, it appears very promising to use optically active terpenoids as starting materials for the production of (−)-menthol, as it is not necessary to introduce optical activity by a resolution step or by asymmetric synthesis. However, the economy of such processes depends highly on certain crucial factors:

1. Availability and price of the natural raw material
2. Its optical purity [see (+)-citronellal and (−)-piperitone]

TABLE 10.7
Flavor Characteristics and Physical Constants of the (1*R*)-Menthol Isomers

Isomer	Flavor Characteristics*	B.P. (°C) M.P. (°C) $[\alpha]_D^{20}$
(−)-Menthol	Fresh 4.2, cool 4.3, minty 2.3, sweet 2.8	216.5 43 −50°
(+)-Neomenthol	Fresh 1.8, cool 1.5, minty 1.5, earthy 1.2, musty 2.0, sweet 2.3	211.7 −15 +20.9°
(+)-Isomenthol	Fresh 2.1, cool 1.6, minty 2.0, carrot 2.1, herbaceous 1.2, earthy 1.8, musty 3.0, camphor 1.2, woody 2.1, sweet 1.5	218.6 82 + 25°
(+)-Neoisomenthol	Carrot 1.4, herbaceous 1.4, earthy 2.8, musty 4.7, camphor 3.0, woody 2.6	214.6 −8 +2.0°

*Scores higher than 1.0 in a 5-point scale: 1 very low, 5 very high. Mean values of seven scores given by a panel of seven flavorists.

TABLE 10.8
Flavor Characteristics and Physical Constants of the (1*S*)-Menthols

Isomer	Flavor Characteristics*	B.P. (°C) M.P. (°C) $[\alpha]_D^{20}$
(+)-Menthol	Fresh 2.7, cool 2.4, minty 1.9, musty 1.3, camphor 1.2, sweet 2.2, bitter 1.3	216.5 43 +50°
(−)-Neomenthol	Fresh 1.7, cool 1.3, minty 2.2, earthy 1.3, musty 2.0, camphor 2.0, sweet 1.8	211.7 −15 −20.9°
(−)-Isomenthol	Herbaceous 1.4, earthy 1.7, musty 3.4, sweet 1.6	218.6 82 −25°
(−)-Neoisomenthol	Fresh 1.7, cool 1.7, carrot 1.1, minty 2.8, hay 1.4, earthy 3.3, musty 3.8, camphor 2.2, woody 2.0, sweet 3.3	214.6 −8 −2.0°

*See Table 10.7 for score information.

3. Most important, synthetic steps of high regio- and stereoselectivities to retain the asymmetry of the starting material and to achieve a satisfactory overall yield

As mentioned earlier, only a few of the processes, developed in the laboratories, were commercialized or are used currently.

10.3.4.2 From Dementholized Cornmint Oil

Dementholized mint oil (DMO) is rich in (−)-menthol (30% to 40%) and other C3-oxygenated *p*-menthanes, which are convertible to (−)-menthol, such as (−)-menthone/(+)-isomenthone (20% to 40%), (−)-menthyl acetate (5% to 15%), and some minor components like (+)-neomenthol, (−)-isopulegol, (+)-pulegone, or (−)-piperitone (see Figure 10.7). Chiral GC analysis indicates that the optical purity of the main constituents is at least 99.5% (Werkhoff et al., 1991).

A simple process to recover additional (−)-menthol from DMO, i.e., the simultaneous reduction of menthones and saponification of menthyl acetate with sodium metal in ethanol at room temperature, was described by Chopra et al. (1972). After crystallization and centrifugation, 30% (−)-menthol was obtained. Alternatively, menthyl acetate is saponified with aqueous caustic soda at a reflux temperature, followed by esterification with boric acid and separation of the solid ester from menthone and terpenes by centrifugation. Hydrolysis of the boric acid ester and crystallization yields 24% to 28% (−)-menthol.

The reduction of menthones with nascent hydrogen predominantly gives the equatorial hydroxyl group, i.e., (−)-menthol from (−)-menthone and (+)-isomenthol from (+)-isomenthone, respectively, whereas the hydrogenation under mild conditions (e.g., with platinum on charcoal) yields not only the axial hydroxyl group (neomenthol/neoisomenthol), but also a considerable amount (20% to 40%) of the all-equatorial menthol (see Solodar, 1976).

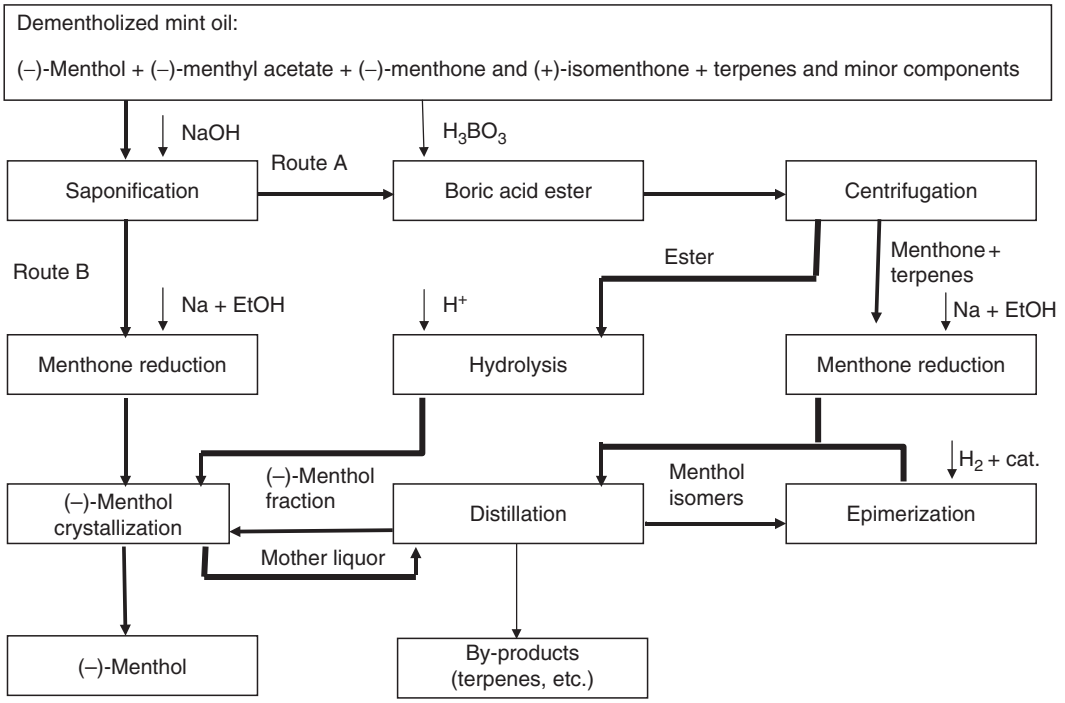


FIGURE 10.7 (−)-Menthol from dementholized mint oil.

Menthol and its isomers can be epimerized by means of sodium mentholate or hydrogen and certain catalysts at higher temperatures (Yoshida et al., 1965). After reaction with 0.8 mole equivalents of sodium metal, (+)-neomenthol or other isomers were heated at 190°C to 210°C for 12 h. According to GC analysis, the equilibrium mixture contained 8% to 9% menthones, 67% to 68% menthol, 8% to −9% neomenthol, 15% isomenthol, and 1% neoisomenthol. No racemization occurred. Isomerization with copper chromite catalyst under hydrogen pressure for 3.0 to 4.5 h at 185°C to 200°C yielded a mixture of 1% to 2% menthones, 54% menthol, 30% neomenthol, 12% isomenthol, and 4% neoisomenthol, which is remarkably different from the composition described earlier. Partial racemization may occur, especially at higher temperatures or with Raney nickel as catalyst.

Liquid menthols, obtained from the mother liquors of menthol crystallization, can be fractionated through high-efficiency distillation to separate (−)-menthol from its isomers. The isomers are epimerized and then fractionated again, thus increasing the overall yield of (−)-menthol from DMO considerably.

10.3.4.3 From (+)-Citronellal

(+)-Citronellal is a major constituent of *Java citronella* oil (up to 45%) and has been used as starting material for the production of (−)-menthol when the supply of natural menthol or DMO was short. A drawback is its low optical purity of approximately 80%. Consequently, cyclization to isopulegol gives a mixture of optically active and 20% racemic isopulegols. Furthermore, the isomeric composition is highly dependent on the catalyst used for cyclization. Selectivity to isopulegol is 62% with silica and 98% with calcined zinc bromide, respectively (Akutagawa, 1999).

If the isomeric isopulegol mixture is hydrogenated to the corresponding mixture of menthols, (−)-menthol can only be separated via crystalline derivatives, such as menthyl benzoate or chloroacetate. A better way is to isolate (−)-isopulegol by low-temperature crystallization of the isopulegol mixture at − 50°C to −78°C (see Sully and Williams, 1963). Hydrogenation gives pure (−)-menthol (see Figure 10.7).

Synthetic (+)-citronellal obtained from (−)-β-pinene via (−)-*cis*-pinane and (−)-citronellene was used by Glidden Organic Chemicals, Jacksonville, U.S., for (−)-menthol production in the 1970s (see Leffingwell and Shackelford, 1974; Dev, 1978). The Takasago process via (+)-citronellal starts from myrcene (see later).

10.3.4.4 From (+)-Pulegone

(+)-Pulegone from pennyroyal oil (80%) can be hydrogenated to (−)-menthone or (+)-isomenthone, followed by reduction with nascent hydrogen to give predominantly (−)-menthol (see Figure 10.8). This process had been used on a smaller scale in Spain, but recent prices are prohibitive for commercial production.

10.3.4.5 From (−)-Piperitone

Eucalyptus dives oil contains 40% to 45% (−)-piperitone, which is usually partially racemized, due to the unstable asymmetric C-4 carbon adjacent to the carbonyl group. Moreover, the

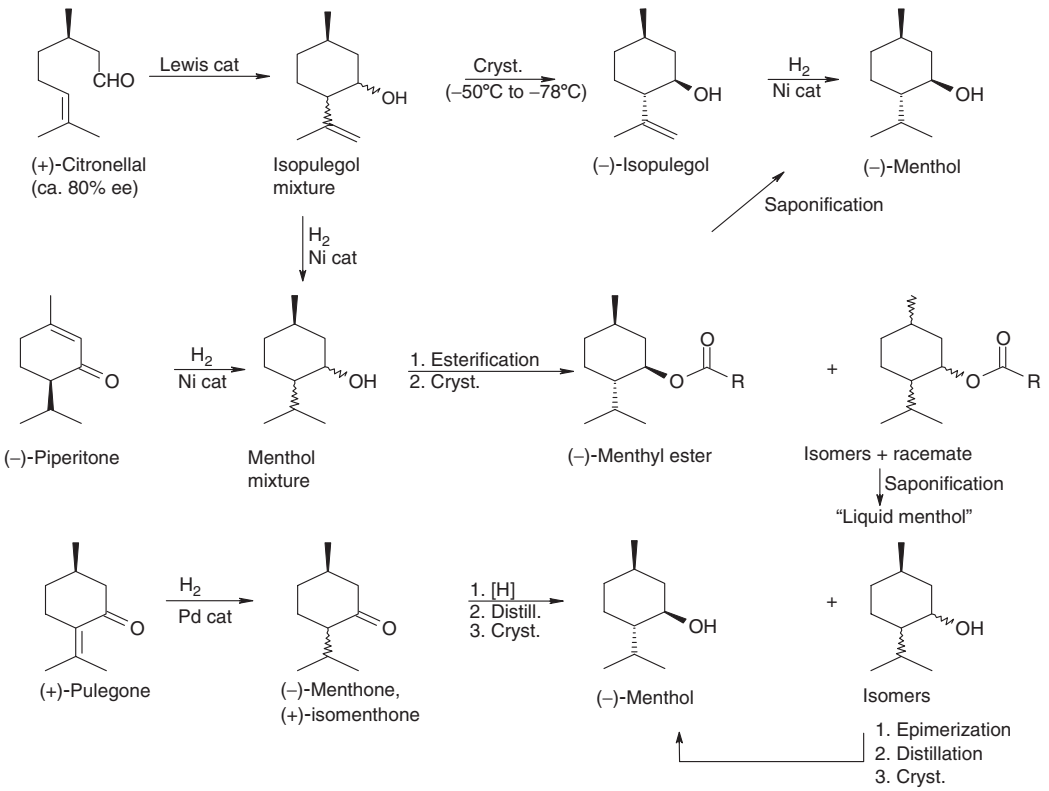


FIGURE 10.8 Semisynthetic menthol processes: the "classical" ways.

hydrogenation to either (+)-isomenthone or an isomeric mixture of menthols is not completely chirospecific with respect to the newly formed chiral center at C-1. (+)-Menthone, which is formed in addition to (+)-isomenthone, might be separated by rectification. Epimerization of (+)-isomenthone and reduction with sodium metal in aqueous trimethyl amine gives 80% (−)-menthol (see Okuda et al., 1973). Because of the racemic portion already present in the starting material, pure (−)-menthol can be obtained only via a crystalline ester, e.g., the chloroacetate (see Figure 10.8). Up to 50 metric tonnes a year had been produced by Keith Harris & Co. in Australia using this method.

10.3.4.6 From Optically Active Terpenes

A method of production from (−)-β-pinene to (+)-citronellal has already been mentioned earlier. Processes starting from (+)-limonene or (−)-α-phellandrene have not been used commercially owing to low overall yield and difficult isomer separation (Leffingwell and Shackelford, 1974).

10.3.5 Malti-Chem Research Centre Process

At Malti-Chem Research Centre (MRC) in India, a multistep process to produce (−)-menthol (see Figure 10.9) was developed based on (+)-δ-3-carene, the main constituent (55% to 65%) of Indian turpentine from *Pinus roxburghii* (Dev, 1978). In 1982, a plant for producing

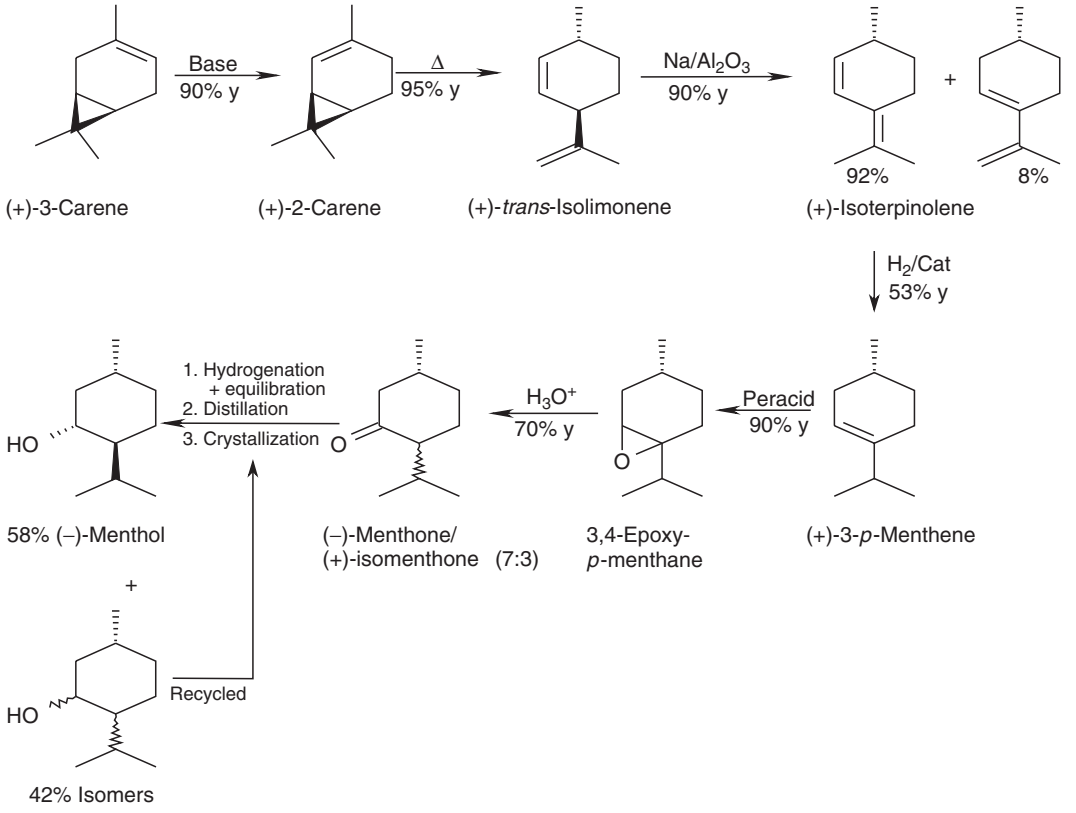


FIGURE 10.9 (−)-Menthol from (+)-3-carene: The MRC process.

200 metric tonnes a year was completed at Camphor & Allied, India. The real capacity was probably lower and the process was not used for many years.

Base catalyzed isomerization of (+)-δ-3-carene provides (+)-δ-2-carene, which is pyrolyzed to (+)-*trans*-isolimonene, followed by isomerization to (+)-isoterpinolene with a strong base (sodium on alumina). This step was recognized as the source of partial racemization of about 10% (Misra et al., 1988). The mixture of 92% (+)-isoterpinolene and 8% *p*-mentha-3,9-diene is hydrogenated to *p*-menth-3-ene (only 53% yield). Approximately 20% of *p*-menth-4(8)-ene is separated by fractionation and the remaining 65:35 mixture of *p*-menth-3-ene and *p*-menth-2-ene is epoxidized preferentially to the required 3,4-epoxy-*p*-menthane. Rearrangement to (−)-menthone or (+)-isomenthone and hydrogenation yields the equilibrium mixture of isomeric menthols, from which (−)-menthol of approximately 90% optical purity is separated by high-efficiency distillation (Sulzer columns of totally 130 theoretical plates) and then purified by crystallization.

Recycling of mother liquor and fractions of menthol isomers to epimerization and rectification yields additional (−)-menthol, although the increased content of racemic menthols in the recycled material may require derivatization, e.g., to the monochloroacetate, to separate pure (−)-menthol (Soman and Dev, 1979).

10.3.6 Haarmann and Reimer Process

In the mid-1960s, the industry anticipated a possible shortage of natural menthol because of decreasing crops of *Mentha canadensis* in Brazil in the next decade. Haarmann and Reimer (H&R) then decided to develop a completely synthetic process in cooperation with Bayer AG, Leverkusen, based on *m*-cresol (Figure 10.10 and Figure 10.11).

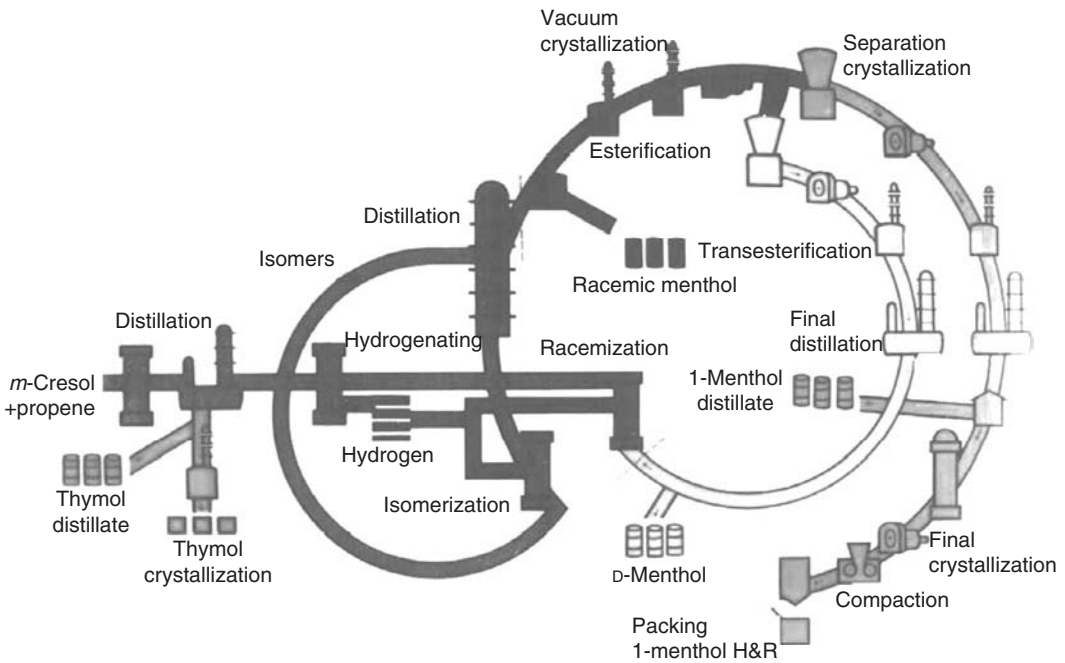


FIGURE 10.10 Flow scheme of the Haarmann and Reimer menthol process.

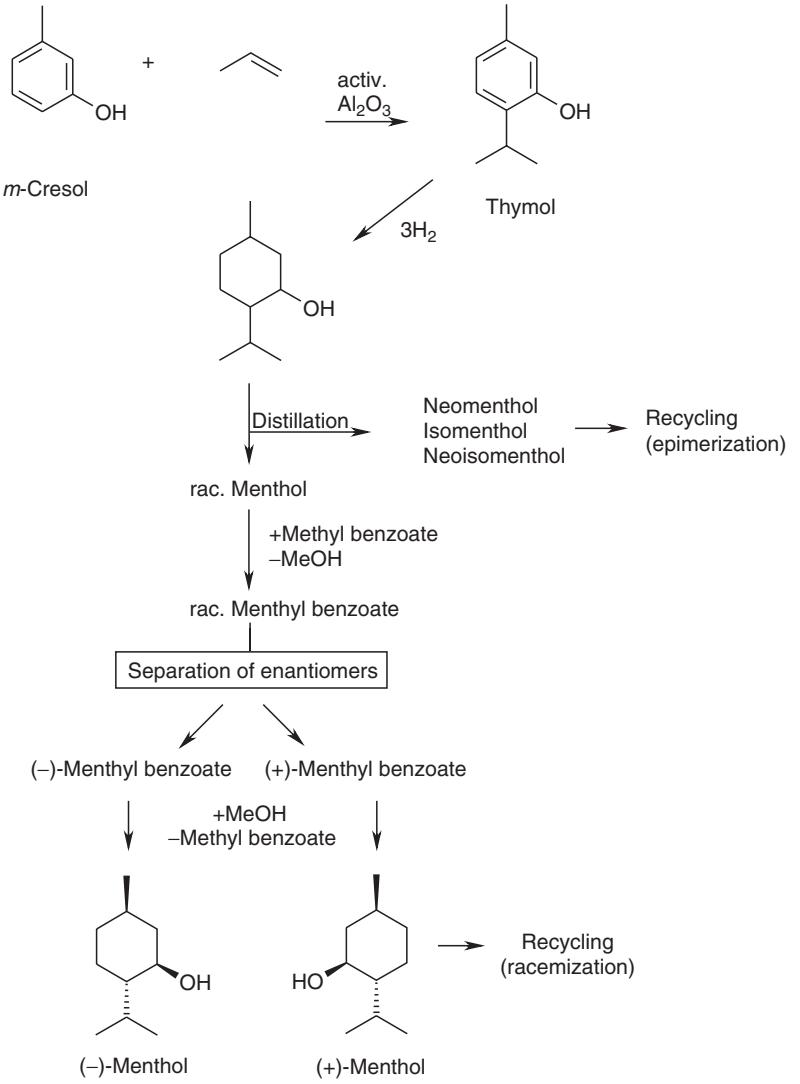


FIGURE 10.11 Total synthesis of (−)-menthol from *m*-cresol: The Haarmann and Reimer process.

10.3.6.1 Thymol and (±)-Menthol

Propylation to thymol and subsequent hydrogenation to a mixture of menthol isomers are well-known processes. Addition of propylene to *m*-cresol is performed continuously by pumping the liquid reactants at 350°C to 365°C through a pressure tube filled with activated alumina. After rectification, thymol of 99.5% purity is obtained; undesired alkylates are recycled. A gas phase process using medium-pore-sized zeolites allows lower reaction temperatures of 230°C to 270°C (Voges, 1991).

Thymol is hydrogenated continuously in a high-pressure tube at 165°C to 180°C and 200 to 300 bar, using a fixed-bed catalyst based on mainly cobalt and manganese oxide (Biedermann, 1973; Darsow and Petruck, 1996) to give an equilibrium mixture of approximately 59% (+)-menthol, 28% (+)-neomenthol, 12% (+)-isomenthol, and 1% (+)-neoisomenthol. This mixture passes through a system of two distillation columns. In the first column, the low boiling isomers, neomenthol and neoisomenthol, are taken overhead. The

bottoms are separated in the second column, in which (+)-menthol of at least 99% purity is taken overhead. The other isomers are recycled to hydrogenation or isomerization.

10.3.6.2 Separation of (+)- and (−)-Menthols

(+)-Menthol is transesterified with methyl benzoate to menthyl benzoate, which is purified by vacuum crystallization. The crucial step of the H&R process is the enantioselective (or direct) crystallization of (−)- or (+)-menthyl benzoate, which is carried out in two separate vessels (one for the (+)-isomer, the other for the (−)-isomer) by seeding a supersaturated solution of (+)-menthyl benzoate with crystals of the (−)- or (+)-antipode, respectively. Temperature has to be carefully controlled to stay below 0.01°C to avoid spontaneous crystallization of the undesired antipode. The crystallizers have a special form (see Figure 10.12) to allow the formation of larger crystals in the cylindric lower part (1) and sedimentation of small crystals from the conical upper part (2) to the crystallization zone (Baumgard et al., 1997). The crystal suspension is continuously discharged from the lower part (3) and centrifuged. A clear solution, which has a slightly lower concentration of the optical antipode recovered at the bottom, is pumped overhead (4) to the second crystallizer for isolation of the other antipode, and from there to the storage vessel to be saturated again.

(±)-Menthol cannot be resolved as such by this procedure, because it crystallizes as a racemic compound containing (+)- and (−)-menthols 1:1 in every crystal, whereas crystalline (±)-menthol benzoate is a conglomerate of crystals of the pure (+)- and (−)-enantiomers, respectively, a prerequisite for optical resolution by direct crystallization.

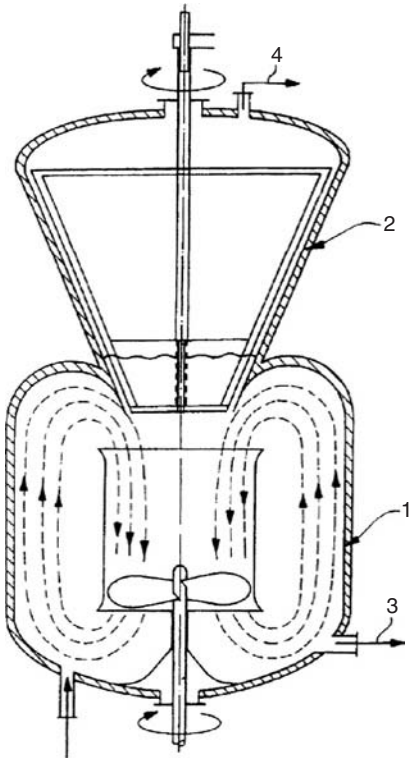


FIGURE 10.12 Crystallizer for the separation of (−)-or (+)-menthyl benzoate (Figure 5 in EP 909–205).

Methanolysis of the centrifuged antipodes yields (−)-menthol, (+)-menthol, and methyl benzoate. The latter is recycled to esterification. (+)-Menthol is distilled and recycled to the racemizer. After distillation and final crystallization, (−)-menthol is pelletized and packed into 20-kg (Germany) or 25-kg (U.S.) cardboard boxes. The overall yield in this process with reference to thymol is more than 90%.

10.3.7 The Takasago Process

In the 1970s, the first synthesis of BINAP (see later) and the discovery of lithium catalyzed addition of diethylamine to myrcene opened the way to enantiopure (+)-citronellal via an asymmetric isomerization of *N*,*N*-diethylgeranylamine (DGA) to the corresponding (+)-citronellal enamine by means of a rhodium–BINAP catalyst (Akutagawa, 1992, 1999) (see Figure 10.13).

10.3.7.1 *N,N*-Diethylgeranylamine

This intermediate is formed in a highly chemo- and regioselective manner on laboratory scale (98%). Under drastic industrial conditions (high temperature, low catalyst ratio), the regioselectivity drops to 92%. Nevertheless, the sensitive homogeneous catalyst requires an extremely pure substrate to achieve a high TON ("turnover number" = moles of enamine produced by one mole of catalyst). Catalyst poisons and isomers, which reduce the optical purity (e.g., *N*,*N*-diethylnerylamine), have to be removed. Treatment with vitride, a toluene solution of $NaAlH_2(OCH_2CH_2OCH_3)_2$, and distillation through a column of 80 theoretical plates yields DGA of 99.98% purity.

10.3.7.2 Enantioselective Isomerization

The process is carried out batchwise in a 15-m^3 reactor, which is charged with 7 metric tonnes of DGA, 6.7 kg of catalyst (a molar ratio of 1:8000), and 3 m^3 of THF. Within 18 h at 100°C isomerization is completed, yielding 99% of the enamine with 98.5% optical purity (ee/enantiomeric excess).

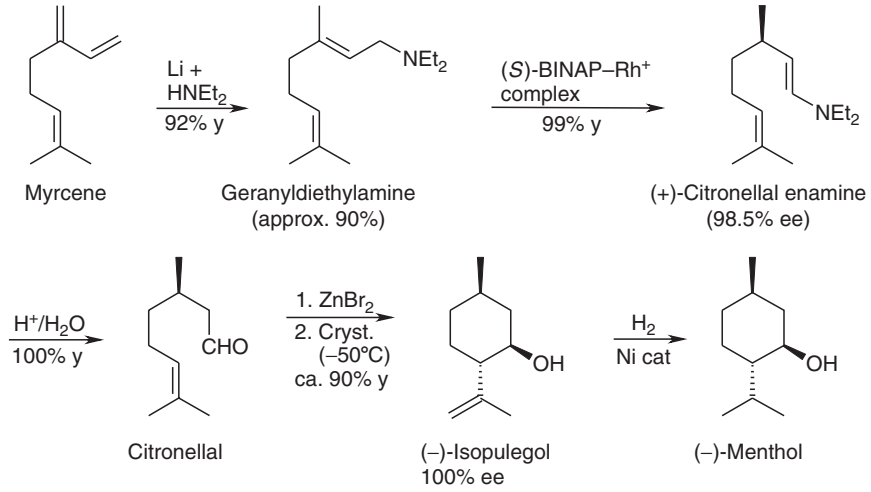


FIGURE 10.13 The Takasago process.

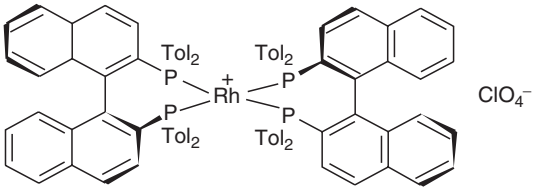


FIGURE 10.14 Rhodium (*S*)–Tol–BINAP complex, the key catalyst in the Takasago process.

From the distillation residue the catalyst is precipitated and recovered (98%) by the addition of *n*-heptane. This corresponds to a total TON of 400,000, which is a result of extensive process development.

The discovery of the thermally stable rhodium bis–BINAP complex made possible a repeated use of the catalyst. Industrially, a Tol–BINAP complex is used instead of the prototype BINAP (see Figure 10.14). The (*S*)–Tol–BINAP complex converts DGA to the enamine of (+)-citronellal, whereas the (*R*)-enantiomer is used to produce (−)-citronellal, an intermediate for the valuable fragrance chemicals (−)-citronellal and (−)-7-hydroxycitronellal.

10.3.7.3 Enamine to Menthol

The enamine is hydrolyzed to (+)-citronellal (98.5% ee) quantitatively and converted to (−)-menthol as described earlier (citronellal). However, synthetic (+)-citronellal has the advantage of high optical purity compared with natural citronellal. Furthermore, the stereoselectivity of the cyclization reaction to isopulegol has been improved to 98% in the course of process development, by using zinc bromide, calcined at 160°C, as the catalyst.

Low-temperature crystallization at −50°C in *n*-heptane yields approximately 90% of (−)-isopulegol, which is simply hydrogenated to pure (−)-menthol.

10.3.8 Cooling Compounds

10.3.8.1 Mechanisms of Cooling Effect

(−)-Menthol is widely used because of its unique cooling and stimulating effect. However, for some applications, its burning and tingling sensation as well as the strong mint flavor are undesirable. Furthermore, there is a strong demand for substances that possess a longer-lasting cooling effect.

It is a fact that menthol does not really cool the skin, as volatile substances do through evaporation, but it is able to convey a sensation of coolness on contact with the skin. Recent studies on the sensory transducer mechanism of cold receptors have demonstrated that menthol activates cold receptors by interfering with the calcium channels of the neuronal sensory membranes. For a detailed review and its pharmacological effects and toxicology, see Eccles (1994).

Before a cooling substance can interact with the cold receptors, it has to penetrate the protective outer skin layer (*stratum corneum*) to diffuse through the skin. Such factors as molecular structure, formulation, or solvent in which the compound is applied, and the properties of the body surface to be penetrated are critical for the transportation. Extremely sensitive areas are eyes, tongue, and the buccal cavity.

10.3.8.2 Molecular Requirements for Cooling Activity

It has been established that four important criteria must be satisfied for a molecule to possess effective cooling activity (Watson et al., 1978).

1. Hydrogen bonding: A hydrogen bonding function, i.e., a nitrogen or oxygen atom capable of acting as a hydrogen bond acceptor, is essential for cooling action. There is no indication that a second hydrogen bond acceptor enhances the cooling effect.
2. Hydrocarbon skeleton: It is assumed that the functional group takes part in hydrogen bonding at a receptor site and that a compact hydrophobic region of the molecule near the site of hydrogen bonding is the key to fit the molecule into the receptor. That stereochemistry plays a crucial part in this lock-and-key relationship is evident, since of the eight menthol isomers only (−)-menthol has a strong cooling effect.
3. Hydrophilic–hydrophobic balance: A correct hydrophilic–hydrophobic balance is important in stimulant–receptor interactions and is also one of the factors that determine the transport rate of compounds through biological membranes, especially the skin. The most common measure is the Hansch log P value, where P is the partition coefficient of the compound between n-octanol and water. Strong cooling compounds have log P values of 1.5 to 4.0; the log P value of menthol is 3.1.
4. Molecular weight: Substances that meet the previous criteria of potential cooling compounds should have a molecular weight in the relatively flexible range of 150 to 350.
5. Most of the cooling agents that have been commercialized are derived from (−)-menthol; only one, WS 23, is an aliphatic compound (see Figure 10.15 and Table 10.9).

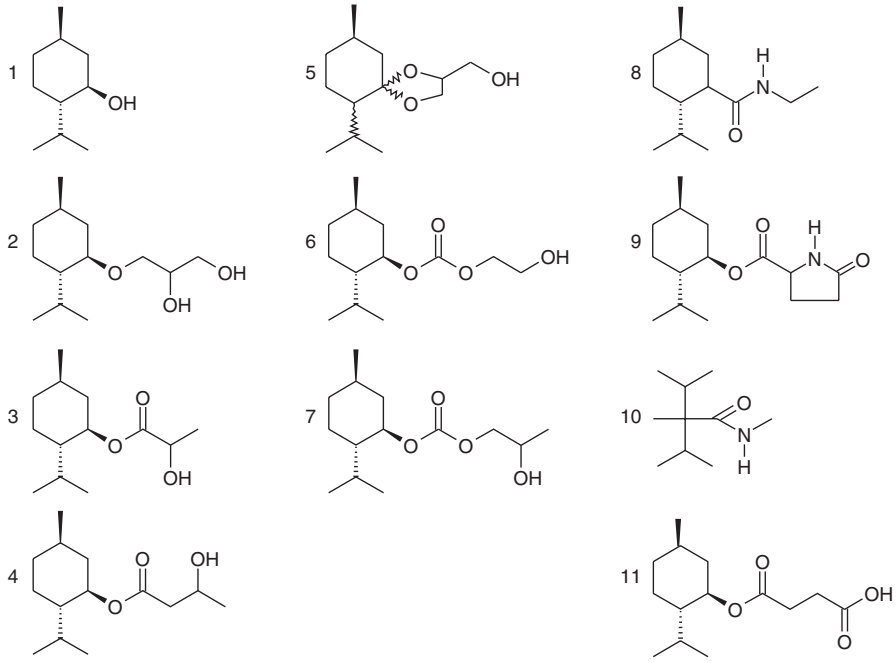


FIGURE 10.15 Structural formulas of commercialized cooling compounds.

TABLE 10.9
Various Cooling Agents

Structure Number (Figure 10.15)	Trade Name	Supplier	Appearance	References
1	L-Menthol	Numerous	White crystals or pellets	See earlier
2	Cooling agent No. 10	Takasago, Japan	Colorless liquid	Amano et al. (1981) (Takasago)
3	Frescolat® ML Frigydil	Haarmann and Reimer, Germany Lab. Produit Hygiene, France	White, crystalline powder Pale, yellowish liquid (mixture)	Bauer et al. (1976) (Haarmann and Reimer)
4	Menthyl 3-hydroxybutyrate	Takasago	Colorless liquid	Takasago (1986)
5	Frescolat MGA	Haarmann and Reimer	Colorless liquid	Grüb et al. (1991) (Haarmann and Reimer) Greenberg (1991) (Wrigley)
6	Optacool MGC	Haarmann and Reimer	Colorless liquid	Pelzer et al. (1993) (Haarmann and Reimer)
7	Optacool MPC	Haarmann and Reimer	Colorless liquid	Pelzer et al. (1993) (Haarmann and Reimer)
8	WS 3	Millenium, U.S.A	White powder	Rowsell and Watson (1974) (Wilkinson Sword)
9	WS 23	Millenium, U.S.A	White powder	Rowsell and Watson (1974) (Wilkinson Sword)
10	Questice L	Quest, Great Britain	White powder	Humbert and Guth (1972) (Unilever)
11	Monomenthyl succinate	Mane Fils, France		Mane and Ponge (1998) (Mane Fils)

10.3.8.3 Desirable Properties of Cooling Compounds

Apart from a strong cooling effect and a degree of flavor potentiation, cooling compounds should produce no noticeable sensations. A longer-lasting cooling effect, compared with menthol, is advantageous for a high-quality product. Any odor or taste, e.g., bitter, as well as burning or stinging sensations, is negative. Cooling agents have to be readily soluble in common organic solvents, but their solubility in water is very limited, owing to the required log *P* value. Liquid products are preferred because of easier handling.

Some menthol derivatives with a free hydroxyl group satisfy these criteria. The structures of commercialized cooling agents are shown in Figure 10.15; trade names, suppliers, and other information are summarized in Table 10.9.

REFERENCES

Akutagawa, S. (1992), A practical synthesis of (−)-menthol with the Rh–BINAP catalyst. In: *Chirality in Industry*, Collins, A.N., Sheldrake, G.N., and Crosby, J., Eds., J. Wiley & Sons, Chichester, pp. 313–323.

Akutagawa, S. (1999), Asymmetric isomerization of olefins. In: *Comprehensive Asymmetric Catalysis,* Jacobsen, E.N., Pfaltz, A., and Yamamoto, H., Eds., Springer-Verlag, Berlin, Vol. 3, pp. 1461–1469.

Amano, A., Moroe, M., and Yoshida, T. (1984), 3-Levo-menthoxypropane-1,2-diol. U.S. Patent 4 459 425, Prior. 1981, Japan (Takasago).

Bauer, K., Brüning, J., and Grüb, H. (1976), Mittel mit physiologischer Kühlwirkung. DE-OS 26 08 226 (Haarmann & Reimer).

Bauer, K., Garbe, D., and Surburg, H. (1997), *Common Fragrance and Flavor Materials*, 3rd edn., Weinheim, Wiley-VCH, pp. 50–54.

Baumgard, H.-D., Haedke, B., Himmelreich, J., and Judat, H. (1997), Kristallisationsvorrichtung und-verfahren. EP 909 205 (Haarmann & Reimer).

Bedoukian, P.Z. (1986), *Perfumery and Flavoring Synthetics*, 3rd edn., Allured Publishing Corp., Wheaton, IL, pp. 283–300.

Biedermann, W. (1973), Kontinuierliches Verfahren zur Herstellung von D,L-menthol. DE 23 14 813 (Bayer AG).

Chopra, M.M., Nigam, M.C., and Rao, P.R. (1972), Production of menthol from dementholized oil. *Parfum. Kosmet*., 55, 282–285.

Clark, G.S. (1998), An aroma profile. Menthol. *Perfum. Flav*., 25(5), 33–46.

Clark, G.S. (1988), A profile of an aroma chemical menthol. *Perfum. Flav*., 13(5), 37–46.

Darsow, G. and Petruck, G.-M. (1996), Verfahren zur Herstellung von D,L-menthol. EP 743 296 (Bayer AG).

Dev, S. (1978), New industrial processes based on carene. In: *11th IUPAC Internat. Symp. Chem. Nat. Prod*., Marekov, N., Ognyanov, I., and Arahovates, A., Eds., Bulg. Acad Sci., Golden Sands, 4(1), pp. 433–447.

Eccles, R. (1994), Menthol and related cooling compounds. *J. Pharm. Pharmacol*., 46, 618–630.

Emberger, R. and Hopp, R. (1985), Synthesis and sensory characterization of menthol enantiomers and their derivatives for the use in nature identical peppermint oils. In: *Topics in Flavour Research*, Berger, R.G., Nitz, S., Schreier, P., and Eichorn, H., Eds., Marzling-Hangenham, pp. 201–218.

Fleischer, J., Bauer, K., and Hopp, R. (1971), Verfahren zur Abtrennung von optisch reinem D- und L-menthol. DE 2 109 456 (Haarmann & Reimer).

Gildemeister, E. and Hoffmann, Fr. (1961), *Die Ätherischen Öle*, Vol. VII, revised by Triebs, W. and Bournot, K., Akademic Verlag, Berlin, pp. 246–421.

Greenberg, M.J. (1991), Enhanced flavors using menthone ketals. EP 485 170, Prior. 1990, U.S.A. (Wrigley, W.M. Jr Comp.).

Grüb, H., Pelzer, R., Hopp, R., and Emberger, R. (1991), Mittel mit physiologischem Kühleffekt und für diese Mittel geeignete wirksame Verbindungen. DE 41 10 973 (Haarmann & Reimer).

Guenther, E. (1949), *The Volatile Oils*. Vol. III, D. Van Nostrand, Princeton, NJ, pp. 652–655.

Hattori, S., Komatsu, A., and Yamaguchi, Y. (1968), Method for the biochemical isolation of (−)-menthol. U.S. 3 620 918 (Takasago Perfumery).

Hiraizumi, T. (1959), Essential oils and synthetic perfumes in Japan. *Perf. Essent. Oil Rec*., 50, 555–591.

Hopp, R. (1996), (−)-Menthol: an example of the various methodologies applied in the production of enantiomers. *Riv. Ital. EPPOS*, 7 (Spec. Num), 111–130.

Humbert, F.E.L. and Guth, G. (1973), Orale, insbesondere kosmetische Zubereitungen. DE 2 339 661, Prior. 1972, GB (Unilever).

Leffingwell, J.C. and Shackelford, R.E. (1974), Laevo-menthol: synthesis and organoleptic properties. *Cosmet. Perfum*., 89(6), 69–78.

Mane, J.M. and Ponge, J.-L. (1998), Coolant compositions. U.S. 5 843 466 (Mane Fils, S.A.).

Misra, A.N., Soman, R., and Dev, S. (1988), On the optical purity of (+)-3-carene from *Pinus roxburghii*, and the source of racemization of (−)-menthol derived there from. *Tetrahedron*, 44, 6941–6946.

Nijjar, G.S. (1990), Optimising *Mentha* oil yield from Shivalik-88 variety of *Mentha arvensis. Ind. Perfum*., 34, 186–189.

Okuda, O., Miyawaki, H., Eno, H., and Taniguchi, Y. (1973), Menthol. Jap. Kokai 73 61, 454 (Taiyo Perfumery Co.); *Chem. Abstr*. (1974) 80, 48 198z.

Pelzer, R., Surburg, H., and Hopp, R. (1993), Process to generate a physiological cooling effect and suitable compounds for this process. EP 583 651, Prior. 1992, DE (Haarmann & Reimer).
Rowsell, D.G. and Watson, H.R. (1974), Flavour modification. GB 1 457 671 (Wilkinson Sword).
Sacco, T. (1967), Essential oil production in Brazil. In: *Proceedings of Essential Oil Production in Developing Countries*. Tropical Products Institute, London, pp. 69–74.
Solodar, J. (1976), Stereoselective reduction of menthone and isomenthone by dissolving alkali metals and by hydrogen with group 8 metals on carbon. *J. Org. Chem*., 41, 3461–3464.
Soman, R. and Dev, S. (1979), A process for the optical purification of partially racemic (−)-menthol to optical pure (−)-menthol. Indian Patent 150 447 (Camphor & Allied Products Ltd.).
Sully, B.T.D. and Williams, P.L. (1963), Verfahren zur Herstellung von L-menthol. DE 1 197 081 (Boake Roberts & Company).
Takasago (1986), Menthyl 3-hydroxybutyrate. F 2 577 922, Prior. 1985, Japan.
Traas, P.C. (1982), Advances in the chemistry of some interesting cyclic monoterpene alcohols. In: *Fragrance Chemistry/The Science of the Sense of Smell*, Theimer, E.T., Ed., Academic Press, San Diego, pp. 165–179.
Vaze, K. (2004), India's mint industry. *FAFAI J*. (January/March), 6, 55–58.
Voges, H.-W. (1991), Isopropylphenols. In: *Ullmann's Encyclopedia of Industrial Chemistry*, Elvers, B., Hawkins, S., and Schulz, G., Eds., 5th edn., VCH, Weinheim (Germany), 19A, pp. 325–328.
Watson, H.R., Hems, R., Roswell, D., and Spring, D.J. (1978), New compounds with the menthol cooling effect. *J. Soc. Cosmet. Chem*., 29, 185–200.
Watts, S. (1997). Menthol and cornmint from China, *Perfum. Flav.*, 22 (1), 1–6.
Werkhoff, P., Brennecke, S., and Bretschneider, W. (1991), Fortschritte bei der chirospezifischen Analyse natürlicher Riech- und Aromastoffe. *Chem. Mikrobiol. Technol. Lebensm*., 13, 129–152.
Yoshida, T., Komatsu, A., and Indo, M. (1965), Isomerization and racemization of menthols, Part II. *Agric. Biol. Chem*., 29, 824–831.

11 The Genuineness of Mint Oils

Brian M. Lawrence

CONTENTS

11.1 INTRODUCTION

For an essential oil to be genuine it must be isolated from a whole plant (fresh or dry), or a plant part of known taxonomic origin by physical means only. Although the physical methods used to isolate oils are distillation (steam, steam and water, and water) or expression, the almost exclusive method for mint oil isolation is steam distillation.

There are several reasons why an essential oil does not have a consistent chemical composition even though the taxonomic origin of the plant from which the oil was isolated is known:

1. Genetic: clone, hybrid, cultivar, or population-specific taxon variation
2. Ontogenetic: variation in the maturity stage of the plant harvested for oil isolation
3. Extrinsic: effect of climate, soil, season, time of harvest, disease or insect damage, geographical origin, etc.
4. Infraspecific: variation in the chemotype of plants harvested (wild plants and also some seed-sown plants)
5. Endogenous: within-plant variation, which can be affected by how much of the plant was harvested
6. Processing: variation in the method of oil isolation
7. Exogenous: addition of components either before distillation or directly into the oil

As the commercially important mints are clonally reproduced, there should be no genetic effects on oil yield and composition unless different cultivars are used to yield the same oil. An example of the effect of the cultivar of cornmint on the composition of selected constituents (Kumar et al., 2000) can be seen in Table 11.1.

TABLE 11.1
Comparative Percentage Composition of Selected Main Constituents of Six Indian Cornmint Cultivars

Compound	MAS-1	Kalka	Shivalik	Gomti	Himalaya	Kosi
Limonene	1.4	2.1	1.1	0.9	1.8	1.2
3-Octanol	0.7	0.6	0.6	1.1	0.9	0.4
Menthone	3.6	3.2	7.4	12.8	0.5	3.4
Isomenthone	1.4	2.0	4.3	3.4	3.1	3.1
Menthyl acetate	0.5	0.8	1.7	3.0	1.0	4.5
Neomenthol	2.6	2.4	1.9	1.8	1.9	1.9
Menthol	85.6	80.7	75.3	71.3	73.3	78.7
Isomenthol	0.4	0.3	0.3	0.4	0.4	0.4

There should not be any infraspecific effects on oil composition because there will not be any chemotypic variation in clonally reproduced plants. Also, there should not be any endogenous effects because any within-plant variation of clonally reproduced plants will be averaged out under normal distillation conditions of plants harvested at their optimum development stage. There will, however, be ontogenetic effects as the oil produced from mints like other herbaceous plants is affected by the development stage of the plant. Two examples of this can be seen in Table 11.2 and Table 11.3. In Table 11.2, a comparison between peppermint oils produced at different growth stages in Indiana was presented (Murray et al., 1988). As can be seen, there are wide variations in the contents of menthone, menthyl acetate, menthol, menthofuran, and isomenthone. A second example of the effect of

TABLE 11.2
Comparison between the Oils Produced from Different Development Stages of Peppermint Grown in Indiana

	Percentage Composition					
Compound	**1**	**2**	**3**	**4**	**5**	**6**
α-Pinene	0.4	0.7–0.8	0.5–0.8	0.6	0.2–0.9	0.1–0.5
β-Pinene	1.0	1.8–2.2	1.4–2.3	1.8	1.0–2.0	0.3–1.0
Limonene	1.6	1.3–2.0	1.6–1.9	2.2	1.5–2.4	0.6–2.0
1,8-Cineole	3.5	6.1–6.9	5.4–7.6	5.9	4.7–6.1	1.9–4.0
Menthone	30.6	16.3–37.2	14.0–20.8	21.8	13.0–15.1	3.3–6.1
Menthofuran	2.0	1.9–2.0	2.1–2.6	7.7	3.4–7.6	5.4–7.0
Isomenthone	3.7	3.5–4.1	3.5–4.3	3.6	2.1–3.5	0.7–1.4
Menthyl acetate	4.7	3.9–13.1	6.5–10.9	3.4	5.6–12.1	18.8–27.8
Neomenthol	4.2	4.1–5.5	4.7–5.3	3.7	4.2–4.5	4.8–5.0
Menthol	40.7	30.3–39.3	36.8–43.1	36.8	43.4–48.2	47.4–49.0
Pulegone	0.6	0.7–1.0	0.6–1.4	2.8	0.7–1.3	1.3–1.9
Piperitone	0.8	0.5–0.8	0.6–0.7	0.6	0.3–0.5	0.2–0.5

Development stages of Mitcham peppermint grown in Indiana: 1, juvenile plants (5 cm); 2, immature plants; 3, just before flowering (normal harvest time for oil production); 4, flowering stage; 5, post-flowering plants; 6, senescent plants.

TABLE 11.3
Comparative Percentage Composition of Selected Constituents of the Oil of the Kosi Cultivar of Cornmint Sampled at Different Development Stages

	Development Stages						
Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Limonene	1.2	1.5	1.7	1.2	1.2	3.6	2.2
3-Octanol	0.5	0.4	0.9	0.4	0.4	0.2	0.5
Menthone	0.8	1.1	10.1	3.4	3.4	7.0	8.2
Isomenthone	0.8	0.7	3.0	3.1	3.2	3.6	4.1
Menthyl acetate	27.4	21.6	1.6	4.5	4.4	3.2	1.2
Neomenthol	4.0	2.2	1.9	1.9	1.9	2.3	2.1
Menthol	59.8	62.8	72.7	78.7	76.8	69.6	76.5
Isomenthol	0.4	0.5	0.3	0.4	0.4	0.4	0.2

1, Oil of whole plants from previous season harvested at early sucker production time; 2, oil of whole plants from previous season harvested at late sucker production time; 3, oil of main crop from early harvest time; 4, oil of main crop from normal harvest time; 5, oil of main crop from late harvest time; 6, oil from second cutting (ratoon crop) early harvest time; 7, oil from second cutting (ratoon crop) late harvest time.

development stage can be seen in Table 11.3, in which the percentage composition of selected constituents of the Kosi cultivar of cornmint can be found. In this example, wide variations can be seen in the menthone, menthyl acetate, and menthol contents (Kumar et al. 2000).

The magnitude of an ontogenetic effect can be quite dramatic as has been demonstrated in the examples; however, it must be realized that this developmental effect is influenced by extrinsic conditions, and it is more pronounced in temperate and subtropical zones, typical of regions in which the mints are grown. The effects of extrinsic conditions such as climate, water availability, sunlight, day length, pressure, nutrient availability, diurnal fluctuation, soil type, disease incidence, and insect damage are the main extrinsic factors that can have an effect on the composition of an oil. An example of the effect of extrinsic conditions on the composition of peppermint oil produced in the Midwest (United States) over three different seasons can be seen in Table 11.4. Even though these oils were produced in a specific region of

TABLE 11.4
Comparison Percentage Composition of Selected Components in Midwest Peppermint Oil over Three Different Seasons

	Season			Three Season
Compound	**1988**	**1993**	**1997**	**Range**
Limonene	1.4–1.7	1.4–1.7	1.0–2.6	1.0–2.6
1,8-Cineole	5.6–6.7	5.6–6.2	3.6–6.7	3.6–6.7
Menthone	18.1–29.1	24.5–29.9	13.2–34.6	13.2–34.6
Menthofuran	0.8–5.3	1.6–3.0	0.2–2.9	0.2–5.3
Isomenthone	3.0–4.3	3.8–4.3	2.0–4.6	2.0–4.6
Menthyl acetate	2.0–5.9	2.9–3.6	1.9–8.8	1.9–8.8
Menthol	33.1–41.1	33.2–37.5	34.1–54.4	33.1–54.4
Germacrene D	2.1–3.5	3.2–2.6	0.9–2.1	0.9–3.5
Piperitone	0.5–0.6	0.5–0.6	0.3–0.7	0.3–0.7

the United States, not only is a between-season difference evident, but there is also a variation in composition within a single season.

The fact that most mint oils are produced by steam distillation means, assuming that the plants are harvested at an optimum time, then other than the moisture content of the plants, the amount and dryness of the steam, and the length of distillation, processing effects are kept to a minimum. As a result, the uncontrollable or partially controllable conditions discussed earlier can have an understandable effect on the composition of mint oils.

If clonally reproduced plants are harvested at the same ontogenetic (development) stage from different regions of the country, oils produced from them will differ chemically because of the extrinsic factors that they have experienced during their life cycle. Such is the situation with peppermint oil produced in the United States.

11.2 PEPPERMINT OIL DIFFERENTIATION

Lawrence et al. (1989) showed that peppermint oil, which was produced from clonally reproduced plants that were grown in different regions of the United States, could be readily differentiated based on their area of cultivation and oil production. The areas of commercial production of these oils were the Midwest, Idaho, Yakima-Kennewick Valley in Washington, and the Madras and Willamette Valleys of Oregon. It was proved that geographical location and microenvironment were influential factors, which affect, albeit slightly, the composition of oils produced in each region. Examination of the data obtained from numerous analyses of oils produced from the same clonally reproduced peppermint grown in different locations in the United States reveals a lot of similarity (Table 11.5).

If maximum and minimum amounts of each major constituent found in the oils are tabulated, it is difficult, if not impossible, to be able to use this data to differentiate between the oils (Table 11.5). As a result, to differentiate between the oils, the choices are to use sophisticated statistical computer-driven programs to analyze the data such as principal component analysis, cluster analysis, etc., or to use a pattern recognition technique. Because the concept of pattern recognition is easier to use, less costly, and easier to comprehend, the latter was originally chosen to analyze the data and generate a ''pattern'' that could be used to readily differentiate between peppermint oils grown in different regions of the United States. As a result, the concept of component ratios, which was originally conceived by Smith and Levi (1961), was used to prove differences between the oils. To simplify the component ratio

TABLE 11.5
Comparative Composition (%) of Selected Components in Various Commercial U.S. Peppermint Oils Produced in 1988

	Midwest	Willamette	Madras	Yakima	Idaho
Limonene	1.42–1.74	1.27–1.48	1.58–1.82	1.52–1.84	1.71–1.96
1,8-Cineole	5.57–6.16	4.93–5.47	4.52–5.27	4.73–5.65	5.08–5.77
Menthone	24.47–29.92	17.75–23.34	13.94–22.64	11.19–19.46	14.77–25.32
Menthofuran	1.55–2.97	1.38–2.01	1.13–2.32	4.29–6.71	1.65–3.59
Isomenthone	3.79–4.31	2.81–3.13	2.03–3.11	2.05–3.08	2.41–3.52
Menthyl acetate	2.91–3.58	4.31–5.68	3.73–7.49	5.25–8.39	2.77–6.36
Menthol	33.16–37.52	41.27–45.39	40.61–47.44	40.27–44.37	38.06–46.59
Germacrene D	2.28–2.61	1.96–2.41	1.89–2.58	0.74–2.85	2.09–2.72
Piperitone	0.45–0.59	0.62–0.68	0.52–0.65	0.49–0.52	0.55–0.61

data obtained from ca. 50 samples of oil from each growing region, the selected component ratios were presented as polygonal representations. Such a presentation of data can be thought of as a pictorial pattern recognition of the oils produced in each region.

The criteria for the choice of components to be used in ratio form to plot as a polygon for a pattern recognition method of differentiation between oils produced from the same plant grown in different regions were as follows:

1. Components must be easily chromatographically separated to allow clean quantitative determination.
2. Quantitative amounts of components chosen must show some minor differences.
3. Some of the components selected must also be available from either synthetic sources or other natural sources, because they could be ideal candidates as adulterants.
4. The major components of peppermint oil must be included in the choice of the ones used for component ratio determinations.
5. A minimum of six components must be chosen.
6. Because circular graphical representation was chosen to present the component ratio data, the ratios in each oil analyzed must be plotted separately.

The original components chosen for differentiation of peppermint oils were limonene, 1,8-cineole, menthone, menthofuran, menthyl acetate, and menthol. These components met all the criteria listed earlier.

The component ratios selected to differentiate between the oils were as follows:

1. 1,8-Cineole/limonene
2. 1,8-Cineole/menthofuran $\times$ 1/2
3. 1,8-Cineole/menthyl acetate
4. 1,8-Cineole/menthol $\times$ 50
5. 1,8-Cineole/menthone $\times$ 25
6. Menthofuran/menthone $\times$ 100/6
7. Menthofuran/menthol $\times$ 50
8. Menthofuran/menthyl acetate
9. Menthofuran/limonene
10. Menthone/menthyl acetate $\times$ 1/2
11. Menthol/menthone
12. Menthol/menthyl acetate $\times$ 1/2

Many of these ratios have multipliers that were used to ensure that the polygons formed would fit the scale of the circular graphical presentation of data. What this means is that only oils produced in a specific region will have their composition ratio data fit within the polygon. Oils whose component ratio data do not fit within the polygon cannot be a pure oil produced in that specific region. Although the polygonal representation could be used to differentiate peppermint oils produced in 1988 by origin, it was decided to expand the technique to include oils produced in Canada and India.

After a second look at the component ratios, it was decided to change the multipliers once the data were calculated for peppermint oil produced in Alberta (Canada) and oil produced in the various regions in India because they would not fit the scale used in the previous study. As a result, in addition to the six previously chosen components for ratio determinations, three other compounds such as isomenthone, piperitone, and germacrene D were added to the list of compounds, the quantitative data of which were determined (Lawrence, 1999).

The full list of component ratios used to examine peppermint oils irrespective of origin were as follows:

1. 1,8-Cineole/limonene
2. 1,8-Cineole/menthofuran
3. 1,8-Cineole/menthyl acetate × 2
4. 1,8-Cineole/menthol × 50
5. 1,8-Cineole/menthone × 25
6. Menthofuran/menthone × 100/6
7. Menthofuran/menthol × 50
8. Menthofuran/menthyl acetate × 5
9. Menthofuran/limonene × 2
10. Menthone/menthyl acetate × 2/3
11. Menthone/isomenthone
12. Menthone/menthol × 5
13. Menthone/piperitone × 1/10
14. Menthone/germacrene D × 6/25
15. Isomenthone/menthyl acetate × 2
16. Isomenthone/piperitone × 2/5
17. Menthyl acetate/piperitone × 1/2
18. Menthyl acetate/germacrene D × 2
19. Menthol/menthyl acetate × 1/2
20. Menthol/piperitone × 1/15
21. Menthol/germacrene D × 6/25
22. Piperitone/germacrene D × 10
23. 1,8-Cineole/piperitone × 1/2
24. 1,8-Cineole/isomenthone × 2
25. 1,8-Cineole/germacrene D × 2/3
26. Menthofuran/piperitone × 1/2
27. Menthofuran/isomenthone × 2
28. Menthofuran/germacrene D

Before examining the effect of blending either Indian or Canadian peppermint oil with oils produced in different regions of the United States, it is pertinent to note the compositional range of the selected constituents (Table 11.6). Using the data summarized in Table 11.6, the component ratios for both peppermint oils were determined as shown in Table 11.7.

Peppermint oil of specific regions is often blended with (a) oils of other regions, (b) oils from other countries, particularly Indian, and (c) dementholized cornmint oil (DMO) of Indian or Chinese origin.

11.3 SPEARMINT OIL DIFFERENTIATION

To further demonstrate the use of this technique of component ratios to differentiate between other oils produced in the United States from different regions, differentiation between Native and Scotch spearmint oils, which are also produced in the Midwest and the Far west, was examined. It should be noted that greater regional differences between the oils could not be used because they are not sold according to the state or region within which they are produced. As a result, only the broad regions of Midwest and Far west spearmint oils could be examined.

TABLE 11.6
Comparative Composition (%) of Selected Constituents of Indian and Canadian Peppermint Oils

Compound	Indian Oils	Canadian Oils
Limonene	2.77–3.67	1.54–2.09
1,8-Cineole	4.42–4.91	4.61–6.20
Menthone	25.21–32.30	22.77–26.34
Menthofuran	4.68–10.75	1.96–2.87
Isomenthone	4.90–5.35	3.15–3.62
Menthyl acetate	3.14–4.84	2.28–4.17
Menthol	18.55–24.63	41.69–46.76
Germacrene D	0.85–1.61	0.48–0.71
Piperitone	0.53–0.71	0.55–0.77

TABLE 11.7
Canadian and Indian Peppermint Oil Component Ratio Ranges

Component Ratio	Alberta	Indian
1,8-Cineole/limonene	2.61–3.77	1.28–1.74
1,8-Cineole/menthofuran	2.12–2.99	0.50–1.05
1,8-Cineole/menthyl acetate × 2	2.21–5.43	1.91–2.82
1,8-Cineole/menthol × 50	4.93–7.30	9.38–12.12
1,8-Cineole/menthone × 25	5.32–6.38	3.56–4.58
Menthofuran/menthone × 100/6	1.29–1.94	2.55–4.96
Menthofuran/menthol × 50	2.18–3.41	10.04–28.98
Menthofuran/menthyl acetate × 5	2.45–5.46	6.26–17.12
Menthofuran/limonene × 2	2.13–3.21	3.02–5.24
Menthone/menthyl acetate × 2/3	2.74–5.70	3.48–6.59
Menthone/isomenthone	6.71–7.59	4.73–6.12
Menthone/menthol × 5	2.33–3.16	5.12–8.37
Menthone/piperitone × 1/10	3.05–4.79	3.55–6.13
Menthone/germacrene D × 6/25	7.52–12.35	4.42–8.69
Isomenthone/menthyl acetate × 2	1.51–2.91	2.12–3.34
Isomenthone/piperitone × 2/5	1.64–2.63	3.00–4.05
Menthyl acetate/piperitone × 1/2	1.81–3.15	2.94–3.53
Menthyl acetate/germacrene D × 2	7.48–14.88	4.66–9.86
Menthol/menthyl acetate × 1/2	5.44–9.30	2.41–2.95
Menthol/piperitone × 1/15	4.05–5.05	2.11–2.64
Menthol/germacrene D × 6/25	4.15–21.04	3.07–6.03
Piperitone/germacrene D × 10	9.26–13.28	3.40–7.24
1,8-Cineole/piperitone × 1/2	2.99–5.54	3.25–4.57
1,8-Cineole/isomenthone × 2	2.92–3.73	1.69–2.02
1,8-Cineole/germacrene D × 2/3	4.67–8.46	2.03–3.64
Menthofuran/piperitone × 1/2	1.33–2.48	3.60–10.14
Menthofuran/isomenthone × 2	1.12–1.74	1.82–4.10
Menthofuran/germacrene D	2.91–5.98	3.22–10.78

The components that were chosen for differentiation of both Native and Scotch spearmint oils were myrcene, limonene, 1,8-cineole, 3-octyl acetate, 3-octanol, *trans*-sabinene hydrate, β-bourbonene, carvone, *cis*-carveol, and *trans*-carveol. This is a larger number of components than used for U.S. peppermint oil differentiations; however, in this case differentiation between one form of spearmint oil produced in two different regions was not the objective. The objective was to differentiate between spearmint oil produced from two different taxonomic origins from two different regions; hence, an increased number of components are needed to obtain sufficient data to readily differentiate between all spearmint oils. The compounds chosen were those whose raw analytical data varied so that a differentiation between Scotch and Native spearmint oils and second cutting Native spearmint oils could be made. For example, Scotch spearmint oil was slightly richer in limonene, 3-octanol, menthone, and carvone than Native spearmint oil, which in turn, was slightly richer in myrcene, 1,8-cineole, 3-octyl acetate, *trans*-sabinene hydrate, β-bourbonene, and *trans*-carveol. However, because it is difficult to look at raw analytical data and readily see slight differences as presented in Table 11.8, component ratios were used to magnify these differences. The same component ratios were used for both Scotch and Native spearmint oil:

1. Limonene/myrcene × 2/5
2. Limonene/1,8-cineole × 1/2
3. Limonene/3-octanol × 2/5
4. Limonene/β-bourbonene × 2/5
5. Myrcene/β-bourbonene × 2
6. 1,8-Cineole/myrcene × 5
7. Carvone/myrcene × 1/10
8. Carvone/β-bourbonene × 1/10
9. 3-Octanol/3-octyl acetate × 2/5
10. 1,8-Cineole/*trans*-sabinene hydrate × 4
11. *cis*-Carveol/*trans*-carveol × 5/3
12. Carvone/*trans*-carveol × 1/100

TABLE 11.8
Comparative Composition (%) of Selected Constituents of Native and Scotch Spearmint Oils

	Native Spearmint			Scotch Spearmint	
Compound	**Midwest Oil**	**Far West Oil**	**Second Cutting Oil**	**Midwest Oil**	**Far West Oil**
Myrcene	2.51–2.85	2.30–4.76	2.72–3.36	0.82–1.13	0.86–1.08
Limonene	9.06–10.90	8.32–13.36	10.39–11.93	13.65–17.27	13.76–21.18
1,8-Cineole	1.77–1.81	1.44–2.11	1.63–2.00	1.40–1.57	1.00–1.56
3-Octyl acetate	0.20–0.29	0.20–0.54	0.24–0.47	0.13–0.23	0.10–0.27
3-Octanol	0.83–0.95	0.82–1.23	0.79–1.02	2.09–2.66	1.75–2.41
trans-Sabinene hydrate	0.84–0.93	0.75–1.68	0.95–1.50	0.95–1.43	0.74–1.42
β-Bourbonene	2.07–2.22	1.78–2.66	1.50–1.88	0.91–1.24	0.83–1.34
Carvone	65.40–66.59	58.47–66.59	65.42–69.44	65.79–71.62	62.84–69.79
trans-Carveol	0.80–1.15	0.32–1.15	0.30–0.58	0.11–0.17	0.10–0.18
cis-Carveol	0.28–0.41	0.26–0.45	0.28–0.47	0.31–0.54	0.23–0.36

TABLE 11.9
Comparative Component Ratios for Midwest, Far West, and Second Cutting Far West Native and Midwest and Far West Spearmint Oils

Component Ratios	MWN	FWN	FWN Second	MWS	FWS
Limonene/myrcene × 2/5	1.36–1.73	0.71–2.20	1.26–1.67	5.59–7.42	6.21–9.03
Limonene/1,8-cineole × 1/2	2.50–3.06	2.41–3.71	2.85–3.25	4.85–5.96	7.04–8.95
Limonene/3-octanol × 2/5	3.94–5.22	2.72–5.74	4.06–5.76	2.30–3.18	2.52–4.66
Limonene/β-bourbonene × 2/5	1.60–2.10	1.38–3.00	2.24–2.84	4.40–6.94	4.68–9.66
Myrcene/β-bourbonene × 2	2.35–2.61	2.42–4.78	3.11–4.48	1.32–2.33	1.30–2.37
1,8-Cineole/myrcene × 5	3.14–3.53	1.61–3.70	2.60–3.62	6.43–8.54	5.30–7.10
Carvone/myrcene × 1/10	2.30–2.65	1.36–2.80	2.02–2.56	5.58–8.14	5.98–8.01
Carvone/β-bourbonene × 1/10	2.93–3.19	2.20–3.71	3.51–4.52	4.91–7.52	5.20–7.39
3-Octanol/3-octyl acetate × 2/5	1.14–1.66	0.91–1.86	0.80–1.60	4.18–8.14	2.78–7.45
1,8-Cineole/*trans*-sabinene hydrate × 4	7.78–8.46	3.62–9.48	5.06–7.58	4.18–6.48	3.34–5.74
cis-Carveol/*trans*-carveol × 5/3	0.51–0.60	0.83–1.82	0.90–1.89	3.97–7.22	2.25–5.45
Carvone/*trans*-carveol × 1/100	0.57–0.83	1.00–2.06	1.16–2.24	3.91–6.23	3.43–7.43

MWN, Midwest Native spearmint oils; FWN, Far west Native spearmint oils; FWN second, second cutting Far west Native spearmint oils; MWS, Midwest Scotch spearmint oils; FWS, Far west Scotch spearmint oils.

The component ratio data for the two taxonomically differentiated oils produced in two regions of the United States are shown in Table 11.9. Again, multipliers were used to ensure that the component ratio data for both spearmint oils would fit on the same circular graph. The results of the component ratio data were obtained for 40 samples of each oil type and 20 samples of a second cutting oil obtained from Far west Native spearmint, which can be seen in Figure 11.1 through Figure 11.3.

The limonene/myrcene ratios for each different spearmint oil are 1.36:1.73 (Midwest Native), 0.71:2.20 (Far west Native), 1.26:1.67 (second cutting Far west Native), 5.59:7.42 (Midwest Scotch), and 6.21:9.03 (Far west Scotch). From these data, it can be seen that it is easy to differentiate Native spearmint oil from Scotch spearmint oil (Figure 11.1 through Figure 11.3). Using the 11 other component ratios merely makes this differentiation task easier. When this data are presented as a polygonal representation, it is evident from these pattern recognition figures (Figure 11.1 through Figure 11.3) that Native and Scotch spearmint oil can be readily differentiated:

1. Far west and Midwest Scotch and Native spearmint oils can be readily differentiated.
2. A second cutting of oil of Far west Native spearmint can be readily differentiated from all the other spearmint oils.

11.4 DEMENTHOLIZED CORNMINT OIL DIFFERENTIATION

The pattern recognition method can also be used to determine whether Chinese and Indian DMO oil can be readily differentiated. This differentiation is slightly more complex because these oils have had a portion of natural menthol removed by freeze crystallization. In addition, many of the dementholized oils have been "topped." This means that the low boiling compounds and, in some cases, including all the monoterpene hydrocarbons, and 1,8-cineole have been removed from the oils. Nevertheless, this meant that the choice of components was reduced because of this redistillation. On examination of the raw data, a

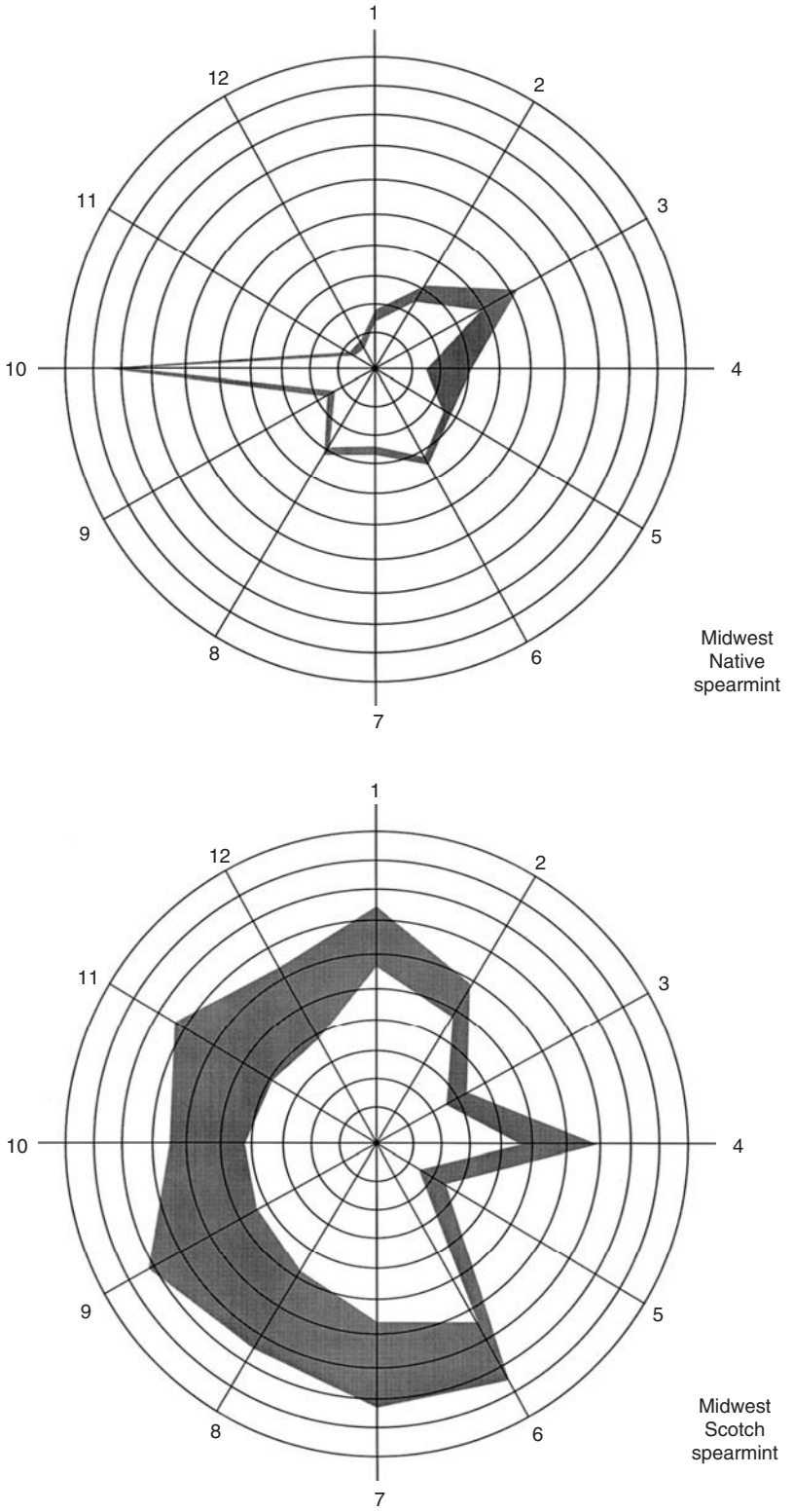


FIGURE 11.1 Pattern recognition (polygonal representation) of the component ratio data for Midwest Native and Scotch spearmint oils.

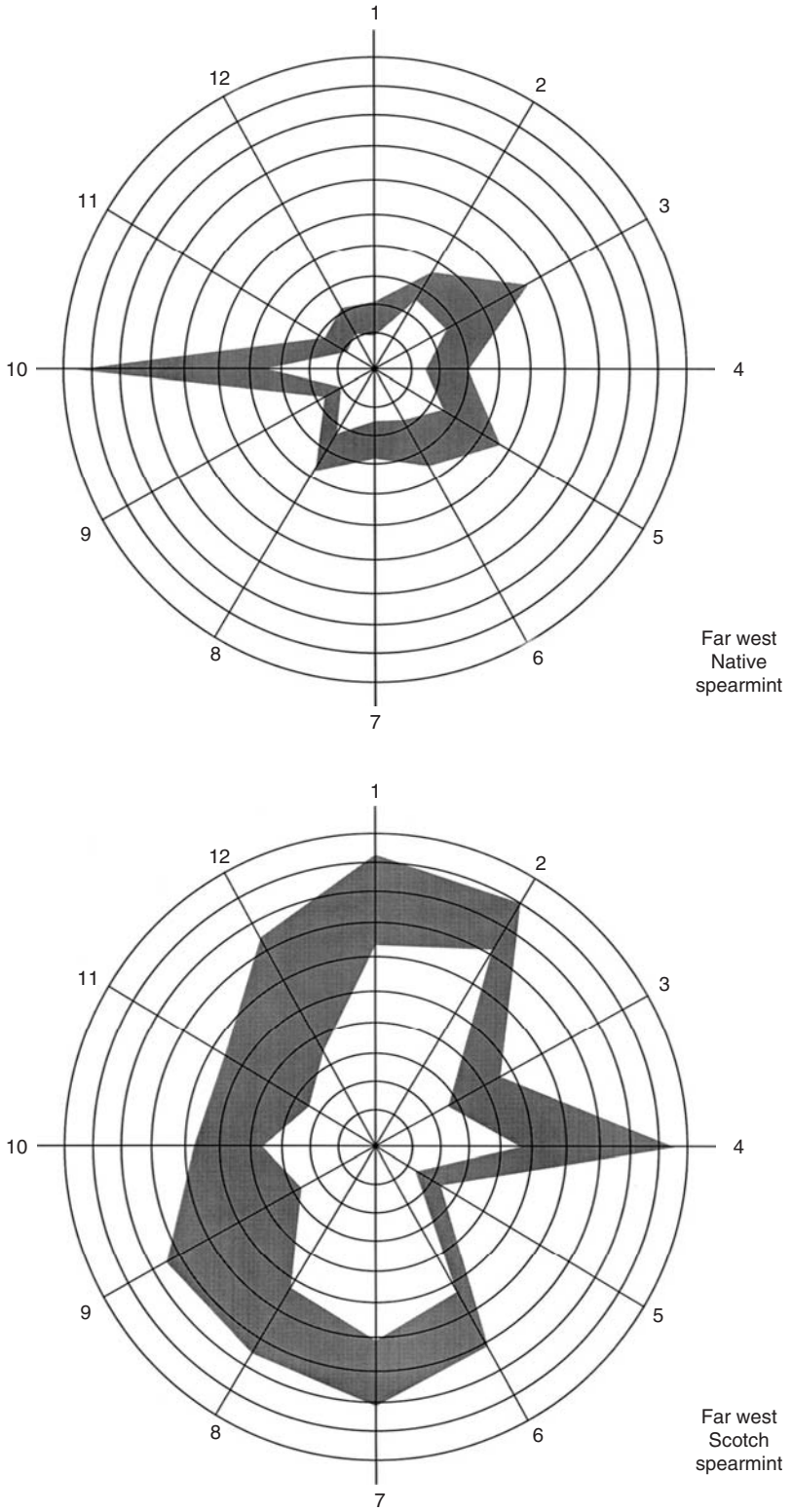


FIGURE 11.2 Pattern recognition (polygonal representation) of the component ratio data for Far west Native and Scotch spearmint oils.

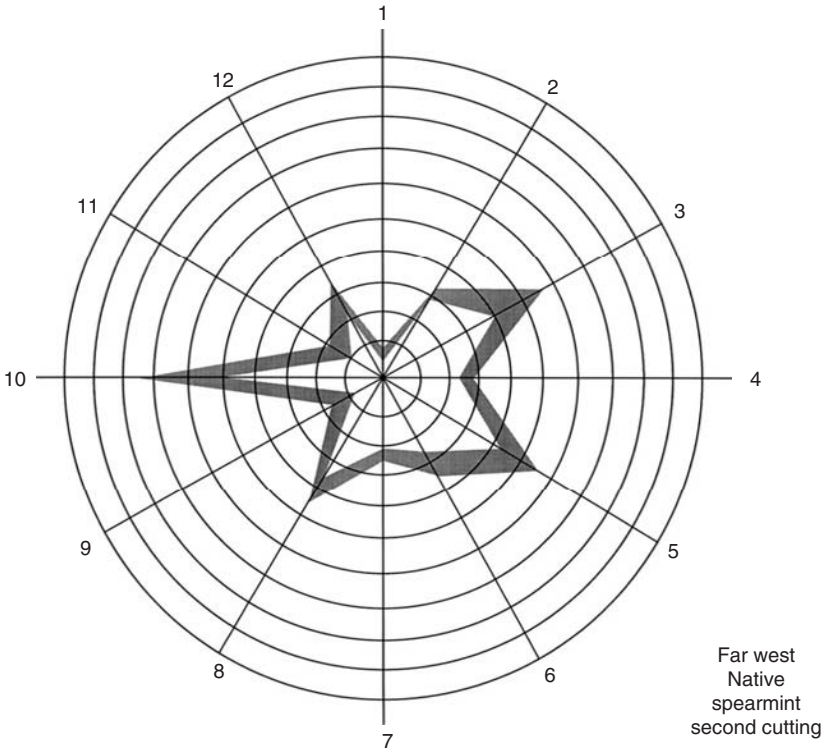


FIGURE 11.3 Pattern recognition (polygonal representation) of the component ratio data for second cutting Far west Native spearmint oil.

summary of which can be seen in Table 11.10, the components that were chosen for differentiation between the Chinese and Indian DMOs were menthone, isomenthone, menthyl acetate, menthol, piperitone, and germacrene D. However, because compounds such as limonene and 1,8-cineole are often reduced by ''topping'' and the amount of menthofuran

TABLE 11.10
Comparative Composition (%) of Selected Constituents of Indian and Chinese Dementholized Cornmint Oils

Compound	Indian Oils	Chinese Oils
Limonene	0–3.09	2.05–4.39
1,8-Cineole	0–0.68	0.28–0.52
Menthone	18.21–24.62	19.25–27.06
Menthofuran	0–0.04	0–0.11
Isomenthone	10.99–16.49	7.04–11.18
Menthyl acetate	1.47–4.36	1.30–4.24
Isopulegol	1.00–2.16	0.89–1.61
Neoiso(iso)pulegol	0.91–2.26	0.86–1.53
Menthol	34.32–46.50	30.10–38.86
Germacrene D	0.11–1.07	0.09–0.59
Piperitone	0.30–1.50	0.90–2.26

TABLE 11.11
Differentiation between Chinese and Indian Dementholized Cornmint Oils Using Component Ratios

Component Ratios	Chinese	Indian
Menthone/isomenthone	1.64–3.84	1.58–1.92
Menthone/menthyl acetate × 1/3	1.52–6.92	1.51–5.33
Menthone/menthol × 5	2.65–4.15	2.05–3.10
Menthone/piperitone × 1/10	1.09–2.27	1.48–7.84
Menthone/germacrene D × 1/25	1.36–7.42	0.86–5.33
Isomenthone/menthyl acetate × 1/2	1.13–9.31	1.44–4.19
Isomenthone/piperitone × 1/5	0.67–3.76	1.87–8.20
Menthyl acetate/piperitone	0.64–3.62	3.15–5.12
Menthyl acetate/germacrene D × 1/3	1.67–9.41	1.24–3.23
Menthol/piperitone × 1/15	0.96–2.88	2.08–9.51
Menthol/germacrene D × 1/50	1.06–7.07	0.78–5.03
Piperitone/germacrene D × 1/2	1.19–7.88	0.31–0.98

is generally very low to trace, the reduced number of component ratios had to be used. These ratios were as follows:

1. Menthone/isomenthone
2. Menthone/menthyl acetate × 1/3
3. Menthone/menthol × 5
4. Menthone/piperitone × 1/10
5. Menthone/germacrene D × 1/25
6. Isomenthone/menthyl acetate × $\frac{1}{2}$
7. Isomenthone/piperitone × 1/5
8. Menthyl acetate/piperitone
9. Menthyl acetate/germacrene D × 1/3
10. Menthol/piperitone × 1/5
11. Menthol/germacrene D × 1/50
12. Piperitone/germacrene D × 1/2

As in the other two examples of this technique, certain multipliers have to be used against the component ratios to ensure that the data would fit within the same type of circular graphical representation that is the hallmark of this pictorial pattern recognition procedure. The results of using these component ratios and their corresponding multipliers to differentiate between Chinese and Indian DMOs can be seen in Table 11.11. The component ratios shown in Table 11.11 can be seen in their pattern recognition form in Figure 11.4 and Figure 11.5. From these figures, it is evident that both Chinese and Indian DMOs can be readily differentiated.

11.5 MINT OIL STANDARDIZATION

The term standardization is often used in the essential oil industry to indicate that a coupage (a blend of components from other sources) has been added to an oil so that the seasonal or geographical variation of the oil blend is minimized and that oil composition consistency is

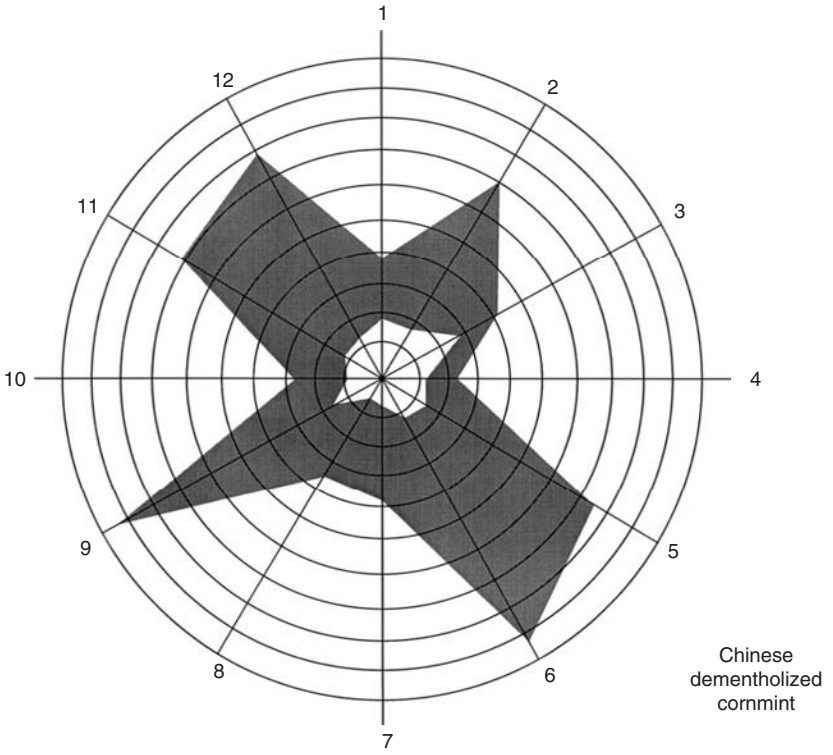


FIGURE 11.4 Pattern recognition (polygonal representation) of the component ratio data for dementholized Indian cornmint oil.

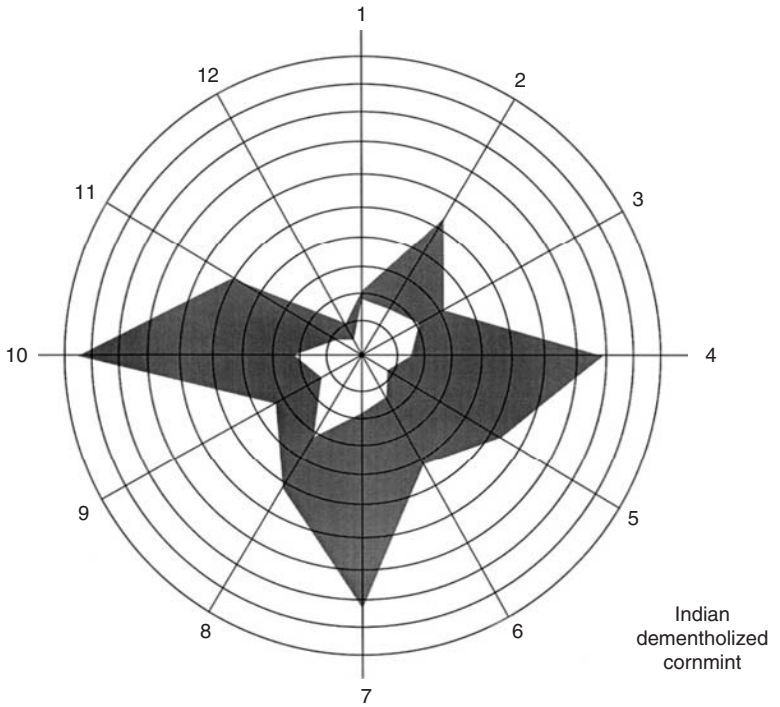


FIGURE 11.5 Pattern recognition (polygonal representation) of the component ratio data for dementholized Chinese cornmint oil.

maintained from shipment to shipment. In some situations, the essential oil seller compounds a coupage so that the end product oil is specific to a customer.

In their discussion on mint oils standardization, Moyler and Moss (1998) noted that commercial gain was not a valid reason for blending mint oils. They stated that the sole purpose of standardization was to achieve consistency in the commercial availability and supply of mint oils irrespective of crop year and origin. As a result, oil blends should not have a geographical origin on the label or they should be labeled as ''with other natural flavors added'' (WONF). Oil standardization for consistency can, therefore, be either

1. A blend of oils produced from the same botanical source irrespective of the geographical origin of the oils
2. An oil with other natural ingredients added
3. A blend of oils with other natural ingredients added

The only difference between these three is that the one listed at no. 1 is a genuine oil whereas the other two are WONF oils.

The blending of oils produced in different geographic locations from the same taxonomic source (although it could well be a different cultivar) is not an uncommon practice. However, if the oil is sold under a geographical origin label, then this is a nefarious practice. Smith and Levi (1961) were able to demonstrate that the use of distribution plots of certain constituent ratios permitted the partial differentiation of oils from various distinctly different origins.

As a follow-up to this, Hawkes and Wheaton (1967) used statistics to analyze the data of Smith and Levi. Although through mathematical manipulation they were able to show how two-part blended oils could be differentiated, probability calculations always have levels of confidence, which, depending upon the data selected can vary substantially. Many years later, Clark and Menary (1981) compared their data on Australian peppermint oils with the data of Smith and Levi. Using principal coordinate analysis, they were able to show differentiation between Australian oils produced in different parts of Tasmania and King Island. They were also able to show some differentiation between U.S. oils and oils of other origins; however, the data used was not their own and, therefore, it was not truly comparable. More recently, Gilly et al. (1986) used analysis of variance to distinguish between peppermint oils of the *pallescens* type (white mint) and the *rubescens* type (Mitcham mint).

Earlier in this chapter, we have seen that oils of peppermint, spearmint, and dementholized cornmint could be differentiated based on a pattern recognition method using component ratios. As noted in the chapter on Indian mint oils, the production of Indian peppermint has increased substantially over the past 10 years. It should be noted that Indian peppermint oil has substantially lower price than North American peppermint oil, so a blend of 10% Indian oil in North American oil offers an economic temptation whereas a blend of higher levels of Indian oil in North American oil has been known to be hard to resist.

Employing model systems, Lawrence (1999) used the component ratio technique to determine that a blend of 10% Indian peppermint oil in oils produced in different regions of the United States could be differentiated based on their component ratios. A summary of the use of these data to differentiate the various oils can be seen in Table 11.12.

11.6 MINT OIL ADULTERATION

Before discussing the specific adulteration of mint oils, it should be noted that adulteration can take many forms, such as:

1. The addition of synthetic (foreign) compounds unrelated to the oil composition
2. The addition of synthetic compounds related to the oil composition

TABLE 11.12
Component Ratios That Can Be Successfully Used to Detect 10% Adulteration of North American Peppermint Oil with Indian Peppermint Oil

Component Ratios	Midwest	Willamette	Madras	Yakima	Idaho
1,8-Cineole/menthol × 50*	+	+	+	−	−
1,8-Cineole/piperitone × 1/2	+	+	+	−	−
1,8-Cineole/germacrene D × 2/3	+	+	+	−	+
Menthone/isomenthone	+	−	−	−	−
Menthone/menthol × 5	+	+	−	−	−
Menthone/piperitone × 1/10	+	+	+	+	−
Menthone/germacrene D × 6/25	+	−	+	−	−
Menthofuran/menthol × 50	−	−	+	−	−
Menthofuran/piperitone × 1/2	−	+	+	−	−
Menthofuran/isomenthone × 2	−	−	+	−	−
Menthofuran/germacrene D	−	−	+	−	−
Isomenthone/piperitone × 2/5	−	+	−	+	−
Menthol/piperitone × 1/15	−	+	−	+	−
Menthyl acetate/piperitone × 1/2	−	+	−	−	−
Piperitone/germacrene D × 10	−	+	−	−	−

+Differentiable component ratio.
*Multipliers used to plot polygons.

3. The addition of oils or fractions of oils of similar composition to all or part of the oil
4. The addition of natural compounds produced enzymatically or from other oil sources
5. The addition of more than one of the above

The addition of both natural and synthetic components to a genuine oil for financial gain is unfortunately not an uncommon practice in the essential oil industry.

Prior to 1960, it was reported that peppermint oil had been adulterated with camphor oil, cedarwood oil, copaiba balsam oil, eucalyptus oil, sandalwood oil, castor oil, mineral oil, paraffin oil, kerosene, anethole, methyl alcohol, α-terpineol, triacetin, benzoate esters, and phthalate esters (Gildemeister and Hoffman, 1961).

With the advent of chromatographic techniques, particularly gas chromatography and gas chromatography coupled with mass spectrometry, the addition of synthetic compounds unrelated to oil composition ceased almost completely.

It is of interest to note that the use of DMO as a bogus replacement for peppermint oil was noted as early as 1913 (Holmes, 1925). Straus and Wolstromer (1974) examined the adulteration of peppermint oil with cornmint oil. They stated that when the limonene content of peppermint exceeded 2% to 2.5% and the 1,8-cineole below 4.0% then cornmint was suspected as an adulterant. Further, they pointed out that because of the commercial availability of monoterpenes, the amounts present in a peppermint oil could easily be manipulated. As a result, the authors proposed the use of *trans*-sabinene hydrate as the key component to determine adulteration of peppermint oil with cornmint oil. The authors stated that the *trans*-sabinene hydrate content of peppermint oil appeared to be consistently in the 1% range irrespective of its geographical origin. With the added fact that cornmint oil contained only trace levels of *trans*-sabinene hydrate or was devoid of it, the convenience of this compound as an adulteration check was obvious.

TABLE 11.13
Percentage Composition of Selected Constituents Found in Peppermint Oil Adulterated with Dementholized Cornmint Oil

		Dementholized Cornmint Oil Addition to Peppermint Oil					
Compound	**Peppermint Oil**	**10%**	**20%**	**40%**	**60%**	**80%**	**100%**
α-Terpinene	0.39	0.36	0.32	0.24	0.14	0.08	0.01
1-Octen-3-ol	0.18	0.17	0.16	0.14	0.08	0.07	0.01
trans-Sabinene hydrate	0.78	0.71	0.63	0.48	0.33	0.19	0.01
Menthofuran	1.45	1.39	1.28	1.11	0.92	0.75	0.38
Viridiflorol	0.30	0.26	0.24	0.17	0.12	0.05	0.01
Menthone/isomenthone	6.9	6.2	5.7	4.9	4.5	4.8	3.9
1,8-Cineole/limonene	3.4	2.4	1.7	1.0	0.5	0.3	0.1

Rojahn et al. (1977) noted that viridiflorol along with *trans*-sabinene hydrate and menthofuran were useful key compounds found in peppermint oil that were present in cornmint oil at trace levels, if found at all. Peyron and Rouzet (1984) reported that if the limonene content of natural peppermint oil was greater than 2% to 2.5%, if the 1,8-cineole and *trans*-sabinene hydrate contents were less than 4.0% and 1.0%, respectively, and if viridiflorol was either only present as a trace component or not present at all, then the genuineness of the oil was suspect. They also noted that common adulterants that had recently been found in commercial peppermint oil were synthetic menthol and menthyl acetate and fractions of cornmint oil.

As a follow-up to the work of Straus and Wolstromer (1974) and Rojahn et al. (1977), Lawrence et al. (1989) examined the composition of more than 50 samples of North American peppermint oil and DMO to determine whether menthofuran, *trans*-sabinene hydrate, and viridiflorol could be used as indicators of adulteration. A summary of their findings can be seen in Table 11.13. As can be seen, in addition to the three compounds previously selected, they found two other constituents and two component ratios that revealed a major difference between peppermint oil and DMO. It should be noted that the range of data found for 1-octen-3-ol and menthofuran was surprising as their levels rarely exceeded 0.02%.

In pursuance of the use of the constituents and component ratios to detect adulteration, Lawrence et al. prepared mixtures of DMO in peppermint oil from randomly selected oils and analyzed the mixtures. A summary of these analyses can be seen in Table 11.14. A comparison between these data and those presented in Table 11.13 revealed that all levels of adulteration up to 10% could be determined, although only the 1,8-cineole/limonene ratio could be used to unequivocally prove adulteration at the 10% level. However, the authors pointed out that if the analyses of these selected constituents were coupled with sensory analysis they believed that adulteration of peppermint oil could be proved.

Spencer et al. (1997) examined the composition of a number of samples of dementholized cornmint of both Indian and Chinese origins to determine whether certain components could be used to detect adulteration of North American peppermint with DMO. The compounds selected, their utility potential, and the reason for the decision of whether they could be useful in determining adulteration are presented in Table 11.15. As can be seen, isopulegol was determined to possess a lot of potential, particularly because the average amount found in peppermint oil by Spencer et al. was 0.07%, whereas in Indian or Chinese DMO the average content was 2.02% and 1.22%, respectively.

TABLE 11.14
Comparative Percentage Composition Range of Selected Constituents Used to Differentiate between Peppermint and Dementholized Cornmint Oils

Compound	Peppermint Oil	Cornmint Oil
	Range*	Range*
α-Terpinene	0.33–0.52	<0.01–0.02
1-Octen-3-ol	0.05–0.22	0.01–0.13
trans-Sabinene hydrate	0.36–1.36	0.01–0.02
Menthofuran	0.72–6.71	0.01–0.05
Viridiflorol	0.16–0.34	<0.01–0.01
Menthone/isomenthone	5.5–9.5	1.3–5.0
1,8-Cineole/limonene	2.6–4.4	0.02–0.25

*Summary of more than 50 analyses.

Lawrence (1999) used component ratios to determine adulteration of peppermint oil of North American origin with DMO of Indian and Chinese origins. A summary of the findings of this study is shown in Table 11.16. As can be seen, adulteration of the Idaho peppermint oil with Chinese DMO could not be determined using the component ratio methodology. Also, it should be noted that the compounds chosen for component ratios did not include either isopulegol or neoiso(iso)pulegol. The main reason for this was

TABLE 11.15
Utility of Specific Components to Detect Adulteration of U.S. Peppermint Oil with Dementholized Cornmint Oil

Compound	Utility	Reason
1. Limonene	Low	(a) Difference in content between oils is small (b) Readily available from other sources (c) Readily modified by distillation
2. 1,8-Cineole	Medium	(a) Large difference in content between both oils (b) Readily available from other sources (c) Readily modified by distillation
3. Menthone	Medium	(a) Large difference in content between both oils (b) Because of moderate presence in peppermint oil is poor indicator of low level adulteration
4. *trans*-Sabinene hydrate	Low-medium	(a) Large difference in content between both oils (b) Readily available from other sources (c) Labile in acidic distillation conditions could give false positives
5. Neoiso(iso)pulegol	High	(a) Should be used as an indicator because large difference between both oils (b) GC resolution problems can exist because of co-elution with β-caryophyllene
6. Piperitone	Low-medium	(a) Difference in content between both oils is not large (b) Easily removed by distillation
7. Isopulegol	Very high	(a) Difference in content between both oils is significant

TABLE 11.16
Component Ratios That Can Be Successfully Used to Detect 10% Adulteration of North American Peppermint Oil with Indian or Chinese Dementholized Cornmint Oil

	MD		WIL		MAD		YAK		ID	
Component Ratio	**IN**	**CH**	**IN**	**CH**	**IN**	**CH**	**IN**	**CH**	**IN**	**CH**
1,8-Cineole/limonene	−	−	+	+	+	+	+	+	−	−
1,8-Cineole/menthofuran	−	−	−	−	−	−	+	+	−	−
1,8-Cineole/menthol × 50	−	−	+	+	−	−	−	−	−	−
1,8-Cineole/menthone × 25	−	+	−	−	−	−	−	−	−	−
1,8-Cineole/isomenthone × 2	−	−	−	−	−	−	−	−	+	−
1,8-Cineole/germacrene D × 2/3	−	−	−	−	−	−	−	−	+	−
Menthone/piperitone × 1/10	+	+	−	−	−	−	−	−	−	−
Menthofuran/menthone × 100/6	−	−	−	−	−	−	+	+	−	−
Menthofuran/isomenthone × 2	−	−	−	−	−	−	+	+	−	−
Menthofuran/menthol × 50	−	−	−	−	−	−	+	+	−	−
Menthofuran/piperitone × 1/2	−	−	−	−	−	−	+	+	−	−
Menthofuran/menthyl acetate × 5	−	−	−	−	−	−	+	−	−	−
Isomenthone/piperitone × 2/5	+	+	−	−	−	−	+	−	−	−
Menthol/germacrene D × 6/25	−	−	+	+	−	−	−	−	−	−
Menthyl acetate/germacrene D × 2	−	−	+	+	−	−	−	−	−	−

+differentiable component ratio; MID, Midwest; WIL, Willamette; MAD, Madras; YAK, Yakima; ID, Idaho; IN, Indian DMO; CH, Chinese DMO.

because on a polar GC column, menthyl acetate and isopulegol, and neoisomenthol and neoiso(iso)pulegol elute closely together, thereby making the choice of the two isopulegols less useful unless analyses are performed on both polar and nonpolar GC columns. As a result, with a combination of component ratios and determination of the isopulegol level, peppermint oil adulterated with DMO can be readily detected.

11.7 NATURAL MENTHOL

As natural (−)-menthol is the most important natural isolate used commercially, a comment on its authenticity and how to prove it is in order. Because racemic menthol exists in equal amounts of (+/−)-forms, chiral GC is the obvious method of choice to determine the addition of racemic menthol to natural menthol, as can be seen in the report by Coleman et al. (1998). They demonstrated that using a chiral GC column and selective ion monitoring mass spectrometry, levels of 0.01% (+)-menthol could be unequivocally quantitatively determined if found in a sample of natural (−)-menthol. To determine the time origin of a natural (−)-menthol sample, a technique known as nonequilibrated solid-phase microextraction method (SPME) coupled with gas chromatography or mass spectrometry was developed (Lawrence, 2000) in which the geographical origin of menthol could be categorically determined. The results of the nonequilibrated SPME–GC/MS analysis of ten samples of menthol from Chinese and Indian sources can be seen in Table 11.17. From these results, it is evident that menthol from the two sources can be differentiated.

In addition to the methods described earlier, other techniques have also been used to determine adulteration of mint oil, particularly peppermint. Such methods are enantioselective capillary gas chromatography, often performed using multidimensional GC. This is a

TABLE 11.17
Nonequilibrated SPME–GC/MS Headspace Analysis of Natural Menthol of Chinese and Indian Sources

Compound	Chinese	Indian
α-Pinene	t–0.4	0.6–1.6
β-Pinene	t–0.7	0.5–3.4
Limonene	1.3–2.9	2.1–6.0
3-Octanol	0.5–0.7	0.3–0.6
Menthone	9.6–16.1	12.3–18.9
Isomenthone	3.3–4.6	7.2–10.1
Menthyl acetate	0.7–1.9	1.0–1.6
Isopulegol	1.7–2.4	1.5–1.9
Neomenthol	2.6–3.5	2.5–3.6
Pulegone + neoisomenthol	0.7–1.1	0.7–1.1
Menthol	67.7–76.4	49.4–67.3

t = trace (<0.01%); menthone/isomenthone Chinese 2.9–3.5; Indian 1.7–2.0.

technique in which a "heart cut" of an oil is separated on a nonchiral column and then rechromatographed on a chiral column so that the enantiomeric distribution of selected chiral components can be determined. Another technique that is used, however, to a much lesser extent is natural stable isotope ratio mass spectrometry. This technique uses the thermal degradation of the selected components to CO_2 and with the use of a modified mass spectrometer, the isotopic ratio of $^{13}CO_2/^{12}CO_2$ is determined.

11.8 CHIRALITY OF MINT OIL CONSTITUENTS

Many components of the mint oils possess one or more asymmetric carbon atoms that exhibit optical activity. A summary of the optically active components that exist in peppermint, cornmint, Scotch and Native spearmint, pennyroyal, and *Mentha citrata* oils can be found in Table 7.1f, Table 7.2ac, Table 7.3y, Table 7.4f, Table 7.5r, Section 7.6.2, and Chapter 10, respectively. These chiral compounds of natural origin are generally found in a characteristic enantiomeric distribution assuming that they have evolved via an enzymatically controlled biosynthetic synthesis rather than a change in chirality caused by processing.

Bicchi and Pisciotta (1990) used a combination of a nonpolar GC column coupled to a chiral phase column to demonstrate how the enantiomers of menthone and menthol could be separated. König et al. (1990) also separated the enantiomers of menthone using a chiral phase GC column. In this study, he also separated the enantiomers of isomenthone and piperitone. One of the difficulties experienced in performing enantioselective gas chromatographic separations is the ability to separate different classes of compounds such as alcohols, esters, and ketones on the same chiral GC capillary column phase. Askari et al. (1992) achieved the separation of all the enantiomers of menthone, isomenthone, menthol, and menthyl acetate using a combination of three chiral columns. Using this technique, they were able to determine when a synthetic menthol or menthyl acetate was added to a peppermint oil as an adulterant. Faber et al. (1994) used a single chiral phase capillary GC column to perform direct enantioselective analysis of all enantiomers of menthone, isomenthone, menthol, neomenthol, neoisomenthol, and menthyl acetate. Using this

procedure the adulteration of peppermint oil with racemic synthetic compounds could be readily determined.

11.9 GAS CHROMATOGRAPHY–ISOTOPE RATIO MASS SPECTROMETRY

Faber et al. (1995) described the use of capillary gas chromatography coupled online with isotope ratio mass spectrometry (GC/IRMS) to examine the components of an authentic peppermint oil with commercial oils, some of which had been adulterated. The technique was developed because chiral GC analysis could not be used to prove adulteration with enantiopure or nonchiral substances, whereas use of the GC/IRMS method could prove adulteration of said substances. Using a specifically designed isotope ratio mass spectrometer coupled to a GC, a selected compound component is first separated, pyrolyzed to CO_2, and the $^{12}CO_2/^{13}CO_2$ ratio is determined. As a result, each natural constituent from peppermint oil has a specific ratio that can be compared with a component isolated from a commercial oil and if the ratio does not match, then the oil can be considered to be adulterated.

Oils prepared from 15 clones of peppermint were subjected to GC/IRMS by Faber et al. (1995) to examine that technique for its potential to detect oil authenticity and oil adulteration. The authors showed that by the method of the internal isotopic standard, a characteristic fingerprint of pure natural peppermint oil could be established. Using this somewhat complex technique the authors were able to show that one of the commercial samples analyzed, the fraudulent addition of synthetic (−)-menthyl acetate, was unambiguously determined.

Furthermore, they recommended that a combination of this technique with enantiomeric distribution data could be readily used to prove or disprove the genuineness of a sample of peppermint oil.

11.10 CONCLUSION

In the present chapter, we have seen how taxonomically authentic oils can possess compositional differences. As mint oil adulteration, and more particularly standardization, is relatively common, the use of component ratios and pattern recognition techniques has been shown through examples to detect and quantify adulteration. Also, the use of chiral gas chromatographic analysis and gas chromatography–isotope ratio mass spectrometry has been shown to be effective in determining differentiation.

Finally, it should be noted that when adulterated peppermint or spearmint oils are encountered in commerce, a combination of specific constituent amounts, component ratios, and chiral gas chromatography should suffice to either prove or disprove the genuineness of the oil.

REFERENCES

Askari, C., Kries, P., Mosandl, A., and Schmarr, H.-G. (1992), Quality evaluation of *Mentha* oils using enantioselective CGC-analysis of monoterpenoid constituents. *Arch. Pharm.* (Weinheim), 325, 35–39.

Bicchi, C. and Pisciotta, A. (1990), Use of two-dimensional gas chromatography in the direct enantiomers separation of chiral essential oil components. *J. Chromatogr.,* 508, 341–348.

Clark, R.J. and Menary, R.C. (1981), Variations in composition of peppermint oil in relation to production areas. *Econ. Bot.,* 35, 59–69.

Coleman, W.M., Perfetti, T.A., and Suber, R.L. (1998), Quantitative analysis of menthol isomer distributions in selected samples. *J. Chromatogr. Sci.,* 36, 318–321.

Faber, B., Dietrich, A., and Mosandl, A. (1994), Chiral compounds of essential oils. XV. Stereodifferentiation of characteristic compounds of *Mentha* species by multi-dimensional gas chromatography. *J. Chromatogr.,* A666, 161–165.

Faber, B., Krause, B., Dietrich, A., and Mosandl, A. (1995), Gas-chromatography–isotope ratio mass spectrometry in the analysis of peppermint oil and its importance in the authenticity control. *J. Essent. Oil Res*., 7, 123–131.

Gildemeister, E. and Hoffman, F. (1961), Oils of *Mentha* species. In: *Die Ätherischen Öle,* Triebs, W. and Bournot, K., Eds., Adademie Verlag, Berlin, Germany, Vol II, pp. 246–521.

Gilly, G., Garnero, J., and Racine, P. (1986), Menthes poivrées. Composition chimique analyse chromatographique. *Parfum. Cosmet. Arômes*., (71), 629–632.

Hawkes, S.J. and Wheaton, M.D. (1967), A statistical method for the analysis of peppermint oil and other complex mixtures. *J. Food Sci.*, 32, 629–632.

Holmes, E.M. (1925), Mint and peppermint. *Chem. Drug.,* 103, 504–505.

König, W.A., Krebber, R., Evers, P., and Bruhn, G. (1990), Stereochemical analysis of constituents of essential oils and flavor compounds by enantioselective capillary gas chromatography. *J. High Resol. Chromatogr.,* 13, 328–332.

Kumar, S., Bahl, J.R., Bansal, R.P., Kukreja, A.K., Garg, S.N., Naqvi, A.A., Luthra, R., and Sharma, S. (2000), Profiles of the essential oils of Indian menthol mint *Mentha arvensis* cultivars at different stages of crop growth in northern plains. *J. Med. Arom. Plant Sci.,* 22, 774–786.

Lawrence, B.M. (1999), Mint oils of commerce. In: *4^{e} Colloque Produits Naturels D'Origine Végétale,* Collin, G. and Garneau, F.-X., Eds., Univ. Quebec à Chicoutimi, Chicoutimi, Que, Canada, pp. 101–131.

Lawrence, B.M. (2000), Commercially important mint oils. Paper presented at 31st International Symposium on Essential Oils, Hamburg, Germany.

Lawrence, B.M., Shu, C.-K., and Harris, W.R. (1989), Peppermint oil differentiation. *Perfum. Flav.,* 14(6), 21–30.

Moyler, D. and Moss. N. (1998), Mint oils: potential for standardizing profiles with natural flavoring substances. *Perfum. Flav.,* 23(2), 37–42.

Murray, M.J., Marble, P., Lincoln, D., and Hefendehl, F.W. (1988), Peppermint oil quality differences and the reasons for them. In: *Flavors and Fragrances: A World Perspective*, Lawrence, B.M., Mookherjee, B.D., and Willis, B.J., Eds., Elsevier Sci. Publ., Amsterdam, The Netherlands, pp. 189–210.

Peyron, L. and Rouzet, M. (1984), Huiles essentielles de Menthe et isolates. Deux décennies d'aspects technico-économiques. In: *Proceedings 3 éms Journées Scientifiques Internaionales,* August 1984, Digne-Les-Bains, France, pp. 40–87.

Rojahn, W., Hammerschmidt, F.J., Ziegler, E., and Hefendehl, F.W. (1977), The occurrence of viridiflorol in peppermint oil (*Mentha piperita* L.), *Dragoco Rept.,* (10), 230–232.

Smith, D.M. and Levi, L. (1961), Determination of geographical origins of peppermint oils by gas chromatographic analysis. *J. Agric. Food Chem.,* 9, 230–244.

Spencer, J.S., Dowd, E., and Faas, W. (1997), The genuineness of two mint essential oils. *Perfum. Flav.,* 22(3), 37–45.

Straus, D.A. and Wolstromer, R.J. (1974), The examination of various essential oils. Paper presented at 6th International Congress of Essential Oils, San Francisco.

12 Biological and Toxicological Properties of Mint Oils and Their Major Isolates: Safety Assessment

Johnnie R. Hayes, Mari S. Stavanja, and Brian M. Lawrence

CONTENTS

12.1 INTRODUCTION

Essential oils isolated from the mint family have a long history of use in products that span the range from foods to pharmaceuticals. These uses provide improved flavor to foods, including confectionaries and beverages, and pleasant fragrances to cosmetics and other commercial products. They have been used as folk remedies for a number of ailments and recently have been used or are under investigation for pharmaceutical use. Their potential for use as pesticides has been investigated and some show significant insecticidal activity, although none is currently in use as a pesticide. Overall, these essential oils provide an increased enjoyment of life to many and it is expected that their use will continue and even expand. There is the potential for greater human exposure to these materials as their uses expand. This requires that these essential oils be under scrutiny for potential adverse effects in the general population and in sensitive individuals.

The toxicology of a number of the mint oils has been reviewed by experts qualified by training and experience and the oils have been deemed safe for their intended uses. However,

these judgments have generally been based on specific uses such as in foods or cosmetics while exposure to these materials can be from multiple sources resulting in increased exposure in individuals with multiple sources of exposure. In addition, some of these oils share common chemical constituents and the total exposure to a specific chemical may come from more than a single essential oil. These factors require that there be constant vigilance for potential increased exposure and adverse effects. Although a thorough review of each commercial essential mint oil and their components is beyond the scope of this chapter, we have attempted to review the published biological and toxicology literature concerning selected components of these oils (menthol and pulegone) and provided a brief summary of published toxicology studies of selected mint oils (cornmint, peppermint, and spearmint oils). A comprehensive critical review of the toxicology of these materials is, again, beyond our intended scope for this chapter. More thorough reviews are provided by expert panels that have assessed the safety of certain of these materials and are referenced in the text.

A major problem associated with the toxicity assessment of essential oils is that they are complex mixtures obtained from plants grown under variable conditions. Different processes may be used in the production of the oils and the production of the article of commercial value may vary from supplier to supplier. Therefore, data from a toxicology study with a specific oil may not extrapolate to another oil produced under different conditions because the concentrations of the major chemical constituents as well as the nature and concentrations of the minor constituents may vary. International standard chemical specifications for the various mint oils used in commerce would be extremely helpful in providing materials suitable for comprehensive toxicological testing. While it is possible and useful to assess the toxicological potential of specific chemical constituents of the oils, the toxicological effects of the whole oil may not be reflected by the toxicology of its most toxic constituent because of possible complex interactions between the various constituents of the oil.

To overcome some of these problems associated with the safety assessment of complex mixtures obtained from natural biological sources, methodologies that are based on rational scientific principles have been proposed. One of these methodologies is outlined in this chapter along with an example of its use. The most valuable data for a safety assessment comes from a coordinated and comprehensive series of toxicology studies based on recommendations provided by regulatory agencies, such as the U.S. Food and Drug Administration (FDA), and other groups that have extensive experience in evaluating toxicology data. Elements of such a toxicology program are discussed in this chapter. An example of a toxicology program that meets many of these criteria is the current efforts of the US National Toxicology Program (NTP) to assess the potential toxicity of pulegone, a major component of pennyroyal oil.

12.2 MINT OILS

Mint, which includes spearmint, peppermint, and cornmint, is probably the third most important flavor used after vanilla and citrus (Maftei, 1992). As a result, mints are among the most important commercial herbs grown. The leaves of spearmint and peppermint are widely used in pharmaceutical products in Europe, whereas in Asia cornmint leaves are used. The specifications for peppermint leaves are found in Table 12.1. Peppermint and spearmint are grown for dry leaf production in Germany, Spain, Poland, Bulgaria, Egypt, Morocco, Greece, Israel, United Kingdom, Turkey, Nigeria, and China. The major countries producing peppermint leaves can be seen in Table 12.2 (Garnon, 1992). The compositions of the major essential oils from mints are shown in Table 12.3.

In 1893, William Wrigley introduced peppermint chewing gum while at the same time Colgate, Pepsodent, and Kolynos introduced toothpastes. The chewing gum and toothpaste

TABLE 12.1
Peppermint Leaves Specifications

Cleanliness and Chemical Specifications	1	2	3
Stem content	<2.0%[a] (3 mm)	<5.0% (1.5 mm)	— —
Damaged leaves (*Puccinia menthae*)	—	<8.0%	5% or 8%[b]
Foreign matter	—	<2.0%	1% or 2%
Thin layer chromatography	—	carvone detected	carvone[b] undetected
Essential oil content	—	12 mL/kg or 9 mL/kg[b]	>10 mL/kg or >8 mL/kg[b]
Total ash	—	<15.0%	13% or 15%[b]
HCl insoluble ash	—	<1.5%	<2.0%

1, U.S. Pharmacopoeia 23rd edn. (1995); 2, European Pharmacopoeia 3rd edn. (1997); 3, ISO 5563 (1984).
[a]Including other foreign matter.
[b]Two quality grades.

industries substantially increased the market need for peppermint oil and spearmint oil (Landing, 1969). Although mint oils are often associated with chewing gum and toothpaste, they have a myriad of other flavor uses. The oils and mint flavors (which generally contain some mint oils or mint isolates) are used to flavor confections such as hard and soft candy (sweets), breath mints including the popular extra strong mint tablets, after-dinner mints, chewing gum, etc. Other than confections, mint oils and their corresponding isolates are used in both nonalcoholic and alcoholic beverages (in particular the liqueur or cordial Crème de Menthe). They can also be found as flavorants in frozen dairy products such as ice cream and ice lollies, baked goods, icings, toppings, cake frostings, puddings, sauces, chutneys, etc. Spearmint oil is used in the preparation of mint jelly (a vinegar- and sugar-based jelly) which is often used in the United States with roast lamb as a replacement for the traditional English mint sauce.

Mint oils and mint flavors are used to deliver a cooling sensation and a minty flavor to a number of oral care products such as toothpastes, mouthwashes, breath fresheners, etc. In

TABLE 12.2
Peppermint Leaf Production (Metric Tonnes) by Various Countries 1985–1991

	Annual Production			
Producing Country	**1985**	**1987**	**1989**	**1991**
Germany	8.7	4.1	22.9	4.2
Spain	123.0	49.9	38.8	62.4
Poland	58.5	24.8	62.2	12.5
Bulgaria	26.6	59.8	62.8	72.0
Egypt	127.0	173.5	107.5	54.4
Morocco	924.5	1,023.5	1,170.5	1,273.8
Other countries*	67.6	242.4	49.4	12.5

*Mainly Greece, China, Turkey, Nigeria, and Israel.

TABLE 12.3
Mint Oil Compositions

Peppermint: *Mentha piperita* L.	
L-Limonene	(1.0%–4.0%)
L-Menthone	(11.0%–32.0%)
D-Menthofuran	(1.0%–7.0%)
D-Isomenthone	(2.0%–5.5%)
L-Menthyl acetate	(2.0%–8.5%)
L-Menthol	(28.0%–47.0%)
Germacrene D	(0.5%–3.0%)
Cornmint: *Mentha canadensis* L.	
L-Menthol	(68%–80%)
Dementholized oil	
L-Limonene	(2.0%–4.5%)
L-Menthone	(18.0%–28.0%)
D-Isomenthone	(7.0%–17.0%)
L-Menthyl acetate	(1.2%–4.5%)
L-Menthol	(30.0%–48.0%)
Native Spearmint: *Mentha spicata* L.	
Myrcene	(2.5%–5.0%)
L-Limonene	(6.5%–11.5%)
cis-Dihydrocarvone	(1.5%–8.5%)
L-Carvone	(55.0%–68.5%)
Scotch Spearmint: *Mentha gracilis* Sole	
Myrcene	(0.8%–1.5%)
L-Limonene	(13.5%–21.5%)
cis-Dihydrocarvone	(0.9%–5.0%)
L-Carvone	(62.5%–70.5%)
Pennyroyal: *Mentha pulegium* L.	
L-Limonene	(0.1%–3.5%)
3-Octanol	(0.5%–3.0%)
L-Menthone	(1.5%–8.0%)
D-Isomenthone	(1.0%–10.0%)
D-Pulegone	(50.0%–80.0%)
Piperitenone	(0.5%–3.5%)
Bergamot Mint: *Mentha aquatica* L. var. *citrata Ehrh.*	
L-Linalool	(10.5%–38.5%)
L-Linalyl acetate	(42.0%–78.5%)

fine fragrances, mint oils and mint aromas are used in perfumes, colognes, lipsticks, lip balms, shaving cream, hair lotions, face creams, soaps, shampoos, etc. In household care products, mint oils and their fragrances are used in room deodorizers (air fresheners), cleaning products (including detergents and fabric softeners), soaps, etc. In the over-the-counter personal care products, mint oils are occasionally used in combination with menthol in inhalants, liniments, creams, etc. Mint oils and mint flavors and, more importantly, menthol are used in cigarettes, snuff, and chewing tobacco (including Pan Masala in India) at levels of 50–20,000 ppm. The use levels of peppermint and spearmint oils and menthol in a range of products according to Walker (1967), Furia and Bellanca (1975), Burdock (2002), Lawrence (2004), and Munroe (2004) can be seen in Table 12.4.

TABLE 12.4
Use Levels (ppm) of Peppermint, Spearmint Oil, and Menthol

Product	Peppermint Oil	Spearmint Oil	Menthol
Hard candy	300–2500	400–1800	500–1000
Soft candy	300–1200	450–900	500–1000
Chewing gum	6500–10,000	6500–10,000	100–5000
Extra strong mints	5000–20,000	5000–15,000	1000–5000
After dinner mints	1000–2000	200–1000	200–500
Nonalcoholic beverages	40–100	100–550	—
Alcoholic beverages	150–250	100–150	—
Frozen dairy products	90–110	50–130	—
Baked goods	140–300	250–1300	—
Puddings	50–200	50–150	—
Icing, toppings, cake frostings	5–650	—	—
Sauces and chutneys	—	40–90	—
Jams and jellies	50–200	40–1900	—
Toothpaste	1000–4500	2,500–3500	500–7000
Mouthwash	200–1200	300–1600	100–2000
Breath fresheners	1000–6000	1500–8000	500–10,000
Perfumes and colognes	5000	5000	10,000
Lipsticks and lip balms	100–1000	—	100–1000
Shaving cream and hair lotion	100–1000	—	20,000
Face creams and soaps	5000	1000–5000	10,000–30,000
Shampoos	1000–2000	—	2000–5000
Room deodorizers	100–1000	—	100–5000
Cleaning products	5000–10,000	10,000	1000–5000
Soaps	5000–10,000	10,000	1000–5000
Inhalants	1000–5000	—	>30,000
Liniments and creams	100–1500	—	>20,000
Preshave and aftershave lotions	100–1000	—	50–2000

Clark (1988, 1998) reported that the use of menthol in various products depends upon the geographic origin of its use as shown in Table 12.6. As can be seen, all use levels increased in every category except North American tobacco products in which there was an 18.75% reduction in the use of menthol. This reflects both lower use levels and a reduction in cigarette smoking over the 10-year period. The major increase in menthol use in tobacco products, particularly in India, reflects its increased use in chewing tobacco. The increased use in oral care and pharmaceuticals is reflected in the wider use of convenience products such as toothpaste and tooth powders and over-the-counter pharmaceuticals such as Tiger Balm, a salve used for headaches and other ailments.

12.2.1 Peppermint

An infusion of dried peppermint leaves was noted by Flück and Jaspersen-Schib (1976) to be useful in the treatment of intestinal spasms of the alimentary canal, atony of the stomach, and flatulence. They also noted that it could be used in the treatment of jaundice and gallstones. In addition to its use as an applicant to wounds (presumably of peppermint oil), it has been used as an inhalant in the treatment of head colds.

According to Schauenberg and Paris (1977) either the oil or an infusion of the leaves of peppermint have a variety of internal uses such as the treatment for nervousness, insomnia,

TABLE 12.5
Oils Rich in Menthol

Species	References
Ligustrum delavayanum Hariot.	Baronikova et al. (2001)
Micromeria fruticosa (L.) Druce ssp. *giresunica* P.H. Davis	Baser et al. (1996b)
Satureja atropatana Bung.	Rustaiyan et al. (2004)
Satureja mutica Fisch. et. C.A. Mey.	Rustaiyan et al. (2004)

cramps, dizziness, nervous sickness, spasmodic cough, migraine, and atony of the digestive system. Other activities purported to be associated with peppermint are antispasmodic, carminative, and cholagogic. In addition, it was also reported that peppermint-based products could be used as stimulants (excitants) and in large amounts are reputed to have aphrodisiacal qualities. Leicester and Hunt (1982) showed that peppermint oil rapidly relieved colonic spasms caused by endoscopy. Peppermint oil in combination with caraway oil has been shown to decrease the number of contractions and the contraction amplitude associated with gastro-duodenal motility measured with stationary manometry in healthy human volunteers (Micklefield et al., 2000).

Owole and Ramson-Kuto (1980) determined that a deficiency in the glucose-6-phosphate dehydrogenase enzyme found in babies could result in jaundice caused by a toxic buildup of menthol in their bodies.

Aromatherapists have used peppermint oil in combination with other oils to treat pulmonary complaints and it has been used in mouthwashes and in inhalants, to treat indigestion, constipation, intestinal infections, debility, and vertigo (Valnet, 1982).

It was reported that in the U.K. either dried peppermint leaves, concentrated peppermint water, or peppermint oil, when taken internally, is used as an antiemetic, carminative, diaphoretic, or spasmolytic and when applied to the skin it is used as an antiseptic and antipruritic (Anonymous, 1983). More recently, the only biological activity found associated with peppermint was that of carminative action (Anonymous, 1990). Chiej (1984) reported that peppermint taken as an infusion, fluid extract, syrup, dry powdered leaves, juice, or oil is reputed to possess tonic, stomachic, antispasmodic, antitussive, and insecticidal properties.

TABLE 12.6
Use of Menthol in Products in Different Geographical Regions (Metric Tonnes)

Product	N. America	Europe	Asia	Central and S. America	Near East, Africa and Australia
Tobacco	800[a]–650[b]	100–200	300–1800	100–150	50–200
Oral care	350–500	500–800	450–1500	300–500	250–400
Pharmaceuticals	200–250	400–600	750–1500	100–200	100–200
Confections	80–110	100–300	200–400	140–200	50–200
Shaving products	50–70	80–150	40–300	50–100	30–100
Miscellaneous	20–70	20–100	10–100	10–50	20–100
Total	1500–1650	1200–2150	1700–5600	700–1200	500–1200

[a]1988.
[b]1998.

Reduced postoperative colic was found among patients who were given peppermint following a colostomy (McKenzie and Gallacher, 1989). Gobel et al. (1991) determined that a weak ethanolic solution of peppermint oil applied topically to healthy humans suffering from induced headache produced a significant analgesic effect.

According to Ody (1996), there are 39 different over-the-counter herbal remedies and supplements in which peppermint oil or an extract of peppermint is one of the components (Table 12.7). Ody also reported that there were four over-the-counter herbal remedies that used menthol as an ingredient. They are "Brumbles Proplis, Menthol, and Eucalyptus Lozenges" for use with sore throat and catarrh; "Gerard House Dragon Balm Ointment" to relieve inflamed or swollen joints; "Phytovarix" for use in massaging tired legs; and "Potter's Dermacreme Ointment" for use as a mild antiseptic cream.

It was reported (Buckle, 1997) that although peppermint has been the classic choice for nausea treatment, overexposure can actually bring on nausea. Fixed combinations containing peppermint oil as a component are sold in herbal pharmacies in Germany (Blumenthal et al., 1998). A list of these can be found in Table 12.8. In Germany, peppermint leaves are classified both as a condiment and also as a drug. According to the published literature (Blumenthal et al., 1998), a number of fixed combinations containing peppermint leaves as a component are sold in herbal pharmacies as can be seen in Table 12.9.

Robbers and Tyler (1999) reported that peppermint was a significant carminative herb and that most of the activity is due to menthol (the main oil component). Although it was reported (Blumenthal et al., 1998) that peppermint was a promoter of gastric secretions, the U.S. FDA banned the use of peppermint oil in nonprescription over-the-counter drugs in 1990 stating that it was not an effective digestion aid (Robbers and Tyler, 1999). The same authors noted that this ruling did not mean that the oil was an ineffective digestion aid; it meant that the evidence of its efficacy was not presented to the FDA. Robbers and Tyler further noted that peppermint is used in peppermint tea (ca. 1.5 g/240 mL H_2O), which according to the manufacturers of such products supports digestion, soothes the stomach, and is comforting when one has a cold.

Menthol is used in cough drops sometimes in combination with peppermint oil or other oils such as eucalyptus, anise, fennel, or thyme. Menthol is also used in emollients in low concentrations to relieve irritation by cutaneous receptor depression (Robbers and Tyler, 1999).

Duke et al. (2002) have reviewed the published literature with respect to the medicinal uses of peppermint. They noted that peppermint has been reported to possess the following biological activities: allergenic, analgesic, aphrodisiac, anesthetic, antibacterial, antiemetic, antihepatic, antiinflammatory, antilactogogue, antiitch, antioxidant, antipyretic, antiseptic, antispasmodic, antitussive, antiulcer, antiviral, apoptotic, astringent, bronchodilation, as a calcium-blocker, cardiotonic, carminative, cholagogue, chloretic, CNS-sedative, counter-irritant, decongestant, detoxicant, diaphoretic, digestive, diuretic, emmenagogic, expectorant, insecticidal, myorelaxant, orexigenic, secretolytic, sedative, stimulant, stomachic, tonic, tranquilizer, vasodilatator, vermifugic, and vulnerary. As can be seen from this list, some of the activities appear to be opposites of others listed. Consequently, unless scientific studies, in which the particular biological activity has been demonstrated using an irrefutable protocol, are performed, many of these activities need to be classified in the "old wives' tales" category.

Pittler and Ernst (1998) have published a critical review with a metaanalysis of the use of peppermint oil for irritable bowel syndrome. The authors note that irritable bowel syndrome is a common gastrointestinal disorder; its cause is not known and the treatment is symptomatic. Treatments currently in use are based on symptoms but are often not completely helpful. These treatments include high-fiber diets, bulking agents, muscle relaxants, psychotherapy, and antidepressants. Peppermint oil has been incorporated into over-the-counter preparations

TABLE 12.7
Over-the-Counter Herbal Remedies and Supplements

1. Bio-Strath Artichoke Formula
2. Bio-Strath Valerian Formula
3. Bioforce Boldocynara
4. Bioforce Imp (Imperthritica)
5. Bioforce Potto Oil
6. Bioforce Potto Ointment
7. Blackmore's Cape Aloes and Cascara
8. Culpepper Elder Flower and Peppermint Mixture
9. Frank Roberts Drops of Life Tablets
10. Gerard House Marshmallow and Peppermint
11. Gerard House Sooth-a-Tea
12. Hactos Cough Mixture
13. Health and Heather Indigestion and Flatulence
14. Herbcraft Fresh Breath Cinnamon Spray
15. Herbcraft Peppermint Formula
16. Höfel's One-a-Day Cardiomax Garlic Pearles
17. Lambert's Peppermint Oil Capsules
18. Lane's Cut-a-Cough
19. Lane's Honey and Molasses Cough Mixture
20. Natraleze
21. Neal's Yard Anti-oxidant Herbs elixir
22. Neal's Yard Elderflower, Peppermint and Composition Essence
23. Neal's Yard Slippery Elm Tablets
24. Obbekjaers Peppermint
25. Olbas Oil
26. Olbas pastilles
27. Potter's Elderflower, Peppermint and Composition Essence
28. Potter's Nine Rubbing Oils
29. Potter's Slippery Elm Stomach Tablets
30. Power Health Head Clear
31. Power Health Peppermint Oil
32. Pure-Fil Romagen Herbal Antioxidant
33. Revitonil
34. Ricola Swiss Herb Lozenges
35. Uvacin
36. Weleda Catarrh Cream
37. Weleda Clairo Tea
38. Weleda Laxadoron Tablets
39. Weleda Oleum Rhinale

Used for ingestion (1); stress (2); digestion carminative (3, 15, 16, 20, 23, 24, 29, 37); external use-arthritis and rheumatisms (4, 25, 28, 31); herbal tea (5, 11); external cream for bruises/abrasions (6); digestion carminative and mild laxative (7); colds, chills, sore throat (8, 9, 22, 27, 33); cough (12, 34); indigestion and flatulence (10, 13); breath freshener (14); irritable bowel syndrome (17); expectorant and catarrh (18, 19); counteractive to cell aging (21, 32); inhalant for hay fever (25, 30); catarrh (26, 36, 39); short term female bladder discomfort (35); mild laxative (38).

TABLE 12.8
Fixed Peppermint Oil Combinations

1 Peppermint Oil and Caraway Oil
2 Peppermint Oil, Caraway Oil, and German Chamomile Flower
3 Peppermint Oil, Caraway Oil, and Fennel Oil
4 Peppermint Oil, Caraway Oil, Fennel Oil, and German Chamomile Flower
5 Peppermint Oil and Fennel Oil
6 Peppermint Oil, Fennel Oil, and German Chamomile Flower
7 Senna Leaf, Peppermint Oil, and Caraway Oil

Used for dyspeptic discomfort, mild gastrointestinal spasms, flatulence and fullness sensations (1–6); constipation with spastic-like discomfort (7).

for treatment of the symptoms of the disorder because of its carminative and antispasmodic properties. The authors reviewed the scientific literature for randomized controlled clinical trials of peppermint oil used for the symptoms of irritable bowel syndrome and the clinical trial data from appropriate studies were combined for metaanalysis. The metaanalysis showed a statistically significant improvement of symptoms compared with placebo. However, the authors warn that the positive results of their analysis must be viewed with caution because of concerns with the methodologies used in the studies that were analyzed in their metaanalysis. These include failure to define patient inclusion criteria in some studies, treatment periods of one month or less when treatment periods should be at least 2–3 months, and problems with some of the cross-over designs. It was noted that adverse effects of peppermint oil were reported by some patients in the various clinical trials and include heartburn, perianal burning, blurred vision, nausea, and vomiting. The authors concluded

TABLE 12.9
Fixed Peppermint Leaf Combinations

1 Dandelion root with herb, peppermint leaf, and artichoke leaf
2 Javanese turmeric root, peppermint leaf, and wormwood
3 Licorice root, peppermint leaf, and German chamomile flower
4 Milk thistle fruit, peppermint leaf, and wormwood
5 Peppermint leaf and caraway seed
6 Peppermint leaf, caraway seed, and fennel seed
7 Peppermint leaf, caraway seed, and German chamomile flower
8 Peppermint leaf, caraway seed, German chamomile flower, and bitter orange peel
9 Peppermint leaf, caraway seed, fennel seed, and German chamomile flower
10 Peppermint leaf and fennel seed
11 Peppermint leaf, fennel seed, and German chamomile flower
12 Peppermint leaf, German chamomile flower, and caraway seed

Used for spastic epigastric discomfort because of functional biliary system disorder (1); dyspeptic discomfort because of functional biliary system disorder (2, 4); chronic inflammation of gastric mucosa with gastrointestinal spastic discomfort (3) dyspeptic discomfort, mild gastrointestinal spasms, flatulence and fullness sensation (5–7, 9–12) dyspeptic discomfort, flatulence, fullness sensation and loss of appetite (8).

that the role of peppermint oil in the treatment of symptoms of irritable bowel syndrome has not been established beyond reasonable doubt and additional studies are warranted.

12.2.2 Menthol

Morton (1977) noted that although menthol was an ingredient in some cough drops and nasal inhalants creating a sense of relief from congestion, the benefit was psychosomatic rather than therapeutic. Menthol-containing ointments and creams are used to treat skin diseases because it is considered to possess antipruritic, antiseptic, counter irritant, stimulant, anesthetic, and skin cooling properties. In Asia, menthol-containing preparations are dabbed on the temples to lessen the pain of an ordinary headache.

In China, mint actually refers to *Mentha canadensis* (Po–Ho or Bo–He). According to Hsu et al. (1986), Po–Ho is used to treat fever caused by exogenous pathogenic factors, headaches, hyperemia, sore throat, oral and dermal lesions, rashes, and toothache. Huang (1998) reported that taken internally, the menthol-rich oil of *M. canadensis* can stimulate the motility of the gastrointestinal tract and reduce flatulence. It can also be a Central Nervous System (CNS) stimulant to increase sweat production and to act as a blood vessel dilator. Application to mucosa or skin results in a cool sensation followed by a slow penetration of the dermis, which improves circulation around the application region. On the skin, it can act as a counter irritant by desensitizing sensory irritation and reducing histamine-induced itching. Furthermore, Huang reported that addition of the menthol-rich oil to ethanol can increase the solubility of most drugs and decrease the skin polarity, resulting in enhanced drug penetration.

Chinese physicians use *M. canadensis* in the form of a water-based infusion ingested to dispel abdominal distention and gastric or colonic spasms. Externally, an oil- and menthol-based ointment is rubbed onto the skin to treat both the common cold and headache.

Hendriks (1998) reported that the use of *M. canadensis* or menthol in powders, creams, or ointments was based on their known cooling properties. It was further reported that the cooling properties of menthol are caused by interaction with specific receptors. Viana et al. (2002) determined the specificity of a cold receptor. McKemy et al. (2002) cloned a menthol receptor from trigeminal sensory neurons. This cold- and menthol-sensitive receptor was found to be a member of the transient receptor potential family of channels and is the main thermal stimulus sensor in the mammalian peripheral nervous system. In addition to menthol, other cooling compounds are also known. A summary of the synthetic structures and sensory properties of these materials can be seen in the reports of Watson et al. (1978), Eccles (1994), and Erman (2004).

12.2.3 Spearmint

Spearmint leaves are more commonly used for culinary purposes, whereas peppermint leaves are used mainly in pharmaceutical products although some do find their way into herbal teas that are extensively consumed in Germany and North Africa. In England, large quantities of spearmint leaves are used in the preparation of mint sauce, a sweet vinegar-based condiment that is used on grilled and roast lamb. Mint leaves are also used on potatoes and peas in England as a typical garnish.

Flück and Jaspersen-Schib (1976) noted that an infusion of one pint of water and one spoonful of ground dried spearmint leaves could be used to treat stomach disorders particularly for diarrhea due to chills. They also noted that it could be used to stimulate biliary secretion in jaundice as well as in the treatment of other liver diseases.

Although spearmint is used to a limited extent as a stimulant, antispasmodic, and carminative (Wren, 1989), it is more widely used as a flavorant and culinary herb. Ody (1996) reported

that spearmint oil is used as a component of Frank Roberts Nervous Dyspepsia tablets, which are taken to relieve indigestion and nausea.

Duke et al. (2002) also reviewed the published literature associated with the medicinal use of spearmint and found that it was reported to possess the following biological activities: allergenic, analgesic, antipyretic, antiseptic, antispasmodic, carminative, decongestant, deodorant, dermatitigenic, diaphoretic, digestive, diuretic, emetic, expectorant, insecticidal, neurodepressant, sedative, stimulant, stomachic, and vermifugic. It is of interest to note that in his treatise on herbal drugs, Wichtl (1994) noted that the only use of spearmint leaves was in spearmint tea.

12.2.4 Insecticidal Activity of Mint Oils

The preceding sections have provided a survey of the numerous uses of mint oils. In addition to these, mint oils have been investigated for their insecticidal activity; although they are not the only essential oils that possess insecticidal activities (Saxena and Koul, 1978; Ahmed and Eapen, 1986; Garg and Banerjee, 1997; Regnault-Roger, 1997). With the exception of the use of pennyroyal oil and pulegone in pet collars designed to kill fleas (pennyroyal oil was voluntarily withdrawn for reregistration as a pesticide in the United States), the authors know of no commercialization of mint oils as insecticides. However, these studies do provide interesting information concerning their species-specific toxicity toward nonmammalian species. Although these studies cannot be extrapolated to humans because they do not use validated toxicological models or toxicological protocols, they do provide data that may be useful for comparative toxicology.

Misra and Kumar (1983) evaluated the use of peppermint oil against the red flour beetle (*Tribolium castaneum*). They found that the use of the oil as a fumigant resulted in LC_{50} values (concentration that is lethal to 50% of the test species) of the oil against the first, second, third, and fifth instar larvae were 0.76, 2.14, 11.88, and 20.4 μL/100 cc volume, respectively. Furthermore, it was found that LC_{50} values for adults after 24-h and 48-h exposure were 3.04 and 3.21 μL/100 cc, respectively. A 90% kill rate was obtained for the emerging first instar larvae when they were exposed to 4.0 μL/100 cc, even though the eggs were unaffected. Tripathi et al. (2000) also investigated the toxicity of oils from *Mentha* species and certain of their constituents as a fumigants against *T. castaneum* and also against the pulse beetle, *Callosobruchus maculatus*. The mint oils were obtained from *M. canadensis*, *M. aquatica var. citrata*, *M. piperita*, and *M. spicata* and fractions high in menthone, menthol, linalool, linalyl acetate, menthofuran, limonene, and carvone were also tested. *C. maculatus* was more sensitive to the mint oils and components when used as fumigants than was *T. castaneum* with menthol as the most effective fumigant. *M. canadensis* and *M. piperita* oils and menthone, linalyl acetate, and menthofuran were also effective fumigants. *Mentha spicata* oil was the most effective larvicide while carvone was most toxic toward adults, and *M. canadensis* and *M. spicata* oils were also highly effective against adults.

Harwood et al. (1990) evaluated the effect of peppermint monoterpenes, including pulegone, menthol, and menthone, on larvae of the variegated cutworm *Peridoroma saucia* Hubner. Larvae treated with menthone and pulegone weighed less than the control because of feeding inhibition and abnormalities found in molting in menthol-treated larvae. Menthol inhibited pupation at doses similar to content in peppermint oil. No effects were seen with limonene or alpha-pinene, although alpha-pinene was toxic to sixth stage instars when topically applied to the larvae.

The efficacy of six essential oils against the land leech (*Haemadipsa zeylanica montivindicis*) found in the rough terrain and thick forest of northeastern India was evaluated by

Nath et al. (1986). Spearmint oil was found to be the second best repellent of the oils screened after cinnamon bark oil, with an exposure concentration of 45–180 ppm/cc.

A patent for a mixture of peppermint, eucalyptus, cedarwood, and citronella oils impregnated in an ethylene-vinyl acetate polymer for use in flea collars for dogs demonstrated usefulness according to Seto (1987), cited in Regnault-Roger (1997).

The nematicidal activity of six essential oils, including *M. canadensis* and their major constituents against second-stage juveniles of the seed-gall nematode (*Anguina tritici*), citrus nematode (*Tylenchulus semipenetrans*), root-knot nematode (*Meloidogyne javanica*), and pigeon-pea cyst-nematode (*Heterodera cajani*), was evaluated by Sangwan et al. (1990). They found that the LC_{50} of both *M. canadensis* oil and menthol was considerably higher than either eugenol-rich or eugenol-free clove oil. Menthol was moderately active against *T. semipenetrans* and *M. javanica*.

Common mint oils, including *M. spicata* oil, prevented egg hatching and provoked pupal malformation with the fly *Drosophila aurea* according to Konstantopoulou et al. (1992), as cited in Regnault-Roger (1997). Menthol may be acting as an acetylcholinesterase inhibitor (Miyazawa et al., 1997), as do several common insecticides.

Menthol, along with some other monoterpenes, was evaluated for its acaricidal or miticidal activity against the mage mite of the rabbit (*Posoroptes cuniculi*), either by direct contact or inhalation, by Perrucci et al. (1995). They found that menthol was very effective in causing 100% mortality of the mites either by direct contact with 0.125% menthol solution or inhalation of 1 μL of menthol (in 1:6 w/w of vaseline oil).

The menthol-rich oil of cornmint was found by Singh et al. (1995) to be an effective fumigant against the sorghum weevil (*Sitophilus oryzae*) on sorghum stored for 3 months. The effective use level of the oil was determined to be 166 μL/L.

Regnault-Roger and Hamraoui (1995) and Regnault-Roger (1997) screened a number of essential oils against the kidney brucid (*Acanthoscelides obtectus*). The LC_{50} for peppermint oil at 24-h and 48-h exposures was 22.4 and 8.7 mg/L, respectively.

Franzios et al. (1997) screened oils of *M. pulegium* and *M. spicata* along with pulegone, menthone, and carvone for their insecticidal and genotoxic activities against the fruit fly (*Drosophila melanogaster*). Both oils were found to exhibit strong insecticidal activity, whereas only *M. spicata* oil was found to be mutagenic. The LD_{50} values for (lethal dose for 50% of the test species) *M. pulegium* oil (76% pulegone) and *M. spicata* oil (32% carvone) were 2.09 μL and 1.12 μL, respectively. In comparison, the LD_{50} for pulegone, menthone, and carvone was 0.17, 1.29, and 0.67 mL, respectively. Pulegone and carvone have been shown to be inhibitors of acetylcholinesterase (Miyazawa et al., 1997), which may be associated with their insecticidal activity.

Sixty-three essential oils produced from Bolivian plants and a number of pure isolates were tested against the kissing or assassin bug (*Triatoma infestans*) for ovicidal and larvicidal properties by Laurent et al. (1997). They found that the menthol-rich oil was fairly active. As a result, they examined the ovicidal, lavicidal (against first and fourth instar larvae) effect of L-menthyl acetate, L-menthone, and L-menthol using an impregnated paper screening test. An ovicidal effect of >2 mg/cm^2 for the acetate and ketone and 0.16 mg/cm^2 for menthol was found. Evaluation of the lavicidal effect resulted in 0.75 mg/cm^2 for menthol against the first instar larvae. The level of the larvicidal effect of menthyl acetate and menthone was found to be 1.5 mg/cm^2 or greater.

Chantraine et al. (1998) further screened 52 oils produced from plants grown in Bolivia against the third instar larvae of *Aedes aegypti*. They found that an oil of *M. canadensis* was not a very effective control measure, because it produced a mortality rate of only 15% when the larvae were exposed to 100 mg/L of oil.

As a result of observing that the green peach aphid (*Myzus persicae*) was not able to reproduce or settle on numerous Labiatae plants (Hori and Harada, 1995), Hori (1999) screened the oils of ten Labiatae on the feeding behavior, settling, diet choice, and toxicity against the aphid. He found that spearmint, peppermint, lavender, and thyme oils inhibited the settling caused by their repellency and probing inhibitory, sucking inhibitory, and locomotion stimulatory activities. However, it was noted that neither spearmint nor peppermint oil was toxic to the aphid as was found with thyme oil; they were merely repellent.

Pathak et al. (2000) screened four oils against the third instar larvae of three mosquito species (*Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti*). They found that the oil of *M. piperita* was less toxic than that of *Tagetes erecta* oil, but more toxic against *A. stephensi* (21.4 ppm) and *A. aegypti* (26.2 ppm) than the other two oils tested. Peppermint oil was further evaluated for its larvicidal activity against *A. aegypti*, *A. stephensi*, *C. quinquefasciatus*, *An. culicifacies*, *An. annularis*, and *An. subpictus*. It was found that when the oil was added to water the mortality rate of the third instar larvae of *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* was 85%, 90%, and 100%, respectively (Ansari et al., 2000). They also found that the oil exhibited strong repellent action against adult mosquitoes when applied to human skin. The percent protection and length of protection against *C. quinquefasciatus*, *An. culicifacies* (the principal vector of malaria in Haryana (India)), and *An. annularis* was 84.5% (6.7 h), 92.3% (9.6 h), and 100% (11.0 h), respectively. Furthermore, the authors found that the repellent action of peppermint oil was comparable to the commercially available repellent known as Mulol oil, which is a mixture of dimethyl and dibutyl phthalates.

Papachristos and Stampoulos (2002) screened a number of essential oils against *Acanthoscelides obtectus* Say (Coleoptera, Bruchidae), a major destructive storage pest of the kidney bean (*Phaseolus vulgaris* L.), one of the most important food pulses used in South America and southern Mediterranean countries. They found that the oil of *M. spicata* was most toxic for males but females were less susceptible. Nevertheless, the authors recommended that a combination of lavender (*Lavandula xintermedia* Emeric ex Loisel), Rosemary (*Rosmarinus officinalis* L.), and *M. spicata* could be used to both repel and curb insect damage on stored kidney beans.

12.3 SAFETY ASSESSMENT OF MINT OILS AND THEIR CONSTITUENTS

12.3.1 General Considerations

As previously noted, essential oils derived from the mint family occur in a wide variety of products at various usage concentrations. These products span the range of food, beverages, pharmaceuticals, cosmetics, and fragrances. Mint-derived oils or extracts may either be used directly or specific components may be purified for use. In some cases, such as menthol, synthetic versions of the natural component are sometimes used. The diversity of the chemical constituents of mint oils and the diversity of their use in products destined for human use emphasize the need for toxicological data concerning these materials. Most of these products come under the regulatory responsibilities of the FDA in the United States and similar regulatory agencies in other countries. In the United States, these products are regulated by different branches of the FDA, for example, food additives, cosmetics, and pharmaceuticals. Each branch has slightly different toxicology testing guidelines and it is important to follow the guidelines proposed by the FDA branch that will be reviewing the data. In the case of food additives, the FDA recognizes many compounds and mixtures as "generally recognized as safe" or GRAS under the conditions of their use in food. This designation originally came about to grandfather substances that had been historically added to food with no evidence of adverse effects. Although the FDA originally published a list of 600 substances it considered

to be GRAS, this list was not comprehensive of the food additives used at that time, but served as an example of substances it considered GRAS. There was no legal mandate against other groups from developing GRAS lists and several groups have developed these lists independent of the FDA. The major criterion for the addition of a substance to the GRAS list was that it be evaluated for safety by "experts qualified by scientific training and experience . . . to be safe under the conditions of its intended use." Although FDA may have previously published regulations concerning a number of mint-derived oils or their components, new uses or increased quantity of use may necessitate providing the agency with new toxicology data.

Because of the wide use of essential oils and specific chemical components derived from them, trade organizations have been established under the sponsorship of manufactures to collate known safety data, to develop new safety data, and to evaluate and distribute these data to the sponsors, regulatory agencies, and other interested parties. An example of such an organization is the Research Institute for Fragrance Materials (RIFM) established in 1966. RIFM has been engaged in the development and evaluation of safety data through the use of an expert advisory panel. This panel consists of individuals who by training and experience are experts in various aspects of the testing and safe use of the materials under evaluation (Bickers et al., 2001). Another such organization is the Flavor and Extract Manufacturers Association (FEMA). FEMA has organized an Expert Panel to evaluate the safety of substances used in foods and determine if they are suitable as GRAS substances. Recently, the Expert Panel has published a scientifically based guide to evaluate the safety of naturally occurring mixtures for their use in foods, the guide emphasizes the evaluation of essential oils (Smith et al., 2005a). Their approach will be discussed further in Section 12.3.16.

The evaluation of existing toxicological data and the design of new toxicology studies must be within the context of the current principles and good practice of toxicology. Toxicological safety assessments are required where existing studies do not provide appropriate data that meet these criteria and where the data are inadequate or no data exist. The major aspects of a toxicological safety assessment are discussed later.

12.3.2 Review of the Scientific Literature

Any toxicological assessment begins with a careful review of the scientific and regulatory literature to evaluate what is known concerning the toxicology of the material of interest and its regulatory status. Another consideration is the relevance and quality of the available data. Certain studies may not meet currently accepted principles for toxicology testing and these will have to be assessed with respect to the need for retesting. A toxicological testing program needs to be designed and initiated in the areas where little data are available or where the available data are not sufficient or not appropriate for the specific material of use or to the specific method of use.

12.3.3 Structure Activity Relationship Assessment

Although a structure activity relationship (SAR) assessment of a complex mixture is not possible, a SAR can be useful in predicting the level of concern of an individual chemical constituent of a mint oil or extract. Commercial computer programs are available to aid in the prediction of toxicological concern associated with a particular chemical structure based on SAR. These programs are generally based on learning sets from previously tested compounds. They develop correlation equations relating specific parameters, such as chemical structure, calculated molecular size and shape, electronic configurations, and known or calculated oil or water partition coefficients, to specific toxicological endpoints. As the size and diversity of the learning sets in these programs increase, so should the accuracy of their predictions. However,

the results from these computer programs can only be considered as predictions and cannot be substituted for actual toxicological testing, although they are sometimes used to determine the priority of testing of a series of compounds.

In respect to food additives, the FDA (1993) has presented SAR guidelines to place a chemical into three structure categories. The FDA SAR categorization is based on chemical structures and functional groups that have been associated with specific types of toxicities. Depending on the structure of the chemical and the structures of its known or predicted metabolites, it can be placed in either Structure Category A that includes chemical structures predicted to be of low toxic potential; Structure Category B that includes structures predicted to have adverse effects excluding mutagenicity and carcinogenicity; or Structure Category C that includes structures associated with mutagenicity and carcinogenicity. The FDA provides further guidance in using the SAR in conjunction with data concerning potential human exposure to develop an initial ''concern level'' for the chemical.

Ford et al. (2000) have published a review of the SAR assessment used by the Expert Panel of the RIFM to assist in their review of fragrance chemicals. It differs a little from the FDA approach for food additives by including structural alerts for topical exposure effects. Ford et al. (2000) have listed 49 molecular groupings and functional groups in their SAR database, 24 of which may be associated with topical effects including potential skin sensitization, photoirritation, and photoallergy. The SAR assessment is combined with consumer exposure and quantity of use to determine the priority for review of specific fragrance materials.

12.3.4 Exposure Assessment

A critical factor in any safety assessment is a determination of the potential human exposure to the material of interest (FDA, 1993; Ford et al., 2000; Paustenbach, 2001). Exposure is determined by the concentration of the material in a product and the quantity and frequency of use of the product. Exposure can be topical (such as with soaps and cosmetics), oral (such as food), oral care products (such as toothpastes and mouthwashes), and drugs and by inhalation (such as fragrances). All these exposures can lead to systemic exposure through absorption. Most toxicity is related to systemic exposure with exceptions, such as dermal sensitization, phototoxicity, and dermal irritation. There are a number of methods to assess exposures that are dependent on the route of exposure.

With a specific product, the concentration of the material of interest in the product is known, along with the anticipated level and frequency of use. However, specific individuals may use the product at a higher frequency and quantity than the average consumer. Because of this, exposure assessments need to be based on the individuals using these products at a higher rate. For instance, RIFM uses the 97.5% level of use for fragrances (Ford et al., 2000), while FDA uses the 90% for consumption of food additives (FDA, 1993). Determining exposure at these high levels of consumption and use leads to conservative estimates that will include the vast majority of consumers. Exposure to materials in drugs can be better estimated because of the recommended dosages used for these products.

Concomitant exposure to the same material through consumption or use of multiple products containing the material complicates an exposure assessment, but must be considered. Total exposure will result not only from use of the product under investigation but also from other products containing the same substance; this additive effect must also be accounted for during the exposure assessment. Various databases exist that may help with exposure assessment. These can include data provided by manufacturers concerning the total quantity of the material manufactured per year, such as the data provided for peppermint leaf production in Table 12.3; data concerning estimated quantity of use in products containing

the material, such as those seen in Table 12.4; and data estimating quantity and frequency of use by individual consumers. The reliability of these data must always be considered because they are based on voluntary data provided by manufactures of the particular material, reported usage levels in products, and consumer provided quantity and frequency of use data. Therefore, conservative estimates and possible correction factors need to be employed to ensure that these estimates represent conservative estimates of total exposure.

12.3.5 Route of Exposure

A critical issue that must be evaluated is the route of exposure of an essential oil, extract, or a specific component of an oil or extract. Because mint oils and extracts may be used in a wide variety of products, consultations between product developers and toxicologists begin this process. A material used to provide flavor in a food or drink product would primarily result in oral ingestion; whereas, a material added to a toothpaste or mouthwash would result in a primary exposure to the oral mucosa resulting in lingual absorption, with a potential secondary exposure by oral ingestion. A material added to a skin care product would result primarily in dermal exposure but could result in systemic exposure through dermal absorption into the blood stream. After careful consideration of the intended use of a product and its potential unintended use or secondary exposures, the route of exposure or multiple routes of exposure to be used during toxicological evaluation can be determined. Because the route of exposure is an important determinate of toxicity, this is one of the most important considerations for the evaluation of the safety of a material.

12.3.6 Test Material

Before a toxicological safety assessment can be initiated, the precise nature of the material to be tested, often called the test article by toxicologists, must be defined. Again, this is a team approach that involves not only product developers, chemists, and toxicologists, but also experts in the manufacture of the product containing the essential oil, extract, or component and those familiar with procuring or sourcing the material to be used in the manufacture of the product. Consultation between these experts should result in a determination of the exact nature of the test article.

The material to undergo toxicological assessment should be as identical to the material to be used in the production of the final product as possible. For example, if an oil or extract from a member of the mint family is to be used, then an oil or extract that is as close as possible in chemical composition to that to be used in the product should be evaluated. An exact chemical match to a plant extract or oil may not be possible because its chemical composition may vary slightly depending on intrinsic and extrinsic factors such as cultivar selection (genetic) or environment and growing conditions, which will vary year to year. Some essential oils are further processed after their initial production to: (a) remove off-notes formed during their isolation by distillation or (b) remove some top-notes (monoterpene hydrocarbons) to ensure that the oil produced meets national or international specifications. Oils produced during a season contain components that can vary quantitatively. To ameliorate any within-season compositional variations, the oils can be blended. Because of this, the chemical composition of the final blended oil will be more consistent than that of individual drums of crude oil. This increases the ability to set specifications for the test article for toxicological testing that will encompass the variability encountered in the oil used for production of the product of interest. Conditions that can be controlled, such as the isolation or extraction process, should represent those to be used in producing the oil or extract. This will ensure that any residual solvents or manufacturing aids that may be in the extract and

any changes in the natural composition produced by extraction will be represented in the test article.

Specifications for the oil or extract to be evaluated need to be set and should be broad enough to encompass the variation found in the oil produced for human consumption. It would be unfortunate if the specifications were set so stringently that they could not be met in practice, invalidating the safety assessment. Specifications should begin with the plant from which the oil is obtained, geographical source(s), specific anatomical locations of the plant used to obtain the oil, and any other characteristics associated with collecting and processing the plant to obtain the crude oil or extract that may be pertinent. In addition, specifications should include any secondary processing of the oil resulting in purification and obtaining the proper balance of constituents to obtain the desired flavor or aroma. The specifications should also include physical characteristics of the oil, such as specific gravity among others, and its chemical composition, especially those constituents important to the flavor and any constituents that may be of toxicological concern and, importantly, the percentage of the oil that contains unidentified chemical constituents. Also, quality control standards are needed to compare different batches of the oil or extract to those used in the safety assessment. These same principles are still appropriate when an individual component of an oil is to be used instead of the natural mixture. When a specific component of mint oil is produced synthetically, the synthetic process, its chirality, and purification methodology should be identical to the isolation process to ensure that the test article contains only chemical residues that would be anticipated to occur in the final product.

An approach that has sometimes been used in the toxicological assessment of essential oils is to determine the potential toxicity of the major component of the oil. Although the overall toxicity of a complex mixture may represent the toxicity of its most toxic constituent, this is certainly not the case with certain mixtures. The overall toxicity of a mixture can be the additive toxicity of some of its components and less frequently synergistic interactions may be encountered where the toxicity of a mixture is greater than simply additive. Another possibility is that the toxicity of a mixture may be less than the expected additive toxicity of its individual components (antagonistic interactions). For example, Franzios et al. (1997) determined the acute toxicity of the essential oil from *M. pulegium* (containing 75.7% pulegone and 10.1% menthone) in fruit flies (*D. melanogaster*) and compared this to the acute toxicity determined for pulegone alone and a 7:1 mixture of pulegone and menthone. LD_{50}s (μL) were found to be 2.09 for the essential oil, 0.17 for pulegone, and 4.06 for the pulegone:menthone mixture. The authors pointed out that these toxicity data were unpredictable and indicative of interactions. For instance, based on the toxicity of pulegone and its content in the oil, the oil would be predicted to be nine times more toxic than the data indicate. The pulegone:menthone mixture was two times less toxic than the oil, which contained a similar ratio of the two constituents, and 17 times less toxic than pulegone alone.

Synergy and less than expected toxicity can be encountered when two or more of the components of a mixture compete with each other for enzymes associated with their detoxication or in cases where a metabolite may be more toxic than its parent compound. These interactions can be complex and in many cases are difficult to predict. These factors, among others, emphasize the need to study the toxicity of the complete mixture as opposed to an individual component of the mixture, even though it may be the major component.

If it is known that the toxicity of a mixture, such as an essential oil, is in most part related to a single component, then it is possible to produce a less toxic mixture by removing that component. An example to be discussed in more detail is pennyroyal oil in which the removal of pulegone results in a substantial reduction in the toxicity of the oil.

12.3.7 Acute Toxicity

Generally, the first set of toxicology data collected for the material assessed for safety is acute toxicity from a single exposure. Acute toxicity data are most valuable for toxicity comparison with other materials. Secondarily, they are useful in setting dose ranges for longer-term multiple dose studies. Previously, acute toxicity studies were designed to provide LD_{50} (lethal dose for 50% of the animals) data that were quantitative and contained statistical information on the uncertainty of the value. Currently, the determination of LD_{50} data is not favored and regulatory agencies discourage its use. This is based on the large numbers of animals of each gender required for the determination and because these data are highly species and strain dependent and may vary between different laboratories. There are a number of accepted designs that provide an acute maximally tolerated dose (MTD) based on the production of severe signs of toxicity but not necessarily causing death. Although general study designs have been presented (DiPasquale and Hayes, 2001), it is important to follow the basic guidelines for acute toxicity testing proposed by the regulatory agency that will be reviewing the data. Acute toxicity data are generally required for both genders of two species, a rodent species and a nonrodent species. The route of human exposure to the material under investigation is generally the same as that used in animal studies. Data from dermal exposures are generally supplemented with an exposure route, such as oral or intravenous, that allows for higher systemic exposure.

Table 12.10 provides acute toxicity data for some of the mint oils and their constituents. These data indicate that these materials are generally not highly acutely toxic, with the acute oral toxicity in the range of g/kg of body weight with the exception of pulegone, and the dermal acute toxicity less than the oral toxicity, as would be expected.

12.3.8 Metabolism Studies

Regulatory agencies require information concerning the metabolism of the material under investigation. Metabolism data are also important to the toxicologist in the appropriate

TABLE 12.10
Acute Toxicity of Mint Oils and Their Components

Essential Oil or Component	Specie	Oral LD_{50}	Dermal LD_{50}	References
Pulegone	Rat	470 mg/kg		ECH & CPDG (2002)
Menthone	Rat	1.6–1.95 g/kg		BIBRA Int. (2000)
Racemic menthone	Rat	2.18 mL/kg	>5 g/kg	BIBRA Int. (2000)
Isomenthone			>5 g/kg	BIBRA Int. (2000)
Peppermint oil	Mouse	2.41 g/kg		CIREP (2001)
	Rat	4.44 g/kg		
	Rabbit		>5 g/kg	RIFM (2004)
L-Carvone	Mouse	5.1 mL/kg		RIFM (2004a)
	Rat	1.64 g/kg		Jenner et al. (1964)
	Guinea pig	766 mg/kg		Jenner et al. (1964)
Cornmint oil	Rabbit	1.24 g/kg	>5 g/kg	RIFM (2004b)
Spearmint oil	Rat	5 g/kg		RIFM (2004c)
	Guinea pig		2 g/kg	
	Rabbit		>5 g/kg	
Menthol	Rat	2.9–4.0 g/kg		BIBRA (1990)
	Rat	3.18 g/kg		Jenner et al. (1964)
Linalool	Rat	2.79 g/kg		Jenner et al. (1964)

design of the toxicology studies. These data include such fundamental information as the chemical structures of the metabolite(s), their rates of formation, and the major enzymes involved in their metabolism. In addition, information concerning the chemical nature and concentrations of the parent compound and its metabolites in plasma, urine, and feces are useful. It has been recommended for decades that such data be available before initiation of critical toxicology studies; however, these data were rarely available because of the time and analytical resources required. Recent advances in analytical chemistry, especially in liquid chromatography mass spectroscopy have made it possible to provide these data during the early stages of the safety assessment (Caldwell et al., 2001). When pulegone, a major constituent of pennyroyal oil, was nominated for carcinogenicity testing by the U.S. NTP, its metabolism was investigated in the species to be used in the carcinogenicity studies at appropriate toxicological doses (Chen et al., 2001, 2003a,b). These studies provided a metabolic profile of pulegone in the rat and mouse strains to be used in the toxicology program and also provided information important for the toxicologists in developing the study designs. For instance, they indicated that mice excreted 85%–100% of the dose in 24 h, whereas rats excreted 59%–81% of the dose in the same time period. This indicates that rats may have a greater potential to accumulate pulegone and its metabolites during multiple dosing than do mice. These studies also indicated that male rats tended to have higher kidney concentrations of pulegone and its metabolites than female rats, a possible important gender difference.

As seen in Table 12.11, there is diversity in the enzymes capable of metabolizing foreign compounds. Of these, the most capable group of enzymes is the cytochrome P450 isozymes that in many cases initiate foreign compound metabolism and often produce metabolites that are further metabolized by enzymes such as the glucuronosyltranferases and sulfotransferases (deBethizy and Hayes, 2001; Parkinson, 2001). Metabolism is generally considered a

TABLE 12.11
Types of Reactions and Enzymes That Participate in Foreign Compound Metabolism

Oxidation	Ester Hydrolysis
Cytochrome P450 isozymes	Carboxylesterases
Xanthine oxidase	Amidases
Peroxidases	
Amine oxidase	Dehydrogenases
Monoamine oxidase	Alcohol dehydrogenases
Dioxygenases	Aldehyde dehydrogenases
Reduction	Methylation
Cytochrome P450 isozymes	O Methyltransferases
Ketoreductase	N Methyltransferases
Glutathione peroxidases	S Methyltransferases
Hydration	Acetylation
Epoxide hydrolase	N Acetyltransferase
	Acyltransferases
Conjugation	
Glucuronosyltransferase	Thiosulfate
Sulfotransferase	Sulfurtransferases
Glucosyltransferase	
Thioltransferase	

detoxification step because the products of metabolism are generally more water soluble and more readily excreted than the parent compound. However, in some cases the metabolite is actually more toxic than the parent compound. For instance, many carcinogens must be activated by these metabolic enzymes before they can produce the DNA damage that results in carcinogenicity.

As would be expected, metabolism of complex mixtures can be intricate and complex, with different components of the mixture metabolized by the same enzyme at different rates or by different enzymes. When the same enzyme metabolizes two or more components of the mixture, one can inhibit the metabolism of the other by several mechanisms. This is a major reason for the toxicity of an essential oil may not reflect the toxicity of its most toxic constituent determined as a pure compound. For instance, if the same enzyme metabolizes the most toxic constituent and another constituent of the mixture, metabolism of the toxic constituent could be decreased by inhibition. This would result in higher than expected systemic concentrations and higher than expected toxicity.

12.3.9 Genetic Toxicology Studies

Damage to DNA and associated replicative processes is an important toxicological mechanism that may lead to initiation of carcinogenicity and other adverse effects (Brusick, 2001). A number of "short-term" assays have been developed to determine the potential of a compound or mixture to produce genetic damage. These assays are generally of short duration and utilize limited resources. Therefore, they can be undertaken during the early phases of a toxicological assessment to provide the toxicologist with important data. Compounds that are "positive" for potential genetic damage in these assays raise the concern level associated with the compound or mixture, whereas those that test "negative" encourage the continuation of the safety assessment. Although the ability of these assays to predict toxicities such as carcinogenicity is not extremely high, compounds with potential genotoxicity must be carefully evaluated.

Although there are a relatively large number of genotoxicity assays available, regulatory agencies have limited their data requirements to those assays that historically have been more reliable and are mechanistically well understood. Generally, an *in vitro* mutagenicity assay capable of detecting specific gene mutations, an *in vitro* assay capable of detecting chromosomal damage, and an *in vivo* assay capable of detecting genetic damage are required. The *in vivo* assays are important because they allow all the aspects of absorption, metabolism, distribution, and excretion that may affect the genotoxicity of a compound or mixture as well as any physiopathology produced by the compound to be expressed. An important aspect of most of the *in vitro* genotoxicity assays is that the cellular systems used generally do not have significant capacity to metabolize test compounds to reactive metabolites capable of interacting with DNA to produce damage and mutation. To overcome this deficiency, preparations that have the ability to metabolically activate many compounds are added to the test systems. The most common system added for metabolic activation is the 9000*g* supernatant obtained from centrifugation of rat liver homogenates. Specific cofactors are added to these "S9" fractions to optimize oxidation by the cytochrome P450 monooxygenase system, most commonly involved in the activation of genotoxicants. However, these systems are not optimized for the production of activated metabolites by other enzymes, and compounds that follow alternative activation pathways may not be detected.

An important aspect of these short-term tests is that the methodology may appear somewhat simple but this can be misleading. Improper design of these studies can lead to false positives, which may result in significant unnecessary testing. The physical and chemical nature of the test material must be carefully considered to ensure that these characteristics

TABLE 12.12
Results of *In Vitro* Genotoxicity Testing of Selected Mint Oils and Components

Essential Oil	Ames (TA98 & TA100) w/wo* Metabolic Activation	Chromosomal Aberration	Sister Chromatid Exchange	Mouse Lymphoma Forward Mutation w/wo Metabolic Activation
L-Carvone (RIFMA, 2004)	−	N/A	N/A	N/A
Cornmint oil (RIFMA, 2004)	−	N/A	N/A	N/A
Peppermint oil (RIFMA, 2004)	−	+	+	−
Spearmint oil (RIFMA, 2004)	−	−	N/A	N/A

*w/wo, with and without; −, negative in the assay; +, positive in the assay; N/A, data not available.

do not affect the outcome of the study. Also, doses that produce excessive cytotoxicity may lead to unreliable results. For these reasons, the toxicologist must be careful that the experimental design of the studies meets all the requirements of the assay and does not result in an invalid test.

Because a wide range of genotoxicity assays are available, care should be taken to ensure that the assays chosen for the safety assessment meets the requirements of the regulatory agencies who will be evaluating the data. Currently, the favored genotoxicity assays include the *in vitro Salmonella* reverse mutation assay, commonly referred to as the Ames assay, in honor of its developer, using at least five tester strains of bacteria, the *in vitro* chromosomal aberration assay, and the *in vivo* micronucleus assay. The *in vitro* assays are always conducted with and without metabolic activation by S9 fractions.

A summary of genotoxicity studies of specific mint oils and their components is presented in Table 12.12. Since the essential oils consist of naturally occurring constituents resulting from biochemical pathways in higher plants, there is no high concern that they may contain DNA-damaging genotoxic constituents. Rosenkranz et al. (1998) has conducted an analysis of the potential of 648 materials used in fragrances to be genotoxic carcinogens using a computer program capable of predicting genotoxic carcinogens based on SARs. Based on the analysis, the authors suggested that the genotoxic risk from these fragrance materials was no higher than that from normal mammalian physiological constituents. However, this does not alleviate the need for adequate genotoxicity testing during a safety assessment.

12.3.10 Short-Term Repeated Dosing Studies

In the current practice of toxicology, data from short-term repeated dosing studies, generally of 2- to 4-week duration are used in the design of more definitive longer-term subchronic toxicity studies (Wilson et al., 2001). These studies are designed to provide the initial data concerning potential adverse effects associated with repeated exposures and can aid in the determination of the target organs (those organs most adversely effected by the test material). Data collected from these studies are not as extensive as that collected from the longer-term subchronic studies but are extensive enough to allow a rational design for the subchronic studies. Variables generally determined during short-term repeated dosing studies include daily observations of the animals for any signs of altered behavior or stress, weekly body weights, food consumption, complete necropsy at study termination, selective organ weights, serum enzymes and electrolytes indicative of organ damage or other physiological changes, hematology, urinalysis, and selective histopathology that is less extensive than that required

for longer-term studies. In cases where evidence of specific toxicities from previous studies is available, additional endpoints can be added to those normally assessed.

An important aspect of the studies of flavors, such as the essential oils fed in the animal's diet, is an assessment of the palatability of the diets. At the high doses used in toxicology studies, strongly flavored materials may become unpalatable to the animals, resulting in decreased food intake with the resulting loss of body weight. This could become dose limiting and attempts should be made to increase the palatability of the diets. If palatability becomes a major problem, dosing using oral gavage where the test material is administered directly into the stomach of the animal must be used.

The animal species used in short-term repeated dosing studies should be the same as those to be used in the longer-term studies. Regulatory agencies generally require toxicology studies in two species. Typically, a rodent species and a nonrodent species are generally employed in toxicology studies. In cases where carcinogenicity studies in two species are planned, data from rats and mice are generally collected. The nonrodent species used in the shorter duration studies is generally not suitable for carcinogenicity studies due to the lack of historical data and the longer lifespans of the nonrodent species, such as dogs. Animals from both genders are always employed to determine any sex differences in toxicity.

Because data from short-term repeated dosing studies are used to determine the appropriate dosing for longer-term more definitive and resource-intensive subchronic studies, dose selection for these studies is critical. Generally, three to four doses are used in short-term repeated dosing studies. Dose selection is based on all the data available to the toxicologist. Data from acute toxicity studies in each species are required and metabolism data are useful. Ideally, the low dose should produce little or no toxicity, the mid-dose should produce some evidence of toxicity, and the high dose should produce definitive evidence of toxicity.

12.3.11 Subchronic Toxicity Studies

The design of subchronic toxicity studies is based on the data obtained from short-term repeated dosing studies and any other pertinent data available, such as metabolism and biodisposition. The subchronic study is a critical component of any safety assessment because of the magnitude of data obtained and the duration of exposure, which is generally 90 days (Wilson et al., 2001). The major objectives of subchronic studies include (1) detection of adverse effects not seen in studies of shorter duration as well as investigation of effects seen in the shorter-term studies, (2) identification of doses that produce no effect and those that produce toxicological effects, (3) confirmation of target organs seen in shorter-term studies as well as detection of target organs not identified in the shorter-term studies, (4) provision of additional data useful to ensure that the proper species are used and to aid in the extrapolation of animal data to humans, (5) provision of data for dose selection and any additional endpoints required for chronic toxicity and carcinogenicity studies, and (6) provision of data useful for human risk assessments.

Many regulatory agencies have issued guidelines for subchronic toxicology studies and the guideline from the pertinent agency should be followed. Studies are generally conducted using both genders and using two animal species, one the rat and the other a nonrodent species. A minimum of three doses of the test article is used with the low dose selected to be a no observed adverse effect level (NOAEL). Usually, 10–20 rodents or gender are used at each dose and in the control group. For nonrodent species, 4–6 animals per gender per group are used. During the in-life phase of the study, twice-daily observations are made for each animal to observe any overt signs of toxicity and once a week each animal is subjected to a more extensive physical examination and individual body weights are obtained. Clinical pathology studies are conducted using blood collected from the animals before the initiation of the study

and at the termination of the study. Often, interim clinical pathology is conducted at the midpoint of the study to assess any progression of detected toxicology. Clinical pathology consists of clinical chemistry comprising assays for variables such as blood glucose, urea nitrogen, total protein, albumin, globulin, calcium, sodium, potassium, bilirubin, triglycerides, cholesterol, triglycerides, among others, and serum enzymes such as alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase, among others. Hematology is also included, which consists of measurement of hematocrit, hemoglobin, erythrocyte count, leukocyte count, differential leukocyte count, reticulocyte count and prothrombin count, among others. Urinalysis is included in the clinical pathology screen with variables such as urine volume, specific gravity, pH, glucose, protein measured, among others. At study termination, each animal is subjected to detailed necropsy and the weight of selected organs is determined. Samples of approximately 47 different tissues from each animal in all treatment groups and controls are collected at necropsy for microscopic examination to detect any microscopic pathology. Additional toxicological endpoints may be added to investigate any toxicological changes noted in previous studies or to address specific questions. These data are statistically analyzed to determine any significant changes compared with the controls.

As is obvious, the subchronic study provides a more extensive data collection than the shorter-term studies. Therefore, it is more valuable in the detection of potential toxicology and in the extrapolation of the animal data to humans. It is also critical in the design of studies of longer duration, including chronic toxicity and carcinogenicity studies. In some cases, the subchronic toxicity studies may complete a toxicological assessment and no further studies may be required; however, in most cases further studies including chronic toxicity and carcinogenicity studies will be required.

12.3.12 Chronic Toxicity Studies

These studies are of longer duration than subchronic studies and generally are of 12-month duration in rodents, which covers half their lifespan. In longer-lived species, such as dogs, study durations of 12 months are also acceptable.

Where chronic toxicology studies are required, the basic study design is similar to that of the subchronic study. Data from the subchronic studies are used to set the doses as well as add any additional toxicological endpoints warranted by the results of the subchronic studies. It is possible to combine the chronic toxicology studies with carcinogenicity studies by using a satellite group of animals that are terminated at the 12-month midpoint of the carcinogenicity study.

12.3.13 Carcinogenicity Studies

Carcinogenicity studies require careful and comprehensive study designs that are to detect the carcinogenic potential of the test chemical (Williams and Iatropoulos, 2001). It is important that the study design meets the criteria established by the pertinent regulatory agency. Because of the cost and duration of these studies, it is important to discuss the study designs with the regulatory agency to ensure that they meet the specific requirements. Studies in both genders of two species are generally required and in most cases rat and mouse studies are acceptable. Study duration is for the lifespan of the animal, which is typically 24 months for rats and mice (studies of 18-month duration may be acceptable for mice).

Dose selection can be difficult for carcinogenicity studies. Three doses are generally required, with the high dose producing some signs of toxicity but not severe enough to shorten the lifespan of the test species. While doses are often established from subchronic or

chronic toxicity studies, other data should also be evaluated, including pharmacokinetics and toxicokinetics. If the high dose reduces the survival of the animals, very few animals may survive to allow appropriate statistical analysis of the data, which would result in the need to repeat the study at a lower high dose. If no toxicity is evident at the high dose, the study may be considered inadequate, because a higher dose may have provided evidence of carcinogenicity. Acceptable toxicological criteria for the high dose may be as simple as a reduction in body weight of at least 10% compared with the controls to changes in clinical pathology.

At the termination of the study, the most critical toxicological endpoint is microscopic examination of the tissues to detect any carcinogenic changes. Because of the duration of the study, it is to be expected that "spontaneous" tumors will develop in the animals as they age and it is critical to be able to distinguish between test-chemical-induced tumors and spontaneous tumors. Therefore, the control groups become extremely important. Potential carcinogenicity is determined by statistical increases in specific tumors that arise in the treatment groups compared with the control groups. Sometimes it is difficult to determine if a small increase in a tumor type in the treated groups is compound related when compared with the control group. It is possible in such situations to compare the incidence of a particular tumor in the treated groups to the historical occurrence of the tumor in controls from other studies. However, comparison with the concurrent control group provides more assurance that the study correctly predicts carcinogenic potential.

12.3.14 Sensitization Studies

Mint oils and their constituents are used in a number of products that result in dermal contact. This raises the potential for allergic contact dermatitis and other dermal damage including phototoxicity and photoallergy. Although direct damage to the skin resulting in irritation, necrosis, and corrosion is possible from products containing essential oils, this type of damage is unlikely because of the low concentrations generally used in these products. However, skin allergies produced by delayed type hypersensitivity are a possibility. These allergies could be produced directly by the oil or its constituents or indirectly by interactions with components of the preparations to which the oils may be added. Based on these possibilities, studies of the potential for allergic contact dermatitis need to be considered on a case-by-case basis.

Allergic contact dermatitis is generally of the cell-mediated type termed delayed contact hypersensitivity. T-lymphocytes are responsible for producing this type of hypersensitivity. For a response to be elicited, the chemical must penetrate into the skin and bind to proteins. The chemical–protein complexes stimulate an allergic response through stimulation of T-lymphocytes. Macrophages or other cells associate with the T-lymphocytes resulting in the release of interleukins that increase the expression of immune proteins and also act to stimulate cell division. Stimulated lymphocytes enter the lymphatic system and move to the draining lymph nodes. Here, the lymphocytes differentiate into immunoblasts, which result in the production of T-effector cells that enter the systemic circulation. When T-effector cells encounter the antigen, lymphokines that initiate a local inflammatory response are released. These processes are complex and a number of factors can influence the potential of the chemical to produce hypersensitivity. For example, the chemical must penetrate the skin to produce a response and the factors that affect percutaneous absorption, such as the solubility and octanol–water partition coefficient, concentration, pH, volatility, anatomical site, etc., must be considered. A detailed discussion of the mechanisms associated with hypersensitivity and factors that influence the elicitation of the dermal response is beyond the scope of this discussion and the interested reader is directed to the studies devoted to these topics such as Maibach and Patrick (2001) and Burns-Naas et al. (2001).

There are a number of assays suitable for the detection of allergic contact dermatitis. The most commonly used assays that have received widespread acceptance are the guinea pig maximization assay, the guinea pig occluded patch assay, and the murine local lymph node assay (OECD, 1992). The guinea pig maximization test utilizes six intradermal injections comprising two injections of the test material, two injections of the test material along with Freud's complete adjuvant, and two injections of the adjuvant alone during the induction period. One week later, this is followed by a single topical application. Controls are treated identically except no test material is used in the injections. Induction is achieved by a 48-h exposure to the test material using an occlusive patch. The test and control animals are treated with the test material for 24 using occlusive patches and reactions are graded for erythema and edema 24 and 48 h after the removal of the patches. The occluded patch assay uses an occluded patch for induction and elicitation of the response. Once a week for 3 weeks, the test material is applied to the skin and occlusive patches used. After 2 weeks, the test-material-treated animals and the controls are challenged with the test material for 6 h and evaluated for erythema and edema 24 and 48 h after patch removal. The lymph node assay uses mice treated with different concentrations of the test material or its solvent only topically on the back of both ears daily for 3 days. The mice are administered [^{3}H]-thymidine 2 days later. They are killed 5 h after thymidine administration, and the ears and the draining lymph nodes are removed. The nodes are processed for radiolabel quantification to determine the mean radiolabel incorporated into each lymph node. The test material is considered to be a sensitizer if there is a threefold or higher incorporation of radiolabel compared with the controls. A number of recommendations have been made to improve these assays and decrease the number of animals required (Steiling et al., 2001).

Generally, if a material is shown to be a sensitizer in animals, it is not tested in humans; however, sometimes in special cases human testing may be warranted. There are a number of tests that have been used to test human responses as discussed by Maibach and Patrick (2001). Many of the same factors that can affect the results from animal studies must also be considered in human studies (Boukhman and Maibach, 2001). One aspect of dermal sensitization that cannot be determined in animal studies is the existence of response thresholds in humans (Boukhman and Maibach, 2001). Human testing requires approval by an Institutional Review Board and a carefully developed informed consent form to ensure that the volunteers are familiar with any risks associated with the studies.

Frosch et al. (2002) reported a study that used six different dermatological centers in Europe and 1606 patients attending patch test clinics to evaluate sensitizers associated with use in fragrances. The following essential oils were included in the tested materials: ylang-ylang I, ylang-ylang II, lemongrass, sandalwood, patchouli, spearmint, dwarf pine needle, cedarwood, and peppermint. The two mint oils were in the lower response percentages. For instance, the highest percentage of observed adverse reactions occurred with ylang-ylang oil I (2.6%), ylang-ylang oil II (2.5%), and lemongrass oil (1.6%), while spearmint oil yielded a 0.8% response and peppermint oil yielded a 0.6% response. Naldi (2002) has provided a discussion of the issues associated with epidemiological studies of fragrance allergy in the general population.

12.3.15 Other Toxicology Studies

The foregoing discussion covers most of the standard toxicology studies associated with the safety assessment of a compound or mixture. However, other studies are also required, including the potential for production of malformation in the developing embryo as assessed by teratology studies and its effects on the reproductive potential of males and females.

Although a discussion of these studies is beyond the scope of this chapter, it should not diminish the importance of these studies. Particular characteristics of the test material or specifics associated with the particular use of the material may result in the need for studies that are not normally a part of a standard safety assessment. In addition, results from the toxicology studies discussed here may result in the need for further studies. Studies concerning the mechanisms associated with a specific toxicological finding may be useful in developing a perspective on the meaning of a particular finding for human safety. A well-known example where mechanistic studies provided a perspective concerning a finding of carcinogenicity in rats is D-limonene. Carcinogenicity studies using rats indicated that D-limonene produced kidney tumors in male rats but not in females and no kidney tumors were produced in mice. Mechanistic investigations revealed that the male rat kidney tumors were associated with a perturbation of a male-rat-specific protein, $\alpha 2\mu$-globulin, which has no relevance to humans.

12.3.16 Guidelines for the Safety Evaluation of Substances for GRAS Status

The preceding discussion of the fundamental elements of a safety assessment outlines steps necessary to determine the safety of a mint oil, or other essential oils or extracts, or a constituent of the oil or extract. The results of such a safety assessment are applicable to the single oil or constituent studied, and unless care is taken, it may only be applicable to the particular material under investigation. Changes in composition based on flavor or processing needs may comprise the extrapolation of the data to a formulation. This type of toxicological testing requires a large commitment of resources and time with the cost potentially ranging from three to four million US$ with a timespan of 4 or more years. An example of an evaluation that meets many of the discussed criteria is the current toxicological evaluation of pulegone by the U.S. NTP. New uses of a particular oil or constituent or a significant increase in the quantity of a particular oil or constituent in a product may result in the need for a comprehensive safety assessment. For example, the use of a mint oil as a pesticide or fumigant would require toxicological studies to ensure its safe use and regulatory approval. However, traditional uses with a long history of use without known adverse effects may allow alternative approaches to the evaluation of safety. An example previously mentioned is the GRAS approach used in the United States for those substances used in food that have been classified as GRAS under the conditions of intended use, as assessed by experts qualified by training and experience to evaluate its safety based on scientific procedures. This approach has been successfully used for several decades in the United States.

Recently, the FEMA Expert Panel has published their procedures for the safety evaluation of essential oils used as ingredients in food (Smith et al., 2005a,b). Although this procedure is limited to essential oils used in foods, it serves as an excellent guide to a GRAS type safety evaluation using current scientific thinking by a panel of highly experienced experts. As such, their approach will be summarized here and an example of their procedure will be provided using a summary of their review of cornmint oil (Section 12.5.1).

The Panel notes that their procedure is limited to a safety evaluation of essential oils derived from higher plants that are to be used as flavorings for food. Other flavoring substances derived from other sources or other intended uses are explicitly excluded from their procedure and must be evaluated using separate criteria suitable for the substances and their intended uses. They also note that the evaluation procedures are not static and will undergo revisions and further refinement based on experience and scientific advances. They emphasize that their procedure is a guidance and not a rigid checklist.

The Panel's safety evaluation guidance begins by listing the data required to initiate the evaluation. These are

1. All recognized commercially practical botanical sources of the oil
2. All relevant geographical sources
3. All commercially used plant parts
4. All commercially used degrees of maturity
5. All commercially used methods of isolation
6. The variability inherent in each method of isolation

With these data, it is then possible to define the essential oil and begin to understand the chemical composition limits that define the commercial oil. The next step is specifying the concentration ranges for each known component of the oil from thorough analytical compositional studies. The physical and chemical specifications for the oil include such factors as specific gravity, refractive index, optical rotation, solubility, among others, as well as the chemical composition, including upper limit of concentration for specific constituents and the structural groupings of which they are a member. It is important to determine the percent of unidentified constituents and list any trace constituents that may be of toxicological concern. This will set the product specifications, if not already set for use in food, and define the identity, purity, and identify any constituent(s) that may be of toxicological concern.

After defining the oil and its specifications, the next step is the exposure assessment in food. As part of the exposure assessment, the history of use of the oil is documented and it is determined if the botanical source of the oil is consumed as a food. Next, the intake of the oil from use as an added flavor in food is estimated. The Panel calculates a conservative consumer intake using the per capita intake (PCI) method. It is assumed that only 10% of the population consumes the total annual production volume of the oil obtained from the flavor manufacturers. To account for potential incompleteness of these data, it is assumed that the annual reported product volume is only 80% of the actual production (use of a 0.8 correction factor). The PCI in mg/day is determined by the following equation provided by the Panel using the U.S. population in 1995:

$$\text{PCI (mg/day)} = \text{kg of oil produced} \times 10^9/260{,}000{,}000 \times 0.8 \times 365,$$

where

the population in 1995 is 260,000,000 million.

The correction factor based on the incompleteness of the poundage survey, assuming that poundage data represent 80% of total annual volume is 0.8, 365 = days in a year

The calculated PCI is multiplied by ten to obtain a conservative estimate of the intake by eaters of the ingredient. If the botanical source of the oil is itself consumed as food, then the PCI from the source plant must be added to that calculated for foods to which the oil is added as a flavor or for some other use (Smith et al., 2005a).

To this point, the sources of the oil are known and the chemical constituents have been speciated with the exception of unknown trace materials, the specifications for the oil have been determined and the exposure from food products assessed. The next step involves broadly classifying each chemical constituent into categories according to toxicological potential. There are three broad categories that relate chemical structure to toxicological potential based on the classification of Cramer et al. (1978). Class I chemicals contain structures that suggest they will be of low potential for oral toxicity. Class II chemicals contain structures that are questionable with respect to toxicological potential, while Class III

chemicals contain structures that are known or suspected to have potential to produce adverse effects. Chemically unidentified constituents are placed in Class III.

The next step is to classify each identified constituent into one of a series of chemically related groups termed "congeneric groups," which are listed in Table 12.13. This important function is based on a number of factors associated with naturally occurring flavors. Essential oils are the predominant type of naturally occurring flavors used in foods and consist, with a few exceptions, of mixtures of volatile, relatively low molecular weight compounds. These compounds provide the unique flavor and aroma of the plants from which they are obtained. The constituents of essential oils do not have an extremely wide variety of chemical structures as do synthetic organic chemicals. This results from their occurrence in higher plants through their formation by enzyme-based biosynthetic pathways. Because these pathways are limited

TABLE 12.13
Chemical Groups of Flavor Materials (from Smith et al., 2005a)

1	Saturated aliphatic, acyclic, linear primary alcohols, aldehydes, carboxylic acids and related esters
2	Saturated aliphatic, acyclic, branched-chain primary alcohols, aldehydes, carboxylic acids and related esters
3	Aliphatic linear and branched-chain alpha, beta-unsaturated aldehydes and related alcohols, acids and esters
4	Aliphatic allyl esters
5	Unsaturated linear and branched-chain aliphatic, nonconjugated aldehydes, related primary alcohols, carboxylic acids and esters
6	Aliphatic primary alcohols, aldehydes, carboxylic acids, acetals and esters containing additional oxygenated functional groups
7	Saturated alicyclic primary alcohols, aldehydes, acids and related esters
8	Saturated and unsaturated aliphatic acyclic secondary alcohols, ketones and related esters
9	Aliphatic acyclic and alicyclic alpha-diketones and related alpha-hydroxyketones
10	Alicyclic ketones, secondary alcohols and related esters
11	Pulegone and structurally and metabolically related substances
12	Aliphatic and aromatic tertiary alcohols and related esters
13	Aliphatic, alicyclic, alicyclic-fused and aromatic fused ring lactones
14	Benzyl derivatives
15	Hydroxyl- and alkoxy-substituted benzyl derivatives
16	Cinnamyl alcohol, cinnamaldehyde, cinnamic acid and related esters
17	Phenyl-substituted primary alcohols, aldehydes, carboxylic acids and related esters
18	Phenyl-substituted secondary alcohols, ketones and related esters
19	Phenol derivatives
20	Hydroxyallylbenzene and hydroxypropenylbenzenes derivatives
21	Phenethyl alcohol, phenylacetaldehyde and related acetals and esters
22	Aliphatic and aromatic ethers
23	Furfuryl alcohol, furfural and related substances
24	Furan derivatives
25	Aliphatic and aromatic sulfides and thiols
26	Sulfur-substituted furan derivatives
27	Sulfur-containing heterocyclic and heteroaromatic substances
28	Aliphatic and aromatic amines and related amides
29	Nitrogen-containing heterocyclic and heteroaromatic substances
30	Pyrazine derivatives
31	Anthranilate derivatives
32	Amino acids
33	Maltol derivatives
34	Epoxide derivatives

in their variety, the chemical structures resulting from them are also limited in their variety. This allows them to be placed into a limited number of congeneric groups. Essential oils generally contain no more than five to ten congeneric groups, while some of these groups may contain a large number of specific chemical constituents but only a few of these constituents predominate. As stated by the Panel "The presence of a limited number of congeneric groups in an essential oil is key to the organization of constituents and subsequent safety evaluation of the oil itself." Because each congeneric group shares chemical structure attributes, it can be assumed that members of each group will share similar absorption, distribution, metabolism, and excretion patterns and also share similar toxicological characteristics. This allows each congeneric group to be classified into the three previously mentioned classes i.e., Class I, Class II, and Class III. When a constituent of the oil under evaluation does not share the same class as its congeneric group, the higher classification is used. For instance, if the constituent falls into Class II but the congeneric group falls into Class I, the constituent is considered to be in Class II (Smith et al., 2005a).

The next step is to calculate a concentration range for each congeneric group. The upper limit (%) of the range is the highest of the sum of each member of the congeneric group that occurs in the oil under evaluation. From this percentage and the reported annual consumption volume, the daily PCI of each congeneric group can be determined using the calculations previously described for the total oil. The same can be done for the unidentified fraction of the oil, which is always placed in Class III, not assigned to a congeneric group.

The next step is to evaluate each congeneric group in the essential oil to determine if its metabolism and disposition, at the current consumption level, are by established detoxication pathways resulting in metabolites considered to have less toxicological potential than the parent compound. If the evaluation leads to the conclusion that members of the congeneric group in the oil would be subject to detoxication by known pathways, the evaluation moves to the next step. If not, it must be determined if sufficient toxicology data exist for a comprehensive safety evaluation. If such data exist, they may help in the determination of a sufficient margin of safety for intake of the congeneric group derived from the oil. If not, additional toxicological data will need to be developed.

If the metabolism and disposition evaluation indicates that known pathways are involved, the next step is to determine if the total intake of the congeneric group is less than the human threshold for its class. Each of the three structural classes have been evaluated quantitatively for toxicological potential based on a database that contains a wide diversity of chemical structures that includes chemicals such as food additives, naturally occurring substances, pesticides, drugs, and industrial chemicals among others (Munro et al., 1996). Based on this evaluation, conservative no observable effect levels (NOELs) have been determined for each structural class. These are converted to human exposure threshold levels by applying a 100-fold safety factor ($100 \times$ NOEL) and then correcting for mean body weight (assuming a mean human body weight of 60 kg). The Panel notes that these conservative exposure thresholds have been adopted by the World Health Organizations Joint Expert Committee on Food Additives and the Commission of the European Communities for evaluation of flavoring agents that have been chemically identified. These human exposure thresholds for each class are as follows: Class I = 1800 μg/person/day; Class II = 540 μg/person/day; and Class III = 90 μg/person/day. Exposures below these thresholds are considered to be of low concern for adverse toxicological effects.

As noted earlier, constituents of the oil that have not been chemically identified are a special case and are placed in structural Class III, which is the class with the highest toxicological concern level. These constituents generally represent less than 5% of the oil and many of them may be members of the congeneric groups already evaluated. If the oil is part of a plant consumed as a food, then the unknown constituents are also consumed with

the food. This decreases the concern level for these materials. However, if the oil is used as a food flavor at higher concentrations than those found in the plant as consumed, then the concern level increases. The Class III designation for these unknown constituents means that consumption at levels below 90 μg/person/day would be of low concern. However, exposures above this level would be of concern and more evaluation would be necessary. Further evaluation could range from more intensive investigation to identify the chemical structures of the unknown constituents to toxicological studies of the oil to determine any adverse effects and determinations of a NOEL. The nature and extensiveness of these evaluations would be determined on a case-by-case basis.

An essential oil that has passed all the previous criteria is next evaluated for potential chemical and biological interactions between its constituents and the congeneric groups. Evaluation consists of estimation of the potential for the constituents to act in an additive or synergistic manner to increase the potential for adverse effects. In most cases with essential oils, the potential for such interactions is low as is the potential for adverse effects because of the low consumption levels of these materials. If the potential for interactions is present, then additional toxicological studies may be required. This step in the evaluation also allows consideration of any scientific concerns that have not been previously addressed.

The final step involves a decision as to whether or not the essential oil can be used as a flavoring agent for food under current conditions of intended use. This decision is based on the ability of the essential oil to pass all aspects of the evaluation of its potential toxicity. If there is agreement among the experts involved in the evaluation that the oil can be safely used in food under its conditions of intended use, then it can be considered GRAS. However, if there are any unresolved issues concerning the safety of the oil, recommendation can be withheld until additional data are available to resolve the concerns (Smith et al., 2005a).

The FEMA Expert Panel has recently provided a summary of the use of this procedure in the evaluation of cornmint oil for GRAS status (Smith et al., 2005b). This evaluation is summarized in Section 12.5.1.

12.4 REVIEWS OF THE TOXICOLOGY OF SELECTED CONSTITUENTS OF MINT OILS

12.4.1 Menthol

L-Menthol ($C_{10}H_{20}O$, mol. wt. 156.27) (CAS No. 2216-51-5; CAS No. 89-78-1) has three asymmetric carbon atoms and occurs as four pairs of optical isomers as shown in Figure 12.1. L-Menthol is the most widely occurring isomer in nature and is generally referred to as menthol. It is also referred to as hexahydrothymol, peppermint camphor, 3-*p*-menthanol, and 5-methyl-2-(1-methylethyl) cyclohexanol. It is a white crystal at room temperature with a melting point of 41°C–43°C and a boiling point of 212°C. It is slightly soluble in water and soluble in alcohol, chloroform, diethyl ether, petroleum ether, and petrolatum (Merck Index, 1989).

Plants containing menthol have been used since the beginning of recorded history, probably as kitchen and medicinal herbs. Plant remains have been found in Egyptian tombs and mint is mentioned as a tithe payment in the Bible (Thomas, 1962), although this reference is more likely to be referring to *Mentha longifolia* (Moldenke and Moldenke, 1952).

Because of its mint-like odor and fresh, cooling taste, menthol is used in a wide variety of commercial products in varying concentrations (Table 12.5). The possible average daily intake (PADI) is 13.419 mg (Burdock, 2002).

Menthol is found in a few natural sources including cornmint (Clark, 1998), peppermint, and other mints, and as a minor or trace component of some commonly consumed food

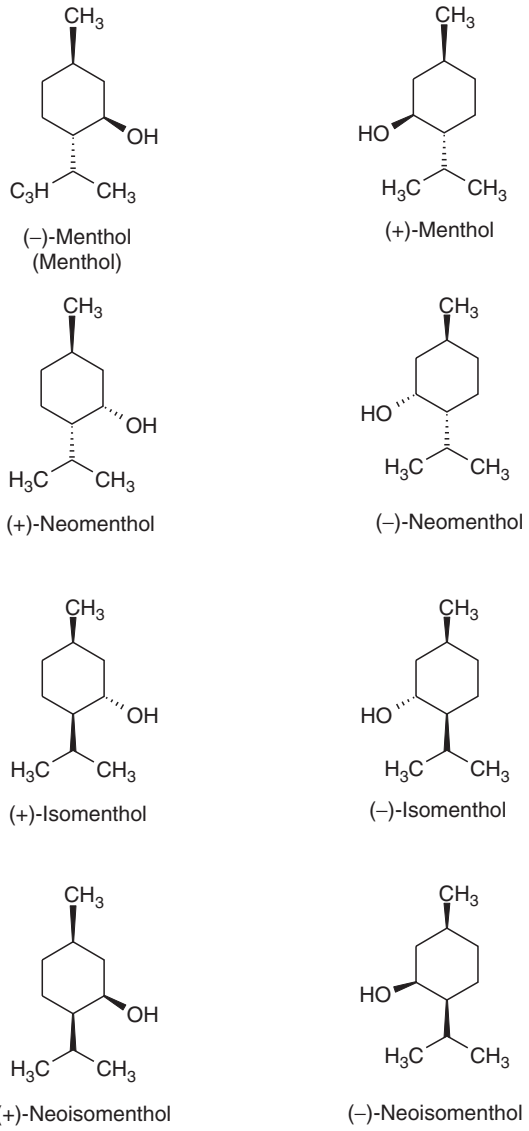


FIGURE 12.1 The four pairs of optical isomers of menthol. (−)-Menthol is commonly referred to as menthol.

products (Burdock, 2002). Essential oils containing menthol are listed in Table 12.6. Menthol is also used in pharmaceutical products (Haarmann and Reimer Corporation, 1985; Hopp, 1993), including drugs (0.1%), compresses (0.1%–0.2%), medicated oils (2.0%–4.0%), analgesic balms (2.0%–3.0%), rubbing alcohol (1.0%–1.2%); in oral products, including toothpaste (0.5%), mouthwashes (1.0%–2.0%), and oral sprays (0.3%); in confectionary goods, including chewing gum (0.5%), hard candies (0.05%–0.1%), cough drops (0.1%), lozenges (0.1%), and licorice (0.05%–0.1%); in perfumed products, including lotions (0.05%), preshave lotions (0.2%–0.3%), handkerchiefs (0.1%–0.2%), foot sprays (0.5%), shampoo (0.2%–0.5%), refreshing towels (1.0%), and cooling gels (1.0%); in tobacco goods, including cigarettes (0.03%), pipe tobacco (0.3%), and chewing tobacco (0.05%–0.1%). However, the tobacco

industry reports maximum use levels between 1.93% and 2.24% (Philip Morris USA, 2005; Reynolds, 2005).

L-Menthol is the largest and most important component of cornmint (*M. canadensis*) and peppermint (*M. piperita*) oils at 46%–80% and 50%–60%, respectively (Leung, 1980; British Industrial Biological Research Association [BIBRA], 1990; Clark, 1998). Menthol is an optically active substance, and its chirality or stereoisomeric forms (L-, D-, or DL-) affect its flavor.

RIFM and the Joint FAO/WHO Expert Committee on Food Additives (JECFA/WHO) have reviewed the available data on the toxicity of menthol and menthol isomers and concluded that they were not genotoxic, teratogenic, or carcinogenic (JECFA/WHO, 1999; RIFM, 2004). In addition, FEMA has assessed the use of menthol as a flavor ingredient and reported that menthol isomers exhibit very low acute, subchronic, and chronic toxicity (Adams et al., 1996). The National Cancer Institute (NCI) has also assessed the available data for menthol and concluded that there was no evidence of carcinogenicity associated with L-menthol and its isomers (NCI, 1979).

12.4.1.1 Regulatory Status

FEMA has assessed the use of menthol as a flavor ingredient (Adams et al., 1996) and has given it "GRAS" status (FEMA No. 2665). Also, the FDA affirmed menthol as GRAS in accordance with 21CFR §182.20 (FDA, 2003a) and approved it for use as a synthetic flavoring substance and adjuvant in foods, with no limitation on use except good manufacturing practices, and no food category restrictions other than those specified in food standards of identity in accordance with 21CFR §172.515 (FDA, 2003b).

The Council of Europe (COE, 2000) has approved menthol as Category A (COE No. 63). The JECFA/WHO has established an acceptable daily dietary intake (ADI) of 0 mg/kg–4 mg/kg body weight/day (JECFA/WHO, 1999).

The use of menthol as a tobacco additive in the manufacture of cigarettes is approved worldwide on the basis of natural or naturally identical status by regulatory authorities in several countries with regulations addressing the design of cigarettes. Table 12.14 lists the countries with applicable regulations that include a list of substances or requirements for substances to be considered for use as a tobacco additive.

12.4.1.2 Human Toxicology

Menthol has a low potential for toxicity in humans. Several databases contain toxicological summaries for menthol, including RIFM (2003). Other reviews and data sets include summaries of menthol health effects and toxicity endpoints (BIBRA, 1990; NCI, 1993; JECFA/WHO, 1999). Data from the National Occupational Survey conducted from 1981 to 1983 (National Institute for Occupational Safety and Health [NIOSH], 1983) reported that approximately 43,997 workers were exposed to menthol (reported as CAS No. 89-78-1) by inhalation and dermal contact in the work place.

Menthol can be absorbed through the skin in a dose-dependent manner (Martin et al., 2004). An irritation study was conducted with 16 individuals (>18 years) exposed to 0.1%, 0.2%, and 0.5% menthol in light liquid petrolatum or in physiological salt solution applied to the nasal passages. Irritation to nasal and mucous membranes was noted at 0.5% (RIFM, 2004). Another study was conducted with 9 individuals (6 females and 3 males) exposed to 25 mL of the vehicle containing menthol at an unspecified concentration. The vehicle solution consisted of 80% ethanol and 20% deionized water. The menthol solution was applied to one forearm and the vehicle to the other in random order. Irritating effects were observed on the

TABLE 12.14
Applicable Regulations for the Use of Menthol in Cigarette Manufacture

Country	Regulatory Reference
Belgium	Royal decree of 13 August 1990 concerning the manufacture and marketing of tobacco-based products and similar products Monday 5 January, 1991 modified by Royal decree of 14 April, 1993, Monday 23 June 1993. The Council of Europe 2000. Chemically-defined flavoring substances. Council of Europe Publishing, Strasbourg, France.
Czech Republic	Ministry of Agriculture. Ordinance Stipulating the Requirements for Tobacco Products. Collection of Regulations of the Czech Republic. No. 344/2003 Coll. Appendix No. 2.
France	Order of the 12th September 1995 relating to additives authorized for use in the manufacture of tobacco products and of their substitutes. Official Journal of the French Republic (1st October 1995). Appendix I.1. Ministry of Economy and Finance/Ministry of Public Health and Social Security, 1995. The Council of Europe 2000. Chemically-defined flavoring substances. Council of Europe Publishing, Strasbourg, France.
Federal Republic of Germany	Tobacco Ordinance of December 20, 1977 No. 360, revised on March 1998. The Federal Republic of Germany, Ministry of Health, 1998; Aroma ordinance of December 22, 1981, modified on June 18, 2001. The Federal Republic of Germany, Ministry of Health, 2001.
Hungary	Minister of Agriculture and Rural Development. *Joint Decree No*. 102/2005 FVM on the Manufacture, Distribution and Monitoring of Tobacco Products. Hungarian Gazette.
United Kingdom	Permitted additives to tobacco products in the U.K., Department of Health, London (2003). <http://www.doh.gov/uk/scoth/technicaladvisorygroup/additives.htm>. [Accessed April 15, 2004].

individuals' skins and the mean time of onset of sensation, including burning and cooling, was 2.59 min (RIFM, 2003). A study was conducted with 17 individuals (>18 years) exposed to menthol on the face and forearms. The vehicle was light mineral oil. The mean sensory irritation threshold on the face was 156.5 mM and on the forearm 1095 mM (RIFM, 2004).

Some evidence of sensitization to menthol has been reported. A 48-h patch test conducted on 20 individuals showed no effects at 1%. Another patch test conducted on 69 contact dermatitis patients showed no effects at 1.5%. A patch study conducted with eczema and dermatitis patients using cosmetic ingredients and fragrance materials, including menthol, reported no positive effects (RIFM, 2004). A case of recurrent oral lichenoid reaction was reported by Fleming and Forsyth (1998), apparently associated with menthol and peppermint, possibly in oral mouthwashes, which resolved after avoidance of all sources of mint for 3 months. They reported a delayed patch test reaction after application of the allergen.

Hayakawa et al. (1996) reported two clinical cases of contact dermatitis from L-menthol from toothpaste and a topical medication. Based on the published literature and previous cases, the authors suggested that dermatitis from menthol should be considered when diagnosing lip dermatitis, perioral dermatitis, or oral enanthema patients.

Olowe and Ransome-Kuti (1980) reported that the use of a mentholated powder to dress umbilical cords of newborns in Nigeria may have been associated with jaundice in babies with a glucose-6-phosphate dehydrogenase deficiency. They suggested that a diminished capacity of the deficient babies to form the glucuronide of menthol resulted in the observed jaundice.

12.4.1.3 Metabolism

Figure 12.2 provides a general outline of menthol metabolism based, in part, on the rat studies of Madyastha and Srivatsan (1988). Rats were orally administered 800 mg/kg body weight for 20 days. The major urinary metabolites were *p*-menthane-3,8-diol and 3,8-dihydroxy-*p*-menthane-7-carboxylic acid. Minor urinary metabolites were 3,8-oxy-*p*-menthane-7-carboxylic acid and *p*-menthane-3,9-diol (Figure 12.2). *In vitro*, rat hepatic

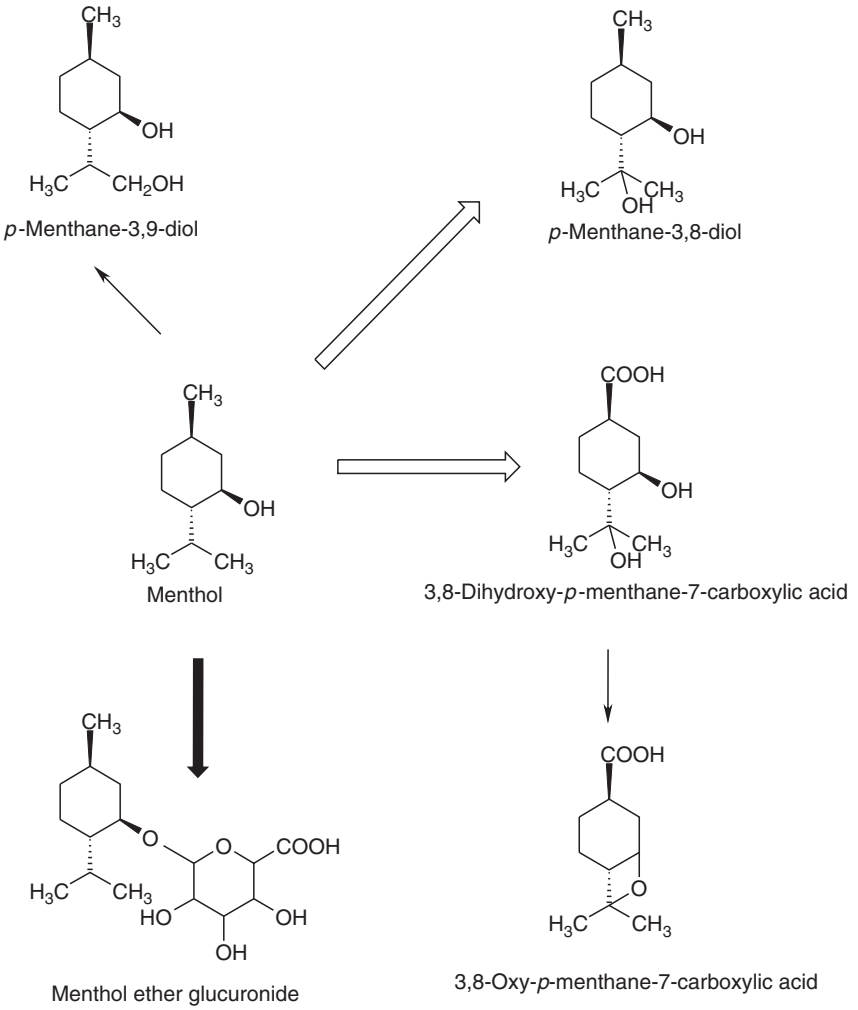


FIGURE 12.2 General outline for the metabolism of menthol by animals. The major metabolites are denoted by the large arrows and minor metabolites by the small arrows. The open arrows denote oxidation reactions catalyzed by the cytochromes P450 monooxygenase system while the filled arrow represents glucuronide formation catalyzed by the UDP-glucuronosyltransferases. Additional glucuronides may be formed from the oxidized metabolites.

microsomes were capable of producing *p*-menthane-3,8-diol in a classic cytochrome P450 monooxygenase catalyzed reaction. Indication that menthol could induce its own metabolism was evident by increased hepatic microsomal cytochrome P450 quantities and increased NADPH-cytochrome *c* reductase activity. Menthol metabolism could be induced by the classic P450 inducer phenobarbital but not by another inducer, 3-methylcholanthrene. Menthol could alter the metabolism of other compounds by its ability to induce cytochrome P450 monooxygenase and also by acting as a competitive inhibitor of monooxygenase metabolism. For example, menthol has been shown to inhibit nicotine and coumarin metabolism (MacDougall et al., 2003; Benowitz et al., 2004). Menthol and menthyl acetate (constituents of peppermint oil), and peppermint oil have been shown to inhibit *in vitro* cytochrome P450 (CYP3A4) metabolism of nifedipine, and peppermint oil may inhibit the *in vivo* metabolism of felodipine by humans (Dresser et al., 2002). Menthol appears to follow expected oxidation reactions catalyzed by cytochrome P450 monooxygenase. These oxidative products are generally more water-soluble than the parent compound and are more easily excreted, reducing their systemic concentrations and their potential toxicity.

Menthol contains a hydroxyl group, as do several of its oxidative metabolites, which is susceptible to glucuronide conjugation catalyzed by the endoplasmic reticulum membrane-bound UDP-glucuronosyltranferases. Glucuronides are generally more water-soluble than the parent compound and are excreted directly in urine and in feces, via the bile. Yamaguchi et al. (1994) orally administered 500 mg/kg body weight radiolabeled menthol to intact and bile duct-cannulated male Fisher rats. In intact rats, 71% of the dose was recovered in 48 h with equal amounts in the urine and feces. In bile duct-cannulated rats, 74% of the dose was recovered with 67% in the bile and 7% in the circulation. The urinary metabolites consisted of a series of mono- and dihydroxy menthols and carboxylic acids, some of which were excreted as glucuronic acid conjugates. Some menthol glucuronide was found in the urine. Menthol glucuronide is apparently subject to enterohepatic circulation in the rat. This results from excretion of the glucuronide into the intestine via the bile. In the intestine, intestinal microfloral β-glucuronidase cleaves the glucuronide freeing the conjugated menthol and it is reabsorbed into the systemic circulation through the intestine. In the liver, again it is conjugated with glucuronic acid and excreted into the intestine. This process can prolong the systemic half-life of the compound.

Humans excrete menthol glucuronide in the urine as demonstrated by Kaffenberger and Doyle (1990). They administered peppermint oil to volunteers and found that 40% of the menthol contained in the oil could be recovered in urine as the menthol glucuronide over the 14 h after administration. Gelal et al. (1999) studied the disposition kinetics of menthol and menthol glucuronide in plasma and urine after oral administration of 100 mg menthol in capsules and compared them to the consumption of 10 mg menthol from candy lozenges and the consumption of 9–10 mg from mint tea. No unconjugated menthol was detected in either plasma or urine. Over the 24-h period after administration, 45.6% of the menthol from the capsule administration was recovered as menthol glucuronide in the urine as was 56.6% of the menthol from the candy and tea.

UDP-glucuronosyltransferases belong to two gene families (UGT1 and UGT2) each of which is subdivided into subgroups that contain a number of isoforms. UGT1A transferases glucuronidate amines, bilirubin, drugs, and steroid hormones while UGT2A enzymes glucuronidate compounds in the olfactive epithelium. UGT2B enzymes are expressed in a number of tissues including the liver, kidney, skin, intestinal tract, brain, uterus, and prostate. UGT2B enzymes glucuronidate foreign compounds and also endogenous steroids. The metabolism of menthol by a number of these isoforms has been investigated. UGT2B9 from monkeys is capable of glucuronidation of both enatiomers of menthol but the rate is faster for (−)-menthol (Green et al., 1997). In contrast, monoterpenoid alcohols, such as menthol,

TABLE 12.15
Studies of the Acute Toxicity of L-Menthol[a]

Species	Route of Administration	LD_{50} (mg/kg Body Weight)	References
	Oral	4380	Litton Bionetics Inc. (1975)
Mouse	Subcutaneous	5000–6000	JECFA (1976)
	Intraperitoneal	2000	JECFA (1976)
	Oral	940	Litton Bionetics Inc. (1975)
Rat	Oral	3330	JECFA (1976)
	Subcutaneous	1000–2500	JECFA (1976)
	Intraperitoneal	710	JECFA (1976)
Guinea pig	Intraperitoneal	4000	JECFA (1976)
	Oral	800–1000	JECFA (1976)
Cat	Intraperitoneal	800–1000	JECFA (1976)
	Intravenous	34	JECFA (1976)

[a] *Source*: As cited in JECFA/WHO (1999), Safety Evaluation of certain Food Additives. WHO Food Additives Series Number 42. Menthol.

were not conjugated by human UGT1A3 (Green et al., 1998). Other human glucuronosyltransferases, such as UGT2B7Y(268) and UGT2B7H(268) (Coffman et al., 1998) as well as UGT2B17, were capable of glucuronidation of menthol (Turgeon et al., 2003).

12.4.1.4 Acute Toxicity

Available acute toxicity data for animals are summarized in Table 12.15. Death appears associated with effects on the CNS and symptoms include the loss of the righting reflex, depression of respiration, and loss of consciousness.

12.4.1.5 Sensitization

A modified Draize sensitization study was conducted using four inbred Hartley strain albino guinea pigs of the same sex. No effects were reported with a 10% concentration of menthol (RIFM, 2003).

12.4.1.6 Neurotoxicity

A neurotoxicity study was conducted to asses the ability of menthol to induce neuropathy (Thorup et al., 1983a). Rats were administered 0, 200, 400, and 800 mg menthol/kg body weight/day for 28 days via gavage. Rats did not exhibit any signs of neurotoxicity but absolute and relative liver weight increases as well as vacuolization of hepatocytes were noted at all doses. The NOEL for menthol was established at <200 mg/kg body weight/day.

12.4.1.7 Short-Term Repeated Dosing Studies

The NCI (1979, 1993) summarized the available oral toxicity data for menthol from studies with Swiss mice that were orally dosed with L-menthol in olive oil. The maximum nonlethal dose was 255 mg/kg.

Rats were administered menthol via gavage for 28 days at daily doses of 0, 200, 400, and 800 mg/kg body weight/day in a soybean oil vehicle. Although there were no alterations in

body weight attributed to menthol, there were significant increases in absolute and relative liver weights at all doses for males and at the high and mid-doses for females. Histopathology indicated vacuolization of hepatocytes in some of the rats but there was no dose dependency in response. The investigators considered that they had not defined an NOAEL but considered it to be less than 200 mg/kg body weight/day (Thorup et al., 1983b).

Groups of 40 rats per sex were exposed to either L- or DL-menthol at up to 200 mg/kg for 5.5 weeks. No adverse effects were observed for weight gain or excretion of glucuronide, water, and electrolytes, and there was no interference in CNS reactions to cardrazol or electric shock, or to intravenous hexobarbital sleeping-time compared with the control (JECFA, 1978).

12.4.1.8 Subchronic Toxicity

In a 13-week study, 10 male and 10 female Fisher 344 rats were fed diets containing menthol at 930, 1870, 3750, 7500, and 15,000 ppm (NCI, 1979). Male rats exhibited a slightly increased incidence of interstitial nephritis with 15,000 ppm menthol. No adverse effects were reported in females.

In a 13-week study, 10 female and 10 male $B6C3F_1$ mice were fed diets containing menthol at 930, 1870, 3750, 7500, and 15,000 ppm (NCI, 1979). Females exhibited an increased incidence of perivascular lymphoid hyperplasia and interstitial nephritis at 7500 ppm and 15,000 ppm menthol. No adverse effects were reported in males.

12.4.1.9 Inhalation Toxicology

An inhalation study was conducted to assess the potential toxicity of volatile components of essential oils (Goldstein et al., 1976). Rats and mice were exposed to vapors of the most common ingredients in cold medications including camphor, menthol, eucalyptol (1,8-cineole), and turpentine via vaporization for 4 and 8 h before challenge aerosols of radiolabeled *Staphylococcus aureus*. Rates of pulmonary bacterial clearance, bacterial transport, and bactericidal activity were recorded. Microscopic examination of lungs showed no biological responses related to exposure to these volatile materials.

Albino Sherman rats (6 per sex) were exposed to 0.087, 0.148, and 0.259 ppm of menthol for at least 6 h per day for 71–79 days. Endpoints included clinical signs, body weight, food and water intake, blood count (hemoglobin, red blood cells, white blood cells, and eosinophils), gross pathology and histopathology, and ciliary activity in fresh preparations of the trachea. Irritant effects of the respiratory tract and hyperemic lungs were reported with 0.259 ppm menthol (Rakieten et al., 1954; RIFM, 2003).

12.4.1.10 Developmental and Reproductive Toxicology

Pregnant rats, mice, hamsters, and rabbits were orally dosed with Brazilian natural L-menthol (CAS No. 89-78-1) in corn oil. Mice were dosed with 0, 1.85, 8.59, 39.9, and 185 mg/kg on days 6–15 of gestation, while rats were dosed with 0, 2.18, 10.15, 47.05, and 218 mg/kg on days 6–15 of gestation. Hamsters were dosed with 0, 4.05, 21.15, 98.2, and 405 mg/kg on days 6–10 of gestation, while rabbits were dosed with 0, 4.25, 19.75, 91.7, and 425 mg/kg on days 6–18 of gestation. Dams were observed daily for clinical signs. The number of implantation sites, resorption sites, live and dead fetuses, and body weights of live fetuses were recorded. Fetuses were grossly examined for external abnormalities. Menthol administration did not affect maternal or fetal survival. The number of fetal abnormalities in the test groups was not different from that in the control (FDRL, 1973).

In another study, rats were administered Rowachol (a mixture of menthol, pinene, menthone, borneol, camphene, and 1,8-cineole) in olive oil at dosing levels of 0.16, 0.80, and 1.60 mL/kg once a day on days 9–14 of gestation. The control group was administered olive oil at a dose of 0.80 mL/kg body weight. No teratogenic effects were reported at any dose of Rowachol (RIFM, 2003).

12.4.1.11 Carcinogenicity Studies

In a carcinogenicity study conducted by NCI (NCI, 1979), 50 rats and 50 mice per sex were fed with DL-menthol in their diets at 3750 or 7500 ppm [average daily intake (ADI) of 187 or 375 mg/kg] and 2000 or 4000 ppm (300 or 600 mg/kg), respectively, for 103 weeks followed by a recovery period of 1 or 2 weeks. Necropsies were conducted at 104 or 105 weeks for all surviving rats and mice. The number of tumors in the test groups was not statistically significantly different from the nontreated controls, in either rats or mice.

12.4.1.12 Cytotoxicity

A cytotoxicity assay was conducted in isolated erythrocytes, hepatocytes, and dipalmitoylphosphatidylcholine (DPPC)-liposomes from Sprague–Dawley rats with menthol at 0.1, 0.4 , 0.6, and 2 mM concentrations. Positive effects included an increase in glutamate oxaloacetate (GOT) leakage in erythrocytes at 0.1–0.6 mM; a decrease in lactate dehydrogenase (LDH) leakage in hepatocytes at 0.1–0.6 mM, a depressed phase transition temperature of DPPC and hypotonic hemolysis at 2 mM. The authors hypothesized that the periapical cellular damage observed in this study was caused by the membrane lysis and surface activity of menthol, and cell penetration by the essential oils was due to membrane affinity and lipid solubility (Manabe et al., 1987; RIFM, 2004).

Isolated rat liver mitochondria were used to determine the effect of 0.5 mM menthol. In the presence of menthol, the respiratory rate was increased and there was osmotic swelling indicating a leakage of the mitochondrial membrane. This concentration of menthol also inhibited the receptor-mediated respiratory stimulation of isolated brown adipocytes even though the intracellular mitochondrial functions were unaffected. These effects were believed to be a direct effect of menthol on biological membranes (Bernson and Pettersson, 1983).

12.4.1.13 Genotoxicity

Menthol was not mutagenic in the Ames test using *Salmonella typhimurium* strains TA2637, TA1537 with and without metabolic activation (S9) at up to 0.5 mg/plate (RIFM, 2003) and TA102, TA100, TA98, and TA97 with and without metabolic activation (S9) at up to 0.8 mg/plate (RIFM, 2004). It also was not mutagenic using *Escherichia coli* at 0.1–0.8 mg/plate (RIFM, 2003).

Andersen and Jensen (1984) tested menthol in *S. typhimurium*/microsome (Ames) assay with and without metabolic activation using tester strains TA1535, TA100, TA1537, and TA98 at doses ranging from 6.4 to 800 μg/plate. There was no evidence of the mutagenic potential of menthol in this study. Carneiro et al. (1997) found no mutagenic potential for menthol using the *S. typhimurium*/microsome assay with and without metabolic activation with tester strains TA100, TA98, TA97a, and TA102.

Menthol did not produce a significant increase in mutant or recombinant frequencies using *Salmonella typhimurium* strain TA1530 up to 145 mg/kg and *Saccharomyces* D3 up to 145 mg/kg. Also, menthol was not mutagenic in a dominant lethal assay at 1150 mg/kg (Litton Bionetics, Inc. 1975).

A sister chromatid exchange (SCE) assay was conducted to assess the ability of several chemicals, including menthol, to induce SCE (Ivett et al., 1989). Chinese hamster ovary (CHO) cells with or without metabolic activation (S9) were exposed to menthol at up to 5 mg/mL. The authors concluded that menthol did not induce SCE with or without metabolic activation.

A chromosomal aberration assay was conducted in cultured CHO cells with and without metabolic activation (S9). Menthol was dissolved in dimethylsulfoxide (DMSO) at concentrations of 0.0313, 0.0625, 0.10, 0.125, 0.2, and 0.3 mg/mL. There were no chromosomal aberrations observed with menthol (RIFM, 2003). A chromosomal aberration assay was conducted in bone marrow metaphase chromosomes of rats at 1150 mg/kg (metabolic activation not specified). The number of aberrations was not significantly different from that of the negative control (saline) (Litton Bionetics, Inc. 1975). Another study was conducted to assess the ability of several chemicals, including menthol, to induce chromosomal aberrations in cells (Ivett et al., 1989). CHO cells with or without metabolic activation (S9) were exposed to menthol at up to 5 mg/mL. No chromosomal aberrations were observed with menthol treatment.

12.4.2 Toxicology of Cigarette Smoke Containing Menthol

12.4.2.1 Genotoxicity

A test battery addressed the genotoxicity of tobacco additives, including menthol (reported as CAS 89-78-1; FEMA # 2665) (Carmines, 2002; Roemer et al., 2002). Cigarette smoke condensate (CSC) was evaluated from test cigarettes with or without 333 common ingredients, including menthol up to 1.8% (18,000 ppm). The Ames assay in *Salmonella typhimurium* bacteria included TA98, TA100, TA102, TA1535, and TA1537 strains, with and without S9 metabolic activation. Results demonstrated no significant differences in the genotoxicity of CSC from cigarette with ingredients compared with CSC from cigarettes without ingredients. The authors concluded that addition of the ingredients tested, including menthol at levels up to 1.8%, did not significantly affect the genotoxicity of CSC.

12.4.2.2 Cytotoxicity

The cytotoxicity of tobacco additives, including menthol (reported as CAS 89-78-1; FEMA # 2665), was reported (Carmines, 2002; Roemer et al., 2002). CSC was evaluated from test cigarettes with or without 333 common ingredients, including menthol at levels up to 1.8% (18,000 ppm). The cytotoxicity test was a neutral red uptake assay using BALB/c 3T3 mouse embryo cells. Results demonstrated no significant differences in the cytotoxicity of CSC from the test cigarette with ingredients compared with CSC from control cigarettes without ingredients. The authors concluded that the addition of the ingredients, including menthol at levels up to 1.8%, did not significantly alter the cytoxicity of CSC.

12.4.2.3 Inhalation Toxicology

The potential effect of 333 ingredients, including menthol, added to cigarettes on the inhalation toxicity of mainstream cigarette smoke (MS) was investigated (Vanscheeuwijck et al., 2002). Male and female Sprague–Dawley rats were exposed nose-only to either the fresh air (sham) or diluted smoke from a test cigarette with ingredients, a control cigarette without ingredients, or a 1R4F reference cigarette at a concentration of 150 μg TPM/L, 6 h/day,

7 days/week for 90 days. Test cigarettes had menthol added at 1.8% (18,000 ppm), whereas the control cigarette had no added ingredients. A 42-day follow-up recovery phase was also included postinhalation. Endpoints monitored included the following exposure parameters: plethysmography, nicotine metabolites and carboxyhemoglobin, hematology, clinical chemistry, body weights, organ weights, gross pathology, and histopathology. Results demonstrated no significant biologically relevant differences between the test and control groups. The authors concluded that the addition of ingredients to cigarettes, including menthol at concentrations up to 1.8%, did not significantly alter the toxicity of MS.

Gaworski et al. (1998) conducted another subchronic nose-only inhalation study of mainstream smoke from cigarettes with and without a mixture of 170 flavor ingredients, including menthol (CAS No. 2216-51-5) at 0.5% (or 5000 ppm) and Brazilian menthol (CAS No. 89-78-1) at 0.0353% (353 ppm) (total concentration of 0.53%), was conducted. Male and female Fisher 344 rats were exposed 1 h/day, 5 days/week, for 13 weeks to MS from test or reference cigarettes at concentrations between 150 and 1200 mg/m^3. The results indicated that the addition of flavor ingredients, including menthol, to cigarette tobacco had no discernible effects on the character or extent of biological responses normally associated with inhalation of MS in rats. It was concluded that the addition of menthol to cigarettes at concentrations up to 0.53% did not alter the toxicological responses to MS.

12.4.2.4 Dermal Tumor Promotion Studies

Four comparative two-stage SENCAR mouse-skin-painting bioassays were conducted with CSC to evaluate the effects of a mixture of cigarette ingredients on dermal tumor promotion (Gaworski et al., 1999). These studies evaluated CSC from test cigarettes with a mixture of 150 ingredients added to cigarette tobacco including menthol at 0.5% (5000 ppm) and Brazilian menthol at 0.0353% (353 ppm) or a total level of 0.53%, and CSC from reference cigarettes with no ingredients were evaluated. The shaved skin of groups of 30–50 female SENCAR mice were initiated with a single 50 μg 7,12-dimethylbenz(*a*)anthracene (DMBA) application in acetone. The initiated skin was promoted three times per week with either 10 or 20 mg "tar" from cigarettes with or without the flavoring mixture for 26 weeks. Control groups included DMBA/acetone (negative control), DMBA/12-*O*-tetradecanoylphorbol-13-acetate (TPA, positive control), and acetone/acetone (promotional control). Mortality was equal or less than 10% for CSC treated groups. There were no statistically significant differences in survival rates between the reference and test cigarette groups. No significant differences in terminal body weights were noted between the reference and test cigarette groups. Even though there were occasional differences between the test cigarette and the reference cigarette in tumor incidence, latency, and multiplicity between test and reference cigarettes, the authors concluded that there were no biologically significant differences in the dermal tumorigenicity responses between groups exposed to CSC from cigarettes with additives, including menthol at up to 0.53%, and groups exposed to CSC from control cigarettes without additives.

In summary, the scientific literature related to the toxicology of menthol indicates that it has a low potential for toxicity. Acute toxicity by oral and dermal routes occurs only at very high doses. At typical levels of use in foods, menthol has not been shown to produce carcinogenic, mutagenic, teratogenic, or reproductive effects, or to be an inhalation toxicant. Addition of menthol to cigarettes did not significantly alter the biological activity of mainstream smoke or smoke condensate compared to cigarettes containing no additives. Most of the infrequent adverse effects reported for menthol are irritation to lips, mouth, and mucous membranes and skin sensitization.

12.4.3 Pulegone

(R)-(+)-Pulegone ($C_6H_{16}O$, mol. wt. 152.23) (CAS No. 89-82-7, FEMA No. 2963) is also referred to as pulegone, D-pulegone, (+)-pulegone, *p*-menth-4(8)-en-3-one, 1-isopropylidene-4-methyl-2-cyclohexanone, among other synonyms. Pulegone is a liquid that boils at 224°C and is insoluble in water but miscible in ethanol, diethyl ether, and chloroform (Merck Index, 1989). It is a hepatotoxic monoterpene that occurs in plant tissues from a number of plant species. Essential oils obtained from *Hedeoma pulegioides* (L.) Pers. (American Pennyroyal), *Bystropogon* (evergreen shrubs), and *Mentha*, including *Mentha pulegium* L. (European Pennyroyal) contain (R)-(+)-pulegone. An isomer (S)-(−)-pulegone occurs in *Agathosma betulina* Berg. (Pillans) from which Buchu leaf oil is obtained. Table 12.16 contains a list of plants from which oils containing pulegone have been obtained. Throughout this discussion, (R)-(+)-pulegone will be referred to as pulegone, unless otherwise noted. Low concentrations of pulegone have been found in foods such as beans and teas.

A major source of human exposure to pulegone is the use of pennyroyal oil obtained from *H. pulegioides* or *M. pulegium*. Boyd (1992) has reviewed the major uses of pennyroyal oil. Historically, pennyroyal oil has been used as a folk remedy as an emmenagogue and an abortifacient and is still sometimes used in folk medicine for these purposes. Leaves containing pennyroyal oil have been used as a flavoring, spice, and for brewing teas and as a mixture with honey and pepper to flavor pork puddings. The roots of the plants, when ground with vinegar, have been used as a tumor remedy while infusions of the leaves used for cramps, spasms, colds, fainting, flatulence, gall bladder ailments, gout, hepatitis, and nervous disorders. None of these uses have been shown to be efficacious by modern scientific methods. The Working Party on Herbal Medicinal Products of the European Agency for the Evaluation of Medicinal Products has released a draft position paper on the use of herbal medicinal products containing pulegone and menthofuran (EMEA/HMPWP, 2004). They reviewed the use of herbal medicinal products containing peppermint oil (*M. piperita*), cornmint oil (*M. canadensis*, syn. *M. arvensis var. piperacens Malinv. ex Holmes*), and pennyroyal oil (*M. pulegium* or *H. pulegoides*). They noted that peppermint oil contains a maximum 4% pulegone, mint oil (partly dementolized) contains a maximum 2% pulegone, and pennyroyal herb contains 1%–2% essential oil with pulegone as the major component (60%–90%). Their conclusions included

> no approval of medicinal products containing pennyroyal oil appear to have been granted in EU and its use in unlicensed products should be discouraged; doses up to a 2.3 mg/kg body weight/day (exceeding the TDI for food) are commonly encountered in herbal medicinal products in Europe. Pharmacovigilance has hitherto revealed no certain cases of liver toxicity in humans caused by peppermint oil or mint oil.

Their proposal for regulatory actions included no immediate action but they suggested increased vigilance for adverse effects from peppermint oil and cornmint oil containing products and the use of pennyroyal should be discouraged.

Nonmedicinal or folklore use of pulegone containing oils as flavoring agents includes alcoholic beverages, baked goods, candy, confectioner's frosting, frozen dairy desserts, gelatins and pudding, jams, jellies, meat products, nonalcoholic beverages, processed fruit, and sweet sauce (Grundschober, 1979).

12.4.3.1 Human Exposure Assessment

The European Commission Scientific Committee on Food (European Commission, Health & Consumer Protection Directorate-General, 2002) has reported that the mean intake of pulegone in the United Kingdom was 0.8 mg/person/day while the 97.5% intake was

TABLE 12.16
Plants Containing Oils Rich in Pulegone

Species	References
Acinos majoranifolius (Mill.) Silic.	Pavlovic et al. (1984b)
Acinos suaveolens (Sibth. et Smith) G. Don f.	Pavlovic et al. (1984a)
Bystropogon plumosus (L.f.) L'Her.	Nahrstedt et al. (1985)
Calamintha arkansana (Nutt.) Shinners	Tucker and Maciarello (1991)
Calamintha nepeta (L.) Savi ssp. *nepeta*	Velasco-Negueruela et al. (1996)
Calamintha pamphylica Boiss. et Heldr. ssp. *davisii* (Quezel et Contandr.) P. H. Davis	Baser et al. (1997b)
Calamintha pamphylica Boiss. et Heldr. ssp. *pamphylica*	Baser et al. (1997b)
Cyclotrichum niveum (Boiss.) Manden et Scheng.	Baser et al. (1994)
Cyclotrichum origanifolium (Labill). Manden et Scheng.	Baser et al. (1996)
Cunila menthoides Benth	de L. Bordignon et al. (1998)
Fumana thymifolia (L.) Spach ex Webb	Couladis and Tzakou (2001)
Hedeoma mandoniana Wedd	Viaseca et al. (2004)
Hedeoma multiflorum Benth.	Koroch et al. (1999)
Hedoma pulegioides (L.) Pers.	Lawrence (1978)
Micromeria albanica (Griseb. ex K. Maly) Silic	Stojanovic et al. (1999)
Micromeria brownei (Swartz) Benth. var. *pilosiuscula* Gray	Tucker et al. (1992)
Micromeria fruticosa (L.) Druce ssp. *giresunica* P. H. Davis	Baser et al. (1996)
Minthostachys andina (Brett.) Epl.	Muñoz et al. (1990)
Minthostachys mollis Griseb.	Rojas and Usubillaga (1995)
Minthostachys verticillata (Griseb.) Epl.	DeFeo et al. (1998)
Monardella undulata Benth. var. *undulata*	Tanowitz et al. (1987)
Agastache rugosa Kuntze	Lawrence (1993)
Agastache schrophulariaefolia (Willd.) Kuntze	Lawrence (1993)
Micromeria fruticosa (L.) Druce ssp. *serpyllifolia* (M. Bieb.) P. H. Davis	Harmandar (1988)
Micromeria fruticosa (L.) Druce ssp. *barbata* (Boiss. et Ky.) P. H. Davis	Fleisher and Fleisher (1991)
Satureja grandiflora (L.) Scheele	Carnat et al. (1991)
Ziziphora tenuior L.	Sezik et al. (1991)
Ziziphora taurica M. Bieb. ssp. *taurica* J. R. Edmondson	Sezik and Tumen (1986)
Micromeria capitella Benth.	Puri and Jain (1988)
Micromeria thymifolia (Scop.) Fritsch.	Stanic et al. (1988)
Ziziphora bungeana Juz.	Dembitskii et al. (1979)
Satureja boliviana (Benth.) Briq.	Vila et al. (1996)
Satureja granatensis (Boiss et Reut.) R. Fernancez	Bellakhdar et al. (1988)
Satureja odora (Griseb.) Epl.	Muschietti et al. (1996)
Satureja pseudosimensis Brenan.	Muhayimana et al. (1998)
Satureja virminea L.	Tucker et al. (2000)
Ziziphora brevicalyx Juz.	Dzhumaev et al. (1990)
Zizphora clinopodioides Lam.	Akgül et al. (1991)

continued

TABLE 12.16 (continued)
Plants Containing Oils Rich in Pulegone

Species	References
Ziziphora hispanica L.	Velasco-Negueruela and Mata Rica (1986)
Ziziphora taurica M. Bieb. ssp. *cleonioides* (Boiss.) P.H. Davis	Kokkalou (1988)
Zizphora aragonensis Pau	Velasco-Negueruela and Mata Rica (1986)
Agathosma betulina (Berg.) Pillans	Collins et al. (1996)
Agathosma crenulata Pillans	Collins et al. (1996)
Minthostachys glabrescens Epl.	Baerheim Svendsen et al. (1987)
Minthostachys setosa (Briq.) Epl.	Senatore (1998)
Micromeria dolichodontha P.H. Davis	Baser et al. (1997a)
Hedeoma drummondi Epl.	Firmage and Irving (1979)
Cunila angustifolia Benth.	Moreira and Krambeck (1976)
Cunila platyphylla Epl.	Bordignon et al. (1998)
Calamintha nepeta (L.) Savi ssp. *glandulosa* (Req.) P.H. Ball	De Pooter et al. (1987)
Micromeria fruticosa (L.) Druce ssp. *fruticosa*	Velasco-Negueruela et al. (1987)
Micromeria fruticosa (L.) Savi ssp. *brachycalyx* P.H. Davis	Kirimer (1992)
Poliomintha incana (Torr.) A. Gray	Lewis and Friedrich (1990)
Pycnanthemum beadlei (Small) Farnald	Lawrence (1999)
Pycnanthemum californicum Trooey ex. Durand	Lawrence (1999)
Pycnanthemum clinopodioides Torrey et Gray	Lawrence (1999)
Pycnanthemum curvipes (Green) Grant et Epl.	Lawrence (1999)
Pycnanthemum floridanum Grant et Epl.	Shu et al. (1994)
Pycnanthemum incanum Michx.	Lawrence (1999)
Pycnanthemum muticum (Michx.) Pers	Lawrence (1999)
Pycnanthemum pilosum Nutt.	Lawrence (1999)
Pycnanthemum pycnanthemoides (Leavenw.) Fernald	Lawrence (1999)
Pycanthemum setosum Nutt.	Lawrence (1999)
Pycnanthemum verticillatum Pers	Lawrence (1999)
Pycnanthemum virginianum Durand et Jackson	Lawrence (1999)
Calamintha sylvatica Jordan ssp. *ascendens* P.W. Ball	Ortiz de Urbina et al. (1988)
Calamintha vardarensis (Greutar et Burdet) Silic	Kitic et al. (2002)
Bystropogon plumosus L'Her.	Nahrstedt et al. (1985)
Bystropogon origanifolius L'Her.	Economou and Nahrstedt (1991)
Bystropogon maderensis Webb	
Bystropogon wildpretii I. La-Sernaibid.	
Bystropogon canariensis (L.) L'Her.	Velasco-Negueruela et al. (1992)
Acinos arvensis (Lam.) Dandy	Soulélès and Katsiotis (1988)
Agastache formosanum Hay.	Fujita and Fujita (1970)
Agastache mexicana (HBK) Link et Epl.	Lawrence (1993)

3.1 mg/person/day with beverages and chewing gum as the major dietary sources. They reported estimated pulegone intake in France based on maximum use concentrations in foods and sugar confectionery, gums and alcoholic and nonalcoholic beverages for 7-day intake surveys and an assumed 100% market share. The mean intake of pulegone would be 43.9 mg/day and the 97.5% intake of pulegone would be 72.7 mg/day. Based on a 1-year Household

Budget Survey, they reported an estimated mean intake of pulegone from sweets, chewing gum, and alcoholic beverages to be 0.05 mg/day and the 97.5% to be 0.5 mg/day. The Committee noted that these estimates were based on accurate food consumption data and information provided by industry on concentrations in foods and not on analysis of the foods themselves. Therefore, they should be interpreted as order of magnitude estimates instead of precise assessments.

Grundschober (1979) reported average concentrations of pulegone in U.S. food products to be 9.07 ppm for nonalcoholic beverages, 10.5 ppm for alcoholic beverages, 28.0 ppm for frozen dairy dessert, 27.4 ppm for candy, 35.4 ppm for baked goods, and 27.3 ppm for gelatins and pudding but precise exposures were not reported.

Because of the potential toxicity of pulegone and the lack of a complete safety assessment, more precise exposure assessments would be useful.

12.4.3.2 Regulatory Status

In the United States, pulegone is listed as a GRAS synthetic flavoring substance (21 CRF §172.515) and American and European pennyroyal oils are approved as natural flavorings (21 CRF § 172.510). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) considered there were no safety concerns for pulegone when used as a food flavoring agent at current levels (JECFA, 2000). The Committee of Experts on Flavoring Substances of the Council of Europe set a tolerated daily intake (TDI) for pulegone at 0.1 mg/kg/body weight. They set provisional limits of 20 mg/kg for foods and beverages except for mint or peppermint flavored alcoholic beverages, which were given a limit of 100 mg/kg and 200–400 mg/kg for liqueurs. Mint or peppermint flavored confectionery was limited to 100 mg/kg and strong mints to 200 mg/kg while mint or peppermint flavored chewing gum was limited to 350 mg/kg. The European Economic Community (EEC) set the maximum concentrations for pulegone in flavored foodstuffs and beverages at 25 mg/kg for foodstuffs, 100 mg/kg for beverages except for 250 mg/kg in mint or peppermint flavored beverages, and 350 mg/kg in mint confectioneries. However, it noted that pulegone could not be added directly to foodstuffs (EEC, 1988). In its recent review, the European Commission Scientific Committee on Food concluded that the currently available data were inadequate for the derivation of an acceptable daily intake and additional data are required.

12.4.3.3 Note on the Toxicology of Pulegone and (R)-(+)-Menthofuran

Although this discussion focuses on pulegone, it must be mentioned that pulegone and menthofuran (CAS No. 494-90-6, FEMA No. 3235) are two compounds closely related in several aspects (structures presented in Figure 12.3). Menthofuran occurs along with pulegone in peppermint and pennyroyal oil and estimates of human exposure for each compound are similar (Council of Europe, 1999; European Commission Health & Consumer Protection Directorate General (ECH & CPDG), 2002).

(R)-(+)-Pulegone (R)-(+)-Menthofuran

FIGURE 12.3 The structure of pulegone and menthofuran, both of which occur in the mint family. Menthofuran is an important metabolite of pulegone in animals.

Within the mint family (*Lamiaceae*), menthofuran is a side product of pulegone synthesis. Mahmoud and Croteau (2003) have shown that menthofuran is an important regulator of pulegone synthesis. In an attempt to improve the quality of peppermint oil, plants were transformed with an antisense menthofuran synthase cDNA. As predicted, these plants produced significantly reduced concentrations of menthofuran but unexpectedly, pulegone concentrations were also decreased (Mahmoud and Croteau, 2001). Additional studies revealed that menthofuran downregulates pulegone reductase at the mRNA level either directly or indirectly. Whether this effect is produced at the transcription or translation level of the reductase gene or through altered messenger stability is not known (Mahmoud and Croteau, 2003). These studies indicate a complex relationship between these two compounds in the plant.

An interrelationship between pulegone and menthofuran is also seen in animals exposed to these compounds. Menthofuran is an important metabolite of pulegone. This metabolic conversion of pulegone to menthofuran in animals represents a metabolic activation because the hepatotoxicity of pulegone is believed to result from its metabolism to menthofuran, as detailed in the pulegone metabolism section of this chapter.

This short discussion of the interrelationships between pulegone and menthofuran should alert the reader that pulegone toxicity must be understood within the context of its relationship with menthofuran.

12.4.3.4 Human Toxicology

The authors found no cases of toxicity associated with ingestion of pulegone. However, there are a number of reports of toxic responses in humans from ingestion of pennyroyal oil and most investigators believe these toxic responses are associated with the pulegone content of pennyroyal oil.

Bakerink et al. (1996) reported cases of two infants who ingested mint tea believed to contain pennyroyal. In one infant, ingestion resulted in death with associated liver failure and cerebral edema. Serum from this infant did not contain detectable concentrations of pulegone but did contain the pulegone metabolite menthofuran (10 ng/mL). Serum from the second infant with symptoms of hepatic dysfunction and epileptic encephalopathy contained concentrations of pulegone (25 ng/mL) and menthofuran (41 ng/mL).

Anderson et al. (1996) reported four cases of pennyroyal ingestion. In one case, a woman repeatedly ingested pennyroyal herbal extract and black cohosh root extract for over 2 weeks in an attempt to induce an abortion. The patient died 46 h after the last pennyroyal ingestion. Symptoms included abdominal cramps, chills, vomiting, syncope, difficulty walking, and cardiopulmonary arrest. At autopsy, hepatic centrilobular degeneration and necrosis were noted along with degenerative changes in the proximal tubules. Blood collected at autopsy 26 h after death contained 18 ng/mL pulegone and 1 ng/mL menthofuran. Western blot analysis of hepatic tissue obtained at autopsy revealed the presence of microsomal protein-bound menthofuran. A second case involved a 22-month-old female baby weighing 10 kg who ingested an unknown quantity of pennyroyal oil. Emergency room treatment started approximately 15 min after the ingestion and consisted of gastrointestinal lavage, charcoal treatment, and administration of oral N-acetyl-cysteine. Serum samples were collected 10 h after ingestion and analyzed for menthofuran, which was found to be 40 ng/mL. The patient completely recovered from the ingestion. Two additional cases involved women who consumed tea made from pennyroyal leaves to induce menses. One woman consumed a cup of tea brewed from one teaspoon of pennyroyal leaves after which she complained of dizziness and weakness but no gastrointestinal symptoms. The other woman consumed a tea prepared from two teaspoons of pennyroyal leaves in a pint of hot water followed by another cup 13 h later, which

resulted in symptoms including nausea and severe abdominal cramping. In both cases, recovery took place with no treatment.

Sullivan et al. (1979) reported toxic effects in two women who consumed pennyroyal oil to induce abortion. In one case, an 18-year-old ingested 10 mL of the oil and reported dizziness. In the other case, a 22-year-old ingested one ounce of oil that produced abdominal pain, nausea, vomiting, lethargy, and agitation. This patient died 7 days after the ingestion from cardiopulmonary arrest showing symptoms of massive hepatic necrosis and disseminated intravascular coagulation.

In their review of the literature, Anderson et al. (1996) found 18 reported cases of adverse effects from the consumption of pennyroyal, all in females, with most cases associated with the consumption of pennyroyal oil. Symptoms of toxicity generally included gastrointestinal upset and CNS effects that included seizures in some cases. Consumptions that resulted in death were often associated with hepatic and renal injury, including centrilobular hepatic necrosis. It is difficult to determine exact doses from these cases of reported human toxicity; however, consumption of 10 mL of pennyroyal oil has been associated with gastritis and mild CNS toxicity, but consumption of 5 mL has been associated with coma and seizures. Consumption of 15 mL or more of the oil has been fatal in some cases. Anderson et al. (1996) recommended gastric cleaning by lavage and activated charcoal and treatment with N acetylcysteine (which decreases depletion of hepatic glutathione) in cases where pennyroyal oil ingestion exceeds 10 mL. Burkhard et al. (1999) have also reviewed the literature concerning the potential of certain essential oils to induce epileptic type seizures and found references to the potential of pennyroyal oil to produce these seizures.

12.4.3.5 Pulegone Metabolism

The metabolism of pulegone in animals is extensive and complex and has been the subject of a number of studies to investigate its role in the hepatotoxicity of pulegone. Metabolism of pulegone involves the phase I enzyme cytochrome P450, which functionalizes it for further metabolism by phase II conjugation reactions. Metabolism may be initiated presystemically by the intestinal mucosa from oral exposures, by the skin from topical exposure, and by the lung epithelium from inhalation. Systemic metabolism is primarily hepatic but other organs may also participate. since pulegone metabolites are excreted into the bile, it appears to undergo enterohepatic circulation where biliary conjugates are cleaved by intestinal microflora hydrolases and the unconjugated metabolite is reabsorbed and reconjugated or further metabolized. An additional source of metabolites may be the metabolism by the intestinal microflora. Metabolites have been detected in the bile, feces, and urine.

Certain electrophilic metabolites of pulegone can covalently bind to nucleophilic cellular sites, such as proteins, and these adducts are thought to be responsible, in part, for the hepatotoxicity of pulegone. Unbound pulegone metabolites are excreted in urine and feces, and both sex and species differences in its biodisposition have been reported. A number of *in vitro* and *in vivo* studies have been reported. Although the *in vivo* studies are most definitive, the *in vitro* studies are important in determining metabolic potential and are useful in developing mechanistic data. An important consideration for *in vivo* studies is the dose administered to the animal. Near-toxic doses, while providing greater quantities of metabolites, may shift metabolism through these pathways resulting in data not reflective of metabolism at the lower doses normally encountered. For this reason, some of the most recent *in vivo* metabolism studies have employed lower, nontoxic doses and have provided data that appear somewhat different from that determined for the higher doses.

Figure 12.4 illustrates the metabolic activation of pulegone to reactive metabolites that can covalently bind to cellular proteins, which is believed to be a critical step in the pathogenesis

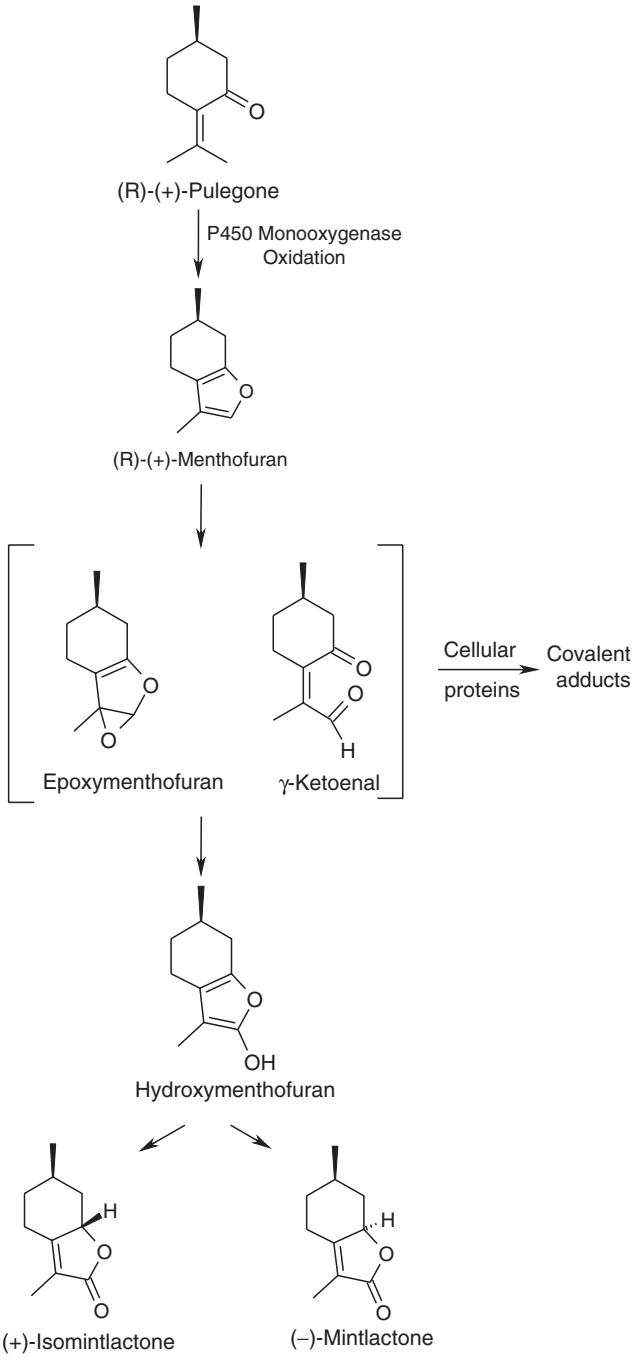


FIGURE 12.4 The metabolic activation of pulegone to electrophilic metabolites capable of covalently binding to nucleophilic sites on tissue proteins. These adducts are believed to be responsible for the hepatotoxicity of pulegone. Pulegone is oxidized by the cytochromes P450 monooxygenase system at one of the allylic methyl groups to produce an alcohol, which undergoes cyclization with the ketone group to form a hemiketal that dehydrates to menthofuran. Further oxidation produces the reactive γ-ketoenal capable of forming adducts with tissue proteins. (Modified from Khojasteh-Bakht, S.C., Chen, W., Koenigs, L.L. Peter, R.M., and Nelson, S.D., *Drug Metab. Dispos.*, 27, 574–580, 1999a. With permission; Khojasteh-Bakht, S.C., Nelson, S.D., and Atkins, W.M., *Arch. Biochem. Biophys.*, 370, 57–65, 1999b.)

of pulegone-related hepatotoxicity in rodents and humans. The first step in the metabolic activation of pulegone is hydroxylation of the C-9 methyl of the isopropylidene by the hepatic P450 monooxygenase followed by cyclization to produce menthofuran. This oxidation reaction is believed to proceed through hydroxylation of the allylic methyl group *syn* to the carbonyl group in pulegone to produce an allylic alcohol, 9-hydroxypulegone, which undergoes an intramolecular cyclization with the ketone group to form a hemiketal that dehydrates to menthofuran. Oxidation of the menthofuran by the P450 monooxygenase results in an electrophilic γ-ketoenal capable of covalent binding to nucleophilic sites on hepatic proteins, altering their structure and activity resulting in hepatotoxicity. A furan epoxide may be an intermediate in the formation of the γ-ketoenal and mintlactones (Gordon et al., 1987; Madyastha and Raj, 1990; Nelson et al., 1992; Thomassen et al., 1992; Nelson, 1995; Khojasteh-Bakht et al., 1999a; Nelson and Trager, 2003). Mizutani et al. (1987) found that inhibitors of cytochrome P450 activity such as SKF-525A, metyrapone, and piperonyl butoxide prevented or decreased the hepatotoxicity of pulegone. Madyastha and Raj (1991, 1992) provided data indicating that menthofuran may be metabolized by pathways that lead to the production of *p*-cresol in rats. Madyastha and Raj (2002) have presented evidence that cytochrome P450 is directly involved in the stereochemical specific hydroxylation of 4-methyl-2-cyclohexenone to produce 4-hydroxy-4-methyl-2-cyclohexenone and *p*-cresol. The importance of the P450 monooxygenase in the hepatotoxicity of pulegone is illustrated by the studies of Sztajnkrycer et al. (2003) who pretreated mice with two inhibitors of P450 activity, disulfiram and cimetidine, before intraperitoneal injection of 300 mg/kg body weight pulegone. Pretreatment with the P450 inhibitors together reduced hepatotoxicity more than pretreatment with each inhibitor individually. The authors concluded that pulegone metabolism by the cytochrome P450 isoform CYP1A2 was more important in the production of hepatotoxic metabolite(s) than metabolism by the CYP2E1 isoform.

SAR studies have indicated that the isopropylidene ketone group is an important structural feature in the metabolic activation of pulegone. Variation in this group that results in the lack of formation of menthofuran, such as reduction of the ketone group or the isopropylidene double bond, eliminates hepatotoxicity. Structural modifications to pulegone, such as isomerization of the double bond to yield isopulegone or modification to piperitenone, decreases hepatotoxicity (Gordon et al., 1982).

McClanahan et al. (1989) investigated the role of covalent adducts between pulegone metabolites and its hepatotoxicity. Phenobarbital administration, which increases the hepatic concentration of certain isoforms of P450, as well as P450 reductase, also increased the covalent binding to hepatic proteins of metabolites of radiolabeled pulegone administered to mice, which correlated with an increase in hepatotoxicity. Piperonyl butoxide, an inhibitor of cytochromes P450, decreased covalent adducts to hepatic proteins and hepatotoxicity. They attributed the covalent binding to the ketoenal formed from the menthofuran produced by oxidation of pulegone. *In vitro* studies of the covalent binding of radiolabeled pulegone to hepatic microsomal proteins followed the patterns found in the *in vivo* studies and were supported by the *in vitro* studies of Madyastha and Moorthy (1989).

Madyastha et al. (1985) found that administration of pulegone to rats resulted in the destruction of cytochromes P450. Incubation of hepatic microsomes with pulegone also resulted in the destruction of P450. Further support for the *in vivo* formation of reactive metabolites of pulegone capable of interacting and modifying hepatic proteins came from Moorthy et al. (1989). They orally administered pulegone at a dose of 400 mg/kg body weight to rats for 5 days and observed significant hepatotoxicity. Hepatic cytochromes P450 concentrations were highly decreased in these rats along with the P450-dependent N demethylation of aminopyrine. No effect on cytochrome P450 reductase activity was noted, although

Khojasteh-Bakht et al. (1998) showed that this reductase activity could be reduced by menthofuran. It was again shown that induction of metabolism by phenobarbital increased pulegone hepatotoxicity and piperony butoxide inhibited the toxicity. Similar studies using menthofuran at a dose of 250 mg/kg body weight administered orally for 3 days yielded similar results, indicating the importance of the metabolism of pulegone to menthofuran in the hepatotoxicity of pulegone (Madyastha and Raj, 1994). Pretreatment of rats with 200 mg/kg body weight phycocyanin, an antioxidant and free radical scavenger, before administration of 250 mg/kg body weight pulegone reduced the depletion of P450 and the decrease in aminopyrine demethylation as well as the hepatotoxicity seen after pulegone administration (Vadiraja et al., 1998). As noted by the authors, it is unclear whether this reduced toxicity was due to inhibition of the P450 monooxygenase or to the free radical scavenger ability of phycocyanin. Administration of diethyl maleate, a compound that depletes hepatic glutathione, increased the hepatotoxicity of pulegone, indicating that glutathione may be involved in the detoxication of reactive pulegone metabolites. Madyastha and Moorthy (1990) used a semipurified, reconstituted P450 system to study the destruction of P450 by pulegone. They reported that pulegone itself, without metabolic activation, could destroy P450 by binding the heme of the enzyme. Yet their *in vitro* studies indicated that inhibitors of metabolism and scavengers of reactive electrophilic compounds inhibited covalent adducts to hepatic proteins. More research concerning the ability of unmetabolized pulegone to destroy P450 may resolve this issue.

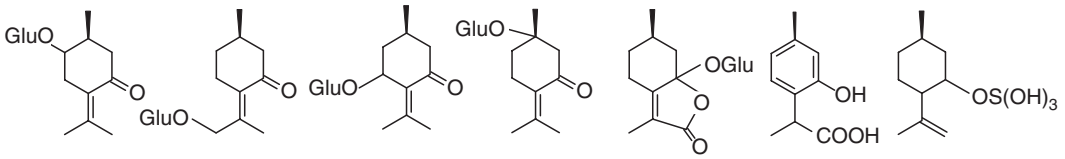
An example of the covalent binding of an activated pulegone metabolite is the inactivation of a cytochromes P450 isoform, human CYP2A6, by menthofuran. Menthofuran is apparently metabolized to an activated form by this P450 isoform and the metabolite covalently binds to the active site of the P450 resulting in its inactivation. Some of the activated metabolite must be released from the P450 because cytochrome P450 reductase activity, the source of reducing equivalents in P450 mediated reactions, is inhibited by covalent adduct formation during oxidation of menthofuran by CYP2A6 (Khojasteh-Bakht et al., 1998). Tisserand and Balacs (1995) postulated that the amount of peppermint oil used in aromatherapy would be insufficient to induce changes in cytochrome P450 activity induced by pulegone because its level was generally less than 1.0%.

An additional metabolic pathway that could lead to metabolites associated with hepatotoxicity has been proposed (Madyastha and Raj, 1993; Thulasiram et al., 2000). This pathway involves the stereoselective hydroxylation of pulegone at the C-5 position followed by dehydration to yield 5-hydroxypulegone (Madyastha and Raj, 1991, 1993). This is metabolized in rats to piperitenone, which can be metabolized to *p*-cresol and 6,7-dehydromenthofuran and other metabolites capable of producing hepatotoxicity (Madyastha and Gaikwad, 1998, 1999). The chiral center at C-5 of pulegone has been known to be important in the toxicity of pulegone for some time. Gordon et al. (1982) determined that S-(−)-pulegone had approximately one third the hepatotoxicity of pulegone. To further investigate the role of the chiral center at C-5, Thulasiram et al. (2000) synthesized the structural analog of pulegone 5,5-dimethyl-2-(1-methylethylidene)-cyclohexanone, which lacks the C-5 chiral center, and studied its metabolism and toxicity in rats. Substitution of the hydrogen at C-5 with a methyl group blocks the P450-mediated hydroxylation at C-5. However, the pathway that leads to the formation of a furanoterpene similar to the formation of menthofuran is still operative. The furanoterpene could undergo intramolecular cyclization to a hemiketal similar to the pathway for menthofuran and like menthofuran; the hemiketal could not be isolated during *in vitro* and *in vivo* studies. The analog was hepatotoxic in a manner similar to pulegone but its toxicity was somewhat less than that of pulegone, leading the authors to propose that the C-5 hydroxylation pathway contributes to the toxicity of pulegone.

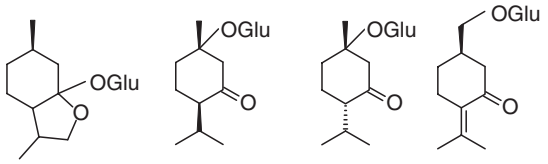
In a further study to investigate the structure activity relations associated with pulegone metabolism, Thulasiram et al. (2001) synthesized R-(+)-4-methyl-2-(1-methylethylidene)-cyclopentanone, a structural analog of pulegone that contained a cyclopentanone ring instead of the cyclohexanone ring and DL-camphorone, which also contains a cyclopentanone ring.

Like pulegone, the methyl *syn* to the carbonyl is hydroxylated but both isopropylidene methyl groups are hydroxylated, with the *syn* methyl group poorly hydroxylated. There is no intramolecular cyclization to yield the corresponding furano compounds and a furanoterpene is not formed. The cyclopentanone did not produce hepatotoxicity and did not reduce cytochrome P450 concentrations, indicating a lack of metabolism to an activated metabolite as seen with pulegone. This study indicates the importance of the cyclohexanone ring in allowing the intramolecular cyclization to form menthofuran from pulegone.

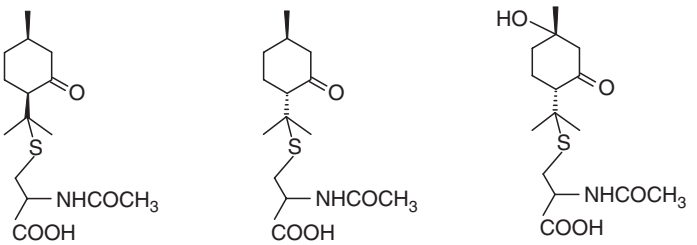
The foregoing discussion has concerned the metabolic activation of pulegone to metabolites believed to be responsible for its hepatotoxicity. However, a number of metabolites that represent detoxification products have been isolated. These metabolites include glucuronides, glutathione conjugate-related mercapturic acids, and other sulfur containing metabolites. As part of the NTP studies on pulegone, Chen et al. (2001) have studied the metabolites that occur in the urine of rats orally dosed with pulegone at lower doses than previously utilized. Lower doses were used because higher doses may saturate certain metabolic pathways shifting metabolite profiles. At doses of 0.8, 8.0, and 80 mg/kg/body weight, approximately 44% of the dose was excreted in the urine of males and approximately 58% in the urine of females with little dose dependency. No pulegone was found in the urine but 14 metabolites were identified, although there were a number of minor peaks that were unidentified. As shown in Figure 12.5,



Metabolites resulting from hydroxylation followed by glucuronidation or further metabolism



Metabolites resulting from reduction to yield menthone/isomenthone followed by hydroxylation/gluronidation



Metabolites resulting from mercapturic acid formation, some of which become hydroxylated

FIGURE 12.5 Metabolites of pulegone identified in the urine of rats orally dosed with pulegone and the major pathways for their formation as proposed by Chen et al. (2001). (Modified from Chen, L.-J., Lebetkin, E.D., and Burka, L.T., *Drug Metab. Dispos.*, 29, 1567–1577, 2001.)

the authors proposed that these metabolites came from three metabolic routes. The first metabolic route yields 7 of the 14 identified metabolites and involves hydroxylation of pulegone, probably by the P450 monooxygenase, followed by glucuronidation and further metabolic steps. The second metabolic route involves reduction to menthone or isomenthone followed by hydroxylation and an intramolecular cyclization or glucuronidation. The third metabolic route involves glutathione S transferase catalyzed formation of glutathione conjugates that result in mercapturic acids, which may be further metabolized by hydroxylation. Similar to the study of Thomassen et al. (1991) who investigated metabolites in the bile of rats dosed with pulegone, equal amounts of hydroxypulegone glucuronides and hydroxymenthone and isomenthone were quantitatively in higher concentrations than the other metabolites. No pulegol, piperitone, or menthofuran were detected in the urine of the rats.

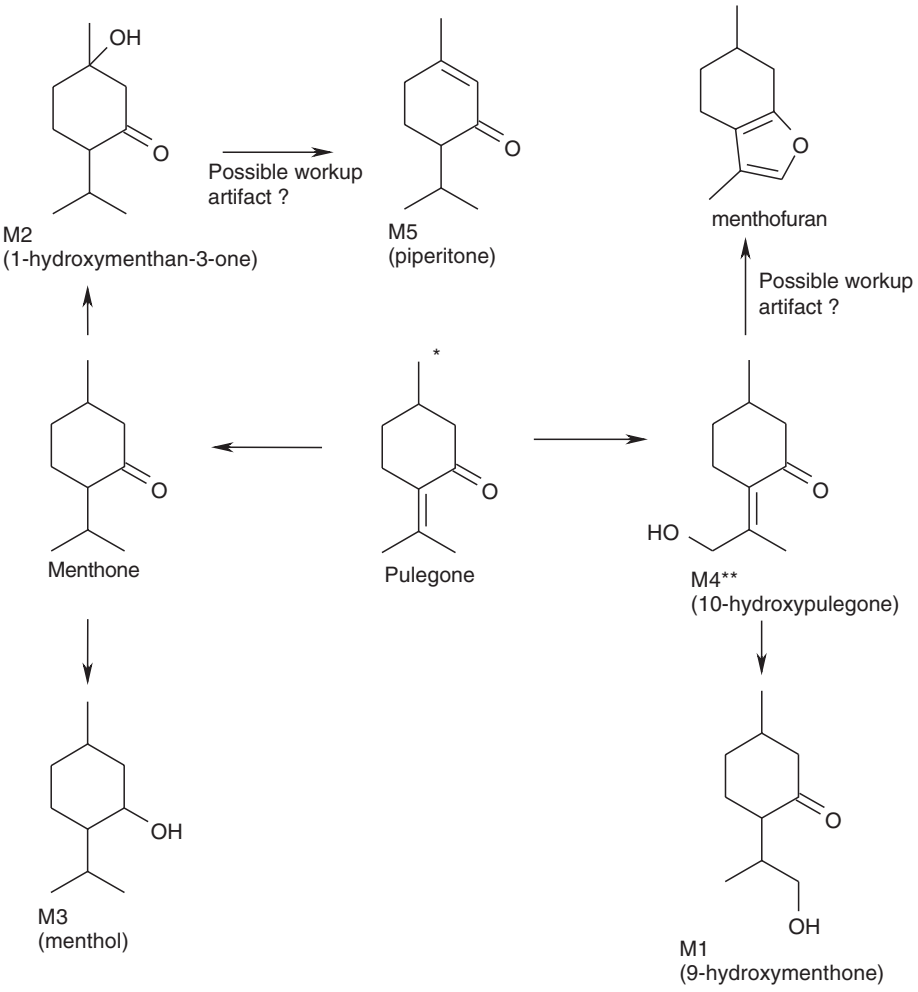
The tautomerization of 2-hydroxymenthofuran (Figure 12.4) to mintlactone and isomintlactone may occur spontaneously and also be catalyzed by glutathione S transferase without the formation of a glutathione conjugate (Khojasteh-Bakht et al., 1999b).

On the basis of menthofuran as an important metabolite of pulegone, Chen et al. (2003a) have investigated its metabolism to urinary metabolites in rats. Two metabolites that were also detected in the urine of rats dosed with pulegone were found. However, a number of metabolites not detected or at concentrations that precluded identification were detected. As with pulegone, menthofuran was metabolized by a number of pathways yielding complex metabolic routes including enterohepatic circulation resulting in multiple metabolic passes.

Many of the metabolism studies with pulegone have been conducted with rats; however, it is important to determine if species differences exist, especially for toxicology studies using more than one species. Chen et al. (2003b) compared the disposition of pulegone in rats and mice associated with a toxicity assessment using these two species. In single oral dose studies with mice and rats, pulegone doses of 0.8, 8.0, and 80 mg/kg body weight were used while 4 oral doses of 80 mg/kg body weight were used in multidosing studies. Mice excreted 85%–100% of the dose in urine and feces at 24-h postdosing while rats excreted 59%–81%, indicating a potential accumulation in rats. Tissue concentrations of pulegone-derived radiolabel at 24 h after dosing were highest in the blood, liver, kidney, and lungs, excluding the digestive tract. Tissue concentrations were lower in mice than in rats, as would be expected by the greater excretion of pulegone-derived radiolabel in mice. Pulegone-derived radiolabel was highest in the kidney of male rats compared with female rats, with the concentration ten-fold higher at the low dose and three-fold higher at the high dose. The authors suggest that this difference is based on the occurrence of α_{2u}-globulin in male rat kidney but not in females. This protein is produced in the liver of male rats and can bind small organic molecules. The bound form of the protein is not broken down in the kidney and thus builds up in this organ. Pulegone or one of its metabolites may bind this protein, which results in the accumulation of radiolabel in the male rat kidney. Mice do not produce this protein. With the exception of the male rat kidney, the radiolabel was highest in the liver, the target organ for pulegone, of both species at 24 h postdosing. This may be associated with the production of covalent adducts between the activated metabolite(s) of pulegone and hepatic proteins. High doses of pulegone decrease P450 content and activity but the multiple doses used in this study resulted in lower pulegone tissue concentrations and higher excretion of radiolabel in the urine than a single dose. This is indicative of an induction of metabolism at these doses. Determination of the metabolites in the urine of mice indicates that they are similar to those found in rats (Chen et al., 2001) with the exception that no mercapturic acids were detected. However, mercapturic acids were found in the bile of mice, indicating that this species is also capable of forming glutathione conjugates, supporting the important role of glutathione in pulegone metabolism.

Engel (2003) has investigated the metabolism of (R)-(+)- and (S)-(−)-pulegone in humans dosed at quantities that might occur in the diet. Three individuals of each gender were dosed

with ~500 μg/kg body weight (R)-(+)-pulegone or 1000 μg/kg body weight of the less toxic (S)-(−)-pulegone and urine was collected for 24 h. Urine was treated with glucuronidase and sulfatase to hydrolyze glucuronide and sulfate conjugates or in some cases, examined directly. On the basis of the analysis of metabolites in the urine, Engel proposed the metabolic scheme illustrated in Figure 12.6. The major metabolite of the (S)-isomer was noted as M1 and determined to be 9-hydroxymenthone. This metabolite was also found in the urine of individuals treated with the (R)-isomer but in much smaller quantities. This led Engel to suggest that metabolism to M1 may be an important detoxication step and may, in part, explain why the (S)-isomer is less toxic than the (R)-isomer. However, it is not known if the (S)-isomer is less toxic in humans than it is in animals. M4 or 10-hydroxypulegone was a



Modified from Engel (2003)
*Stereochemistry not noted to indicate metabolite could be from (R)-(+)- or (S)-(−)-pulegone
**Metabolite indentification key used by Engel

FIGURE 12.6 Metabolic routes for (R)-(+)- and (S)-(−)-pulegone in humans based upon chemical analysis of urine as proposed by Engel (2003). The stereochemistry is not noted to indicate metabolite could be from either the (R) or (S) isomer. (Modified from Engel, W., *J. Agric. Food Chem.*, 51, 6589–6597, 2003.)

minor metabolite of the (S)-isomer but was one of the major metabolites of the (R)-isomer. Engel suggested that it is the precursor of 9-hydroxymenthone and might be the source of menthofuran found in some studies. He determined the M4 would degrade to menthofuran under the workup conditions for the chemical analysis. Pulegone may also be reduced to menthone in humans; trace concentrations were found in the urine. Engel suggested that M3 or menthol, produced in greater quantities from the (S)-isomer than from the (R)-isomer, is formed by a reduction of the carbonyl group of menthone. Metabolite M2 or 1-hydroxy-menthan-3-one could be formed from hydroxylation of menthone. The role of *p*-cresol in pulegone toxicity could not be clarified in this study because it was detected in the urine before and after administration of pulegone. The author suggests that it may be possible that M4 can be further oxidized to pulegone-10-aldehyde, which may be quite reactive. The greater quantity of M4 formed from the (R)-isomer could lead to greater quantities of the aldehyde and to the higher toxicity of this isomer. Further studies will be needed to support this hypothesis.

Although there have been numerous studies on the role of metabolism in the toxicity of pulegone, there are still questions to be answered. For instance, Thomassen et al. (1990) have proposed that there may be a second menthofuran independent pathway for the metabolic activation of pulegone. The existence of this pathway and its nature may require further studies.

12.4.3.6 Genotoxicity of Pulegone

Pulegone has not been extensively tested in genotoxicity assays but there are data available that allow an initial assessment of its genotoxic potential. Andersen and Jensen (1984) determined the mutagenicity of pulegone using the *Salmonella typhimurium* reverse mutation assay (Ames assay). They employed tester strains TA98, TA100, TA1535, and TA1537 with and without metabolic activation using hepatic S9 fractions from rats. Their data indicate that pulegone was nonmutagenic both with and without metabolic activation in this highly validated assay. Pulegone has been tested in the U.S. NTP's genotoxicity screen as part of its ongoing toxicological evaluation. Although no details of the *S. typhimurium* assay with pulegone have been provided, NTP typically uses tester strains TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, and TA1538 with and without metabolic activation by hepatic S9 fractions. NTP has reported that pulegone was negative for mutagenicity in this assay (National Toxicology Program, 2005), confirming the studies of Andersen and Jensen (1984).

NTP has also reported the results of an *in vivo* micronucleus assay using pulegone. This is a validated assay that is capable of detecting chromosomal damage. An advantage of this *in vivo* assay is that all physiological and biochemical processes are intact, as well as absorption, distribution, metabolism, and excretion are active. Although no specific details of the micronucleus assay have been published, NTP typically uses the rat or mouse assay and both genders of the test species. Animals are dosed one to three times at 24-h intervals at doses that extend up to the maximum tolerated level. Micronuclei are determined in bone marrow (or peripheral blood with longer-term studies) 24 h after the last dose. NTP found pulegone to be negative in the micronucleus assay with males and females (National Toxicology Program, 2005).

Franzios et al. (1997) studied the genotoxic potential of the oil from a Greek *M. pulegium* and pulegone using fruit flies (*D. melanogaster*) in an assay capable of detecting mutations and recombinations. They found that the essential oil demonstrated no genotoxic effects; whereas, pulegone produced a weakly positive result. Menthone produced positive genotoxic effects in their assay.

12.4.3.7 Acute Toxicity

Grundschober (1979) has cited studies by Wenzel and Ross that indicated the subcutaneous administration of pulegone to mice yielded an LD_{50} of 1709 mg/kg/body weight, although the gender and mouse strain were not provided. A study by Plazak and Doull is also cited, which indicated that the LD_{50} for an intraperitoneal administration to rats was 150 mg/kg body weight (again the gender and strain were not provided).

Gordon et al. (1982) studied the single-dose, acute toxicity of pennyroyal oil, pulegone, and a number of related compounds in male mice (Swiss–Webster and BALB/c strains). Mice were administered various terpenes dissolved in corn oil as a single intraperitoneal injection, a route of administration not commonly employed in safety assessments. Three doses were used that generally ranged from 400 to 600 mg/kg body weight. Liver, lungs, and kidneys were subjected to histopathological examination and plasma glutamic pyruvic transaminase (GPT) and hepatic glutathione concentrations were determined. A pennyroyal oil dose of 500 mg/kg body weight produced extensive hepatic centrilobular necrosis as well as pulmonary bronchiolar necrosis. The constituents of the oil were analyzed and then fractionated. Only the fractions containing pulegone caused significant organ damage. Hepatic necrosis was more extensive than bronchiolar necrosis and bronchiolar necrosis occurred at all dose concentrations in mice administered menthofuran. These data led the authors to conclude that there was a requirement for the presence of the α-isopropylidene ketone unit for the production of liver and lung toxicity. Reduction of either the ketone group, such as that in pulegol, or the isopropylidene double bond, such as that in menthone, eliminated the liver and lung toxicity. Isomerization of isopropylidene double bond to the acyclic position, as in isopulegone, decreased the toxic effects and isomerization to an endocyclic position as in piperitone eliminated the effects. Piperitenone, which contains both exocyclic and endocyclic double bonds decreased the toxicity, whereas removal of the isopropylidene group, as in 3-methylcyclohexanone, eliminated the liver and lung toxicity. Removal of the methyl group, as in 2-isopropylidenyl-cyclohexanone, decreased the toxicity. Pulegone epoxide was not toxic, while isopulegone epoxide had equivalent toxicity to pulegone. Menthofuran was most toxic to the liver and lung. Differing mouse sensitivity to the terpenes was noted with BALB/c c mice significantly more sensitive to the organ toxicity.

Hepatic glutathione, which can protect the liver from the damaging effects of many metabolically activated electrophilic compounds, was reduced in concentration by 75% within 3 h after the administration of pennyroyal oil and pulegone. Glutathione depletion is generally considered to be an indication of its reaction with an activated metabolite. Glutathione depletion was followed by an increase in plasma GPT, an indication of hepatic damage. When glutathione was depleted by 70% before the administration of pennyroyal oil or pulegone, their toxicity was significantly increased. Therefore, when the protective effect of hepatic glutathione is overcome, hepatic damage becomes apparent. Administration of menthofuran to the mice resulted in only a 25% depletion of glutathione, whereas a 70% depletion of glutathione before the administration of menthofuran did not alter its toxicity.

In an attempt to determine the contribution of menthofuran to the hepatotoxicity of pulegone in Sprague–Dawley rats, Thomassen et al. (1988) compared the hepatotoxicity of pulegone and menthofuran at doses that resulted in equivalent quantities of plasma menthofuran measured as total area under the plasma disappearance curve after an intraperitoneal administration. Under the condition of equivalent area under the disappearance curve, hepatotoxicity was equivalent. Even though the areas under the plasma concentration curves were equivalent, the kinetics were not. Menthofuran produced by the metabolism of pulegone peaked much earlier and the peak was higher than that produced after the administration of menthofuran. When pulegone was administered to the rats in a manner to match the kinetics found after menthofuran

administration, pulegone produced more than twice the hepatotoxicity of administered menthofuran. The authors concluded that pulegone hepatotoxicity was not directly related to its metabolism to menthofuran and other mechanisms may also be involved.

Application of high concentrations of pennyroyal oil to the skin may cause significant absorption and result in acutely toxic systemic concentrations of pulegone. Although not a toxicology study, Sudekum et al. (1992) reported the case of a 30-kg dog that died after receiving a topical treatment for fleas of 60 mL of pennyroyal oil, obtained from a health food store. Less than 1 h after the treatment, the dog appeared listless and was shampooed in an attempt to remove the oil. Vomiting began 2 h after application and within 30 h diarrhea, along with other effects, was apparent. Death followed seizures even though supportive veterinary treatment was provided. Necropsy revealed hemorrhage into the thoracic cavity and congested and edematous lungs with areas of subplueral hemorrhages with evidence of liver damage. Histopathology revealed hepatic cellular necrosis. Pulegone was chemically identified in the liver.

12.4.3.8 Short-Term Repeated Dosing Studies

Thorup et al. (1983a) assessed the toxicity of pulegone in a 28-day repeated dosing study using rats. Groups of 10 rats of each gender were dosed by oral gavage with 0, 20, 80, or 160 mg/kg body weight pulegone in a soybean oil vehicle. Variables measured included clinical observations, clinical chemistry, hematology, urinalysis, and histopathological examination of selected tissues. During the in-life phase, a dose-dependent atonia was noted along with increased water consumption at the high dose. Body weight gain was reduced by 20% at the high dose and 10% at the intermediate dose. Blood creatinine was decreased in both genders at the high dose while neutrophilic granulocytes were increased at the high dose. Histological examination revealed vacuolization of hepatocytes at doses of 80 and 160 mg/kg body weight and ''cyst-like spaces in the white matter of the cerebellum.'' The authors considered the NOAEL to be 20 mg/kg body weight.

Molck et al. (1998) treated 28 6-week-old, female Wistar rats per group with either soybean oil (vehicle control) or 160 mg/kg body weight pulegone in soybean oil for 28 days by oral gavage. Plasma alkaline phosphatase, alanine aminotransferase, albumin, calcium, creatinine, glucose, total protein, urea, and uric acid were determined at study termination, along with body and liver weight. Liver and brain were prepared for histological examination using two different tissue fixation techniques. Pulegone dosed rats appeared listless and depressed and demonstrated reduced food intake but higher water intake and body weight gain than the vehicle controls during the in-life phase of the study. At study termination, plasma glucose and creatinine were decreased, as was body weight. Plasma albumin and total protein and liver weight were increased, as was alkaline phosphatase. Histopathology revealed no liver changes at the dose used in this study. The cyst-like spaces in the white matter of the cerebellum reported by Thorup et al. (1983a) and Olsen and Thorup (1983) were not seen in this study in brain tissue.

The U.S. NTP is currently investigating the toxicity of pulegone using protocols that conform to the currently acceptable principles of toxicity evaluation. It has completed 14-day repeated dosing studies using gavage administration to male and female Fischer 344 rats and B6C3F1 mice. Rats were dosed at 0, 37, 5, 75, 150, 300, or 600 mg/kg body weight while mice were dosed at 0, 18.75, 37.5, 75, 150, or 300 mg/kg body weight. The results from this study have not been published at this time.

12.4.3.9 Subchronic Toxicity

No reports of toxicology studies that have used dosing periods longer than 28 days have been found in the literature. However, the U.S. NTP has completed a 13-week subchronic toxicity study using gavage administration to male and female Fisher 344 rats and B6C3F1 mice. Rats and mice were dosed at 0, 9.375, 18.75, 37.5, 75, or 150 mg/kg/day. The results from this study have not been published at this time. However, an recent publication (Smith et al., 2005b) has reported an NOAEL from the NTP 90-day toxicity studies as 9.375 mg/kg body weight/day. No species was denoted and since this was the low dose for both species perhaps it applies to the rats and mice.

12.4.3.10 Carcinogenicity Studies

No reports of carcinogenicity studies with pulegone were found in the scientific literature. However, the U.S. NTP has completed the in-life portion of a 2-year carcinogenicity study of pulegone using gavage administration to male and female Fisher 344 rats and B6C3F1 mice. Currently, the program is conducting the histopathology phase. Male rats were dosed at 0, 18.75, 37.5, or 75 mg/kg body weight and female rats and mice of both genders were dosed at 0, 37.5, 75, or 150 mg/kg/body weight. No results from this study are available at this time.

12.4.3.11 Other Toxicology Studies

No data were found in the scientific literature concerning the effects of pulegone on reproduction, including teratology, embryotoxicity, and immunotoxicity.

12.5 SHORT SUMMARIES OF THE POTENTIAL TOXICOLOGY OF SPECIFIC ESSENTIAL OILS FROM THE MINT FAMILY

12.5.1 Cornmint Oil

Cornmint oil has recently been given GRAS status for use as a flavoring agent in foods by the FEMA Expert Panel. Their evaluation of cornmint oil has been summarized in a recent publication and will be presented here as an example of their evaluation process and to indicate the known potential toxicity of this oil (Smith et al., 2005b). The reader is referred to the original publication (Smith et al., 2005b) for a more thorough explanation of the Panel's evaluation, supporting data, and references.

The FEMA Expert Panel notes that the steam distillate of *M. canadensis* (as *M. arvensis*) contains 70% or greater menthol, which is reduced by low temperature crystallization to produce dementholized cornmint oil. It is further distilled and blended to produce the commercial oil (Table 13.3). Because dementholized cornmint oil is not consumed as a food, the unidentified constituents must be considered in the evaluation of its potential GRAS status using the FEMA Panel's evaluation criteria.

The Panel considers the principal congeneric structural group of cornmint oil to be terpene alicyclic secondary alcohols, ketones, and related esters represented by menthol, menthone, isomenthone, and menthyl acetate among others, which represent up to 95% of the commercial oil. There are toxicology data available on several of these constituents with menthol as the most thoroughly studied (Section 12.4.1 of this chapter). The Panel considers this congeneric group to be in Structure Group II (Section 12.2.16 of this chapter).

With respect of the exposure assessment of dementholized cornmint oil, the Panel believes that the anticipated potential market in the United States could be 200,000 kg/year, which would correspond to a daily PCI for individuals consuming the oil of approximately 20 mg/person/day or 0.333 mg/kg/day. The Panel calculates the intake of the congeneric group to be 19 mg/person/day, which is higher than the exposure threshold of 0.54 mg/person/day for Structural Class II, resulting in the need for toxicity data for this congeneric group. The Panel's review of the toxicology literature indicated that menthol, menthone, and other members of the congeneric group exhibited NOAELs at 1000 times the daily PCI. Genotoxicity assays of members of this congeneric group provided no indication of interactions with DNA capable of producing mutations. Based on its evaluation of these data, the Panel concluded: "Therefore, the intake of this congeneric group from consumption of *M. arvensis* is not a safety concern." (Smith et al., 2005b).

The Panel noted that one constituent of dementholized cornmint oil, pulegone, is a terpene alicyclic ketone related to the major congeneric group in cornmint oil but has a unique structure (2-isopropylidene cyclohexanone) and can be metabolically activated to more toxic metabolites (see Section 12.4.3.5 of this chapter). Pulegone can produce hepatotoxicity at doses at least an order of magnitude less than the NOAELs for structurally related alicyclic ketones and secondary alcohols. This led the Panel to consider pulegone and a metabolite, menthofuran, which account for less than 2% of commercial dementholized cornmint oil, separately from the congeneric group. The Panel calculated the daily PCI of 0.40 mg/person/day or 0.0067 mg/kg body weight/day, which exceeds the 0.09 mg/kg body weight threshold for Class III. Using data from an unpublished 90-day subchronic NTP sponsored study of pulegone toxicity that indicated a NOAEL of 9.375 mg/kg/day, the Panel concluded that this was approximately 1000 times the intake of pulegone and its metabolites in dementholized cornmint oil. The Panel also considered a rat 28-day repeated dosing study with peppermint oil that contained approximately 4% pulegone and menthofuran. From the NOAEL of 400 mg/kg body weight/day for females and 200 mg/kg body weight/day for males, the Panel considered this to correspond to an NOAEL of 8 mg/kg body weight/day for pulegone and menthofuran. The Panel also considered a rat 90-day subchronic study of the toxicity of a mixture of *M. piperita* and *M. canadensis* (as *M. arvensis*) peppermint oils that established an NOAEL of 100 mg/kg body weight/day for the mixture and calculated that this corresponded to an NOAEL of 4 mg/kg body weight/day for pulegone and menthofuran.

A second congeneric structural group considered by the Panel was terpene hydrocarbons, including (+)- and (−)-pinene and (+)-limonene among others that account for up to 8% of dementholized cornmint oil but can be reduced to less than 3% in the commercial oil. The Panel used the 8% composition to calculate a conservative estimate of intake, which results in an intake of 1.6 mg/person/day or 0.027 mg/kg body weight/day. The Panel considered long-term studies of the principal members of the group sponsored by the NTP that indicated NOAELs of 300 mg/kg body weight/day to be orders of magnitude greater than the daily PCI of terpene hydrocarbons. This led the Panel to state "Therefore, all congeneric groups in cornmint oil are considered safe for use when consumed in mint oil" (Smith et al., 2005b).

During its evaluation of cornmint oil, the Panel considered that commercial dementholized cornmint oil can contain unidentified constituents in the 2.9%–4% range. The Panel calculated that this represented a daily PCI for consumers of the oil as high as 0.80 mg/person/day. As unidentified constituents are placed in the highest concern class (Class III), this level of intake is above the 0.09 mg/person/day intake for this class and results in the need for toxicology data. Although data were not available for dementholized cornmint oil, the Panel considered data from a 28-day repeated dosing study with peppermint oil containing essentially the same constituents as dementholized cornmint oil. This study revealed an NOAEL of 100 mg/kg/day, which the Panel considered to be equivalent to 5 mg/kg body weight/day for

the unidentified constituents. This was considered to be at least 100 times the intake of 0.0133 mg/kg body weight/day calculated for the intake of unidentified constituents in dementholized cornmint oil. This led the Panel to conclude: ''Hence the total of unidentified constituents is also not a safety concern'' (Smith et al., 2005b).

The final step in evaluation of dementholized cornmint oil by the Panel was an evaluation of the total oil in respect to all the congeneric groups and unidentified constituents. The Panel noted that members of the terpene alicyclic secondary alcohols, ketones, and related esters along with some monoterpene hydrocarbons and peppermint oil have the potential to produce α-2μ-globulin related kidney pathology in male rats (Smith et al., 2005b). This toxicity is not seen in female rats or in other species, including humans. Evaluation of this male-rat-specific kidney toxicity from a number of studies has led to the conclusion by toxicologists that this male-rat-specific pathology is not relevant to humans. The Panel considered that interactions among the congeneric structural groups would be expected to be minimal because the NOAELs for the congeneric groups and the commercial mint oils are on the same order of magnitude.

The final conclusion based on the Panel's evaluation is as follows:

> Based on the above assessment and the application of the scientific judgment of the FEMA Expert Panel, cornmint oil is concluded to be 'Generally Recognized As Safe' under conditions of intended use as a flavoring substance. Given the criteria used in the evaluation, recommended specifications should include the following chemical assay: (1) <95% alicyclic secondary alcohols, ketones and related esters, typically measured as (−)-menthol; (2) <2% 2-isopropylidenecyclohexanones and their metabolites, measured as (−)-pulegone; and (3) <10% monoterpene hydrocarbons, typically measured as limonene (Smith et al., 2005b).

Unfortunately, the vast majority of the toxicology studies conducted on *M. canadensis* (as *M. arvensis*) oil have not used a cornmint oil that resembles the commercial oil. Reproduction studies using extracts from the plant and other studies with other types of extracts have been published. However, the relevance of these studies to the commercial oil is questionable. Toxicology studies using a commercial oil that meets the chemical specifications recommended by the FEMA Expert Panel would be highly useful in the further evaluation of the safety of dementholized cornmint oil as used in food and other products. Menthol is the major constituent of dementholized cornmint oil (30%–48%) and has been the subject of a variety of toxicology studies that are reviewed in Section 12.4.1 of this chapter.

12.5.2 Peppermint Oil

Peppermint oil is obtained from *M. piperita* by steam distillation of the fresh flowering plant and its composition is presented in Table 12.3. Menthol is its major constituent (28%–47%) followed by menthone (11%–32%). The many uses of peppermint are discussed in Section 12.2.1 of this chapter.

A number of acute toxicity studies employing peppermint oil with different species and routes of administration have been reported. These are summarized in Table 12.17. Clinical signs at the lethal doses were generally salivation, ataxia, convulsions, loss of righting reflex, and depression. These data indicate that peppermint oil has low toxicity in these commonly used species because the LD_{50}s are in the grams per kilogram of body weight range.

Thorup et al. (1983b) conducted a 28-day repeated dosing study with peppermint oil by oral gavage using male and female Wistar rats. The doses used in this study were 10, 40, and 100 mg/kg body weight/day. No adverse effects were found at 10 mg/kg body weight/day but histopathological changes described as cyst-like spaces were found scattered in the white matter of the cerebellum at doses of 40 and 100 mg/kg body weight/day. Mengs and Stotzem

TABLE 12.17
Reported Acute Toxicity (LD_{50}) of Peppermint Oil in Various Species

Species (strain)	Route of Administration	LD_{50} (g/kg body weight)	References
Rat (Wistar)	Oral	Male 2.65 (CI = 2.30–3.00)	RIFM (1972)
Rat (Wistar)	Oral	4.0	Mengs and Stotzem (1989)
Rat	Oral	4.44	VonSkramlik (1959)
Rat (Wistar)	Oral	4.44	Eickholt and Box (1965)
Rat (Wistar)	Intraperitoneal	0.82 ± 0.13	Eickholt and Box (1965)
Mouse (ddY)	Oral	Male 2.41 (CI = 2.17–2.68)	Ohsumi et al. (1984)
Mouse (NMRI)	Oral	4.0	Mengs and Stotzem (1989)
Rabbit	Dermal	>5	RIFM (1972)

(1989) dosed Wistar rats with peppermint oil for 5 weeks by oral gavage at doses of 20, 150, and 500 mg/kg body weight/day. No effects were seen at 20 and 150 mg/kg body weight while only nonspecific effects were seen at 500 mg/kg body weight, such as increased water intake. Mengs and Stotzem (1989) also reported data from a study where beagle dogs were dosed for 5 weeks with enteric-coated gelatin capsules containing 25 or 125 mg/kg body weight peppermint oil. An array of variables were determined that included body weight, clinical signs, hematology, urinalysis, necropsy, and histopathological examination of tissues. No evidence of significant adverse effects was reported at either dose.

A 90-day subchronic toxicity study of peppermint oil using Wistar rats orally dosed with 10, 40, or 100 mg/kg body weight/day has been reported by Spindler and Madsen (1992). Body weight, food and water intake, hematology, clinical chemistry, and gross and microscopic pathology were assessed at necropsy. No peppermint-oil-related effects were observed at 10 and 40 mg/kg body weight/day. At 100 mg/kg body weight/day, histopathological alterations in the brain of both sexes that consisted of cyst-like spaces in the white matter of the cerebellum were observed. These were apparently similar to those reported by Thorup et al. (1983b). There were also histopathological changes in the kidneys of male rats at the high dose.

There have been no published reports of chronic toxicity studies with peppermint oil; however, a study where peppermint oil was placed into a toothpaste matrix for chronic toxicity testing with male and female mice by the oral route has been reported (Roe et al., 1979). The toothpaste matrix containing peppermint oil to yield 4 or 16 mg/kg body weight/day was administered by oral gavage at a standard dose volume of 1 mL/kg body weight for 6 days per week for 80 weeks. Body weights as well as clinical observations and food consumption were determined during the study. At necropsy, organ weights were determined and histological examination of tissues and any observed tumors was conducted. A number of tumors were detected but these were considered to be tumors that commonly occur in mice and were not considered to be test article related. Although peppermint oil was incorporated into the toothpaste matrix and the data compared with that collected from mice receiving the toothpaste matrix only and untreated controls, this study cannot be considered a chronic toxicity study of peppermint oil. The toothpaste matrix contained a number of constituents including glycerol, carrageen gum, precipitated calcium carbonate, sodium lauryl sulfate, sodium saccharin, mineral oil, and water. It is possible that these constituents could have altered the absorption, distribution, metabolism, and excretion of the constituents of the peppermint oil. Other interactions could have taken place that could have modified the effects

of the peppermint. Therefore, there is still a need to investigate the effects of peppermint oil in a chronic toxicology study designed to meet current toxicological standards.

The genotoxicity of peppermint oil has been assessed in a number of *in vitro* assays and fewer *in vivo* assays. Peppermint oil was evaluated for mutagenic potential in the *Salmonella*/microsome assay (Ames assay) using tester strains TA98, TA100, TA1535, and TA1537 with and without metabolic activation (Andersen and Jensen, 1984). Doses that ranged from 6.4–800 μg/plate did not present evidence of mutagenic potential in the presence and absence of metabolic activation. Peppermint oil was studied in the chromosomal aberration assay and the SCE assay using human lymphocytes in whole peripheral blood from healthy volunteers with no external metabolic activation (Lazutka et al., 2001). Peppermint oil induced chromosome aberrations with chromatid and chromosome breaks. The dose response was not linear and peaked at a value eight-fold higher than the control and then decreased, possibly due to cytotoxicity. Peppermint oil produced increases in SCEs compared with the control. The dose response curve indicated a rapid increase in exchanges followed by a lack of increased exchanges as the dose increased. As with the chromosomal aberration assay, peppermint oil demonstrated considerable cytotoxicity in the SCE assay. As peppermint oil produced its clastogenic effects at concentrations that produced high cytotoxicity, the authors suggested that it may be producing its effects due to secondary cytotoxicity mechanisms and possibly not by a direct genotoxic interaction with DNA and suggested that additional studies are needed to resolve this issue. Unpublished studies and those that may have used peppermint mint extracts or other forms have produced either negative or equivocal results in *in vitro* genotoxicity studies.

Peppermint oil has been studied in an *in vivo* assay using *Drosophila melanogaster* wing spot assay (Lazutka et al., 2001). In this assay, 72-h-old larvae were exposed to peppermint oil added to the surface of the medium upon which the larvae develop and treatment lasted until pupation, resulting in a 48-h treatment period. Wings from the flies were examined microscopically to assess the presence of spots that represent somatic mutations or mitotic recombination. Peppermint oil induced mutations but there did not appear to be a dose dependency and the mutations were less frequent than those from the positive control, benzo(a)pyrene. The authors suggest that peppermint oil should be further studied for genotoxic potential using *in vivo* mammalian test systems.

The potential immunotoxicity of peppermint oil has been determined in animal models. Gaworski et al. (1994) used a screening protocol based on a tier testing strategy to assess humoral and cell-mediated immune responses in female mice. Peppermint oil was administered intragastrically for 5 days at three doses. Cell-mediated immunity was determined by the host resistance assay and humoral immunity was determined by the antibody plaque-forming cell response to sheep erythrocytes. Peppermint oil increased the mortality rate and reduced survival in the host resistance assay but did not alter the plaque-forming cell response. Doses of 625 and 1250 mg/kg body weight/day resulted in immunotoxicity, whereas a dose of 313 mg/kg body weight/day had no effect.

No published reports concerning the developmental and reproduction toxicology of peppermint oil were found in the scientific literature. However, a number of reports concerning other activities have been published. It appears that peppermint oil may be a sensitizer in a small proportion of the population but generally responses have been mild. As would be expected, certain effects of peppermint are based on its constituents. A few studies have investigated the metabolites excreted after exposure to peppermint and these reflect the major constituents of the oil. For example, menthol metabolites (see Section 12.4.1.3 of this chapter) are commonly encountered depending on the menthol content of the oil. Dresser et al. (2002) have evaluated the effect of peppermint oil on cytochrome P4503A4 (CYP34A)-mediated *in vitro* metabolism of nifedipine and its oral bioavailability in humans. Peppermint oil was a

"moderately potent" reversible inhibitor of CYP3A4 and increased the oral bioavailability of nifedipine, possibility by inhibition of CYP3A4-mediated presystemic metabolism. The authors suggest that these effects of peppermint oil may be associated with its menthol content.

Certain studies have implicated activities such as kidney damage in rats produced by aqueous extracts or "tea" obtained from peppermint plants (Akdogan et al., 2003). However, the relevance of studies using peppermint extracts to effects produced by the essential oil from peppermint is questionable because the constituents of extracts or other forms of peppermint are generally different from those of the essential oil.

A review of the safety of peppermint oil, leaf extract, leaf and leaf water has been published by the Cosmetic Ingredients Review Expert Panel (2001).

12.5.3 Spearmint Oil

Native spearmint oil ex *M. spicata* L. has been approved for food use in the United States for many years. Scotch spearmint oil ex. *M. gracilis* Sole has recently been given GRAS status for use as a flavoring in foods by the FEMA Expert Panel (Smith et al., 2005b). Prior to this, it was used under the name spearmint oil, although that was specifically identified as sourced from *Mentha spicata* L. or incorrectly identified as *Mentha sativa* L., which is a synonym of the natural hybrid *M. verticillata* L.

Few toxicology studies using spearmint oil were found in the published scientific literature. Unpublished studies estimate an LD_{50} in rats of approximately 5000 mg/kg body weight. A few studies were found where "teas" (aqueous extracts of leaves) were used but these are not relevant to the oil.

A few genotoxicity studies were found. Spearmint oil was tested in the Ames assay for mutagenicity to *S. typhimurium* tester strains TA100 and TA98 with and without metabolic activation with no evidence of mutagenic potential (Marcus and Lichtenstein, 1982; Crebelli et al., 1990). Spearmint oil was found to be negative in the Ames assay and an assay for clastogenic potential in the chromosomal aberration assay using CHO cells in culture (Ishidate et al., 1984). It was also found to be negative in the mouse micronucleus assay (Hayashi et al., 1988). A spearmint oil was reported to produce mutations in the *Drosophila melanogaster* wing spot test (Franzios et al., 1997).

Spearmint oil at high concentrations can produce dermal irritation in animals and probably humans. It may be a sensitizer in some individuals (Larsen et al., 2001).

(−)-Carvone, the major constituent of Scotch spearmint oil, has been tested for mutagenicity in the Ames *S. typhimurium* assay using tester strains TA98, TA100, TA1535, and TA1537 with and without metabolic activation and was found to have no mutagenic potential in this assay (Florin et al., 1980). (−)-Carvone was a weak inducer of chromosomal malsegregation in *Saccharomyces cerevisiae* (Zimmermann et al., 1989) but showed no genotoxicity in the Wing Somatic Mutation and Recombination Test using *D. melanogaster* (Franzios et al., 1997).

Carvone has been examined for its acetylcholinesterase (AChE) activity by Miyazawa et al. (1997). They found that monoterpene ketones possessed stronger activities than their corresponding alcohols. In addition, they found that the strength of inhibition of AChE was decreased with an isopropenyl group on the *p*-menthane molecule rather than an isopropyl group. However, (−)-carvone was found to possess a weaker activity than (+)-pulegone, even though it possesses a conjugated double bond. The antimicrobial properties of (−)-carvone can be found in Chapter 12.

Engel (2001) has investigated the metabolism of (+)- and (−)-carvone in humans administered the carvones at doses close to those anticipated to represent a daily intake. Urine was

collected 24 h before and after oral ingestion of 1 mg carvone/kg body weight by six volunteers. The urine was treated with glucuronidase and sulfatase to free glucuronide and sulfate conjugates and yield the phase 1 metabolites. The major metabolites detected were reported to be α-4-dimethyl-5-oxo-3-cyclohexene-1-acetic acid (dihydrocarvonic acid), α-methylene-4-methyl-5-oxo-3-cyclohexene-1-acetic acid (carvonic acid) and 5-(1,2-dihydroxy-1-methylethyl)-2-methyl-2-cyclohexen-1-one (uroterpenolone) while minor metabolites were identified as reduced forms of carvone (carveol and dihydrocarveol). No difference was detected between the (+)- and (−)-carvone although the stereochemistry of the metabolites was not reported. Jager et al. (2000) studied the *in vitro* microsomal metabolism of R-(−)- and S-(+)-carvone using rat and human hepatic microsomes. They found stereoselective biotransformation with each enantiomer resulting in the production of 4R,6S-(−)-carveol from R-(−)-carvone and 4S,6S-(+)-carveol from S-(+)-carvone. Microsomes from both species demonstrated a lower Michaelis constant (*K*m) for the production of 4R,6S-(−)-carveol. Glucuronide formation was detected only from 4R,6S-(−)-carveol. Jager et al. (2001) investigated the metabolism of these two carvone isomers after dermal exposure of 300 mg to humans. Both isomers were rapidly absorbed but S-(+)-carvone resulted in higher plasma concentrations and a longer distribution half-life. No metabolites were detected in plasma but analysis of urine after glucuronidase treatment indicated the metabolism of R-(−)-carvonte to 4R,6S-(−)-carveol and its glucuronide. No urinary metabolites were found after S-(+)-carvone application.

Differences in biological activity have been reported for R-(+)- and S-(−)-carvone. Buchbauer et al. (2005) reported that there were differences between the two chiral isomers when assessed by locomotion activity in mice. The form found in spearmint [(−)-carvone] produced more sedative and relaxing effects than did the form found in caraway oil [(+)-carvone], which demonstrated both sedative and activation effects.

12.5.4 Bergamot Mint Oil

Bergamot mint or Mentha citrata oil, which is obtained from *M. aquatica* var. *citrata*, has not been approved for food use in the United States; however, it is used to a very limited extent as a minor component of fragrances. To date, no toxicological assessment of Mentha citrata oil has been published although toxicological and dermatological reviews of linalool (Powers and Beasley, 1985; Bickers et al., 2001; Letizia et al., 2003) and linalyl acetate (Bickers et al., 2003), the major constituents of the oil, have been published. The antimicrobial properties of linalool and linalyl acetate are found in Chapter 12.

REFERENCES

Adams, T.B., Hallagan, J.B., Putnam, J.M., Gierke, T.L., Doull, J., Munro, I.C., Newberne, P., Portoghese, P.S., Smith, R.L., Wagner, B.M., Weil, C.S., Woods, L.A., and Ford, R.A. (1996). The FEMA GRAS assessment of alicyclic substances used as flavour ingredients. *Food Chem. Toxicol.*, 34, 763–828.

Ahmed, S.M. and Eapen, M. (1986). Vapour toxicity and repellency of some essential oils to insect pests. *Ind. Perfum.*, 30(1), 273–278.

Akdogan, M., Kilinc, I., Oncum M., Karaoz, E., and Delibas, N. (2003). Investigation of biochemical and histopathological effects of *Mentha piperita* L. and *Mentha spicata* L. on kidney tissue in rats. *Hum. Exp. Toxicol.*, 22, 213–219.

Akgül, A., De Pooter, H.L., and De Buyck, L.F. (1991). The essential oils of *Calamintha nepeta* subsp. *glandulosa* and *Ziziphora clinopodioides* from Turkey. *J. Essent. Oil Res.*, 3, 7–10.

Andersen, P.H. and Jensen, N.J. (1984). Mutagenic investigation of peppermint oil in the Salmonella/mammalian-microsome test. *Mutat. Res.*, 138, 17–20.

Anderson, I.B., Mullen, W.H., Meeker, J.E., Khojasteh-Bakht, S.C., Oishi, S., Nelson, S.D., and Blanc, P.D. (1996). Pennyroyal toxicity: measurement of toxic metabolite levels in two cases and review of the literature. *Ann. Intern. Med*., 124, 726–734.

Anonymous. (1983). *British Herbal Pharmacopoeia*. British Herbal Medicine Association, Bournemouth, UK, p. 142.

Anonymous. (1990). *British Herbal Pharmacopoeia 1990*. British Herbal Medicine Association, Bournemouth, UK, p. 72.

Ansari, M.A., Masudevan, P., Tandon, M., and Razdan, R.K. (2000). Lavicidal and mosquito repellent action of peppermint (Mentha piperita) oil. *Bioresour. Technol*., 71, 267–271.

Baerheim Svendsen, A., Scheffer, J.J.C., and Looman, A. (1987). Composition of the volatile oil of *Minthostachys glabresens* Epl. *Flav. Fragr. J*., 2, 45–46.

Bakerink, J.A., Gospe, S.M., Dimand, R.J., and Eldridge, J.W. (1996). Multiple organ failure after ingestion of pennyroyal oil from herbal tea in two infants. *Pediatrics*, 98, 944–947.

Baronikova, S., Grancai, D., Nagy, M., and Duris, M. (2001). Essential oil composition of *Ligustrum delavayanum* Hariot. flowers. *J. Essent. Oil Res.*, 13, 194–195.

Baser, K.H.C., Sarikardasoglu and Tümen, G. (1994). The essential oil of *Cyclotrichum nimeum* (Boiss.) Manden et Scheng. *J. Essent. Oil Res*., 6, 9–12.

Baser, K.H.C., Kirimer, N., Kürküoglu, M. Özek, T., and Tumen, G. (1996a). Essential oil of *Cyclotrichum origanifolium* (Labill.) Manden et Scheng. from Turkey. *J. Essent. Oil Res.*, 8, 569–570

Baser, K.H.C., Kirimer, N., Özek, T., Tümen, G., and Karaer, F. (1996b). Essential oil composition of three Labiatae endemic to Turkey [*Micromeria fruticosa* (L.) Druce subsp. *giresunica*, P.H. Davis, *Sideritis lycia* Boiss. et Heldr., and *S. arguta* Boiss. et Heldr.]. *J. Essent. Oil Res.*, 8, 699–701.

Baser, K.H.C., Kirimer, N., and Duman, H. (1997a). Composition of the essential oil of *Micromeria dolichodontha*. P.H. Davis. *Flav. Fragr. J.*, 12, 289–291.

Baser, K.H.C., Özek, T. Kürkcüoglu, M., Tümen, G., and Duman, H. (1997b). Essential oils of *Calamintha pamphylica* Boiss. et Heldr. subsp. *pamphylica* and subsp. *davisii* (Quezel et Contandr.) Davis. *J. Essent. Oil. Res.*, 9, 371–373.

Bellakhdar, J., Aiklgri, M., Rombourg, M., and Berrada, M. (1988). Sur la composition chimique de-l'-huiles essentielles de *Satureja vulgaris* (L.) Fritzch, *Satureja grantensis* (Boiss. et Reut.) R. Fernandes et *Satureja calamintha* (L.) Scheele du Maroc. *Biruniya*, 4(1), 67–73.

Benowitz, N.L., Herrera, B., and Jacob, P. (2004). Mentholated cigarette smoking inhibits nicotine metabolism. *J. Pharmacol. Exp. Ther.*, 310, 1208–1215.

Bernson, V.S., and Pettersson, B. (1983). The toxicity of menthol in short-term bioassays. *Chem. Biol. Interact*., 46, 233–246.

Bickers, D.R., Calow, P., Greim, H.A., Hanifin, J.M., Rogers, A.E., Saurat, J.-H., Sipes, I.G., Smith, R.L., and Tagami, H. (2001). The safety assessment of fragrance materials. *Regul. Toxicol. Pharmacol.*, **37**, 218–273.

Bickers, D.R., Calow, P., Greim, H.A., Hanifin, J.M., Rogers, A.E., Saurat, J.-H., Sipes, I.G., Smith, R.L., and Tagami, H. (2003). A toxicologic and dermatolic assessment of linalool and related esters when used as fragrance ingredients. *Food Chem. Toxicol*., 41, 919–942.

British Industrial Biological Research Organisation (BIBRA) (1990). Toxicity Profile Menthol, pp. 1–9.

Blumenthal, M., Busse, W.R., Goldberg, A., Gruenwald, J., Hall, T., Riggins, C.W., and Rister, R.S. (1998). *The complete German Commission E Monographs Therapeutic Guide to Herbal Medicines. Am. Bot. Council*, Austin, TX, USA, pp. 5–684.

Bordignon, S.A. De L., Schenkel, E.P., and Spitzer, V. (1998). The essential oil composition of *Cunila platyphylla* Epling (Lamiaceae). *Acts Pharm. Bonaerense*, 17, 143–146.

Boukhman, M.P. and Maibach, H.I. (2001). Thresholds in contact sensitization: Immunologic mechanisms and experimental evidence in humans-an overview. *Food Chem. Toxicol*., 39, 1125–1134.

Boyd, E.L. (1992). *Hedeoma pulegioides* and *Mentha pulegium*. In De Smet P.A.G. M., Keller K., Hansel R., and Chandler R.F., Eds., Adverse *Effects of Herbal Drugs*, 1, Springer-Verlag, New York, pp. 151–156.

Brusick, D. (2001). Genetic toxicology. In: Hayes, A.W., Ed., *Principles and Methods of Toxicology 4th edn*., Taylor & Francis, Philadelphia, PA, pp. 819–852.

Buchbauer, G., Jager, W., Gruber, A., and Dietrich, H. (2005). *R*-(+)- and *S*-(−)-Carvone: influence of chirality on locomotion activity in mice. *Flav. Fragr. J*., 20, 686–689.

Buckle, J. (1997). *Clinical Aromatherapy in Nursing*. Singular Publ. Group Inc., San Diego, CA, pp. 289.

Burdock, G.A. (2002). *Fenaroli's Handbook of Flavor Ingredients 4th edn*. CRC Press, Boca Raton, FL, pp. 1023–1025.

Burkhard, P.R., Burkhardt, K., Haenggeli, C.-A., and Landis, T. (1999). Plant-induced seizures: reappearance of an old problem. *J. Neurol*., 246, 667–670.

Burns-Naas, L.A., Meade, B.J., and Munson, A.E. (2001). Toxic Responses of the Immune System. In C.D. Klaassen., Ed., *Casarett and Doull's Toxicology: The Basic Science of Poisons 6th edn*., McGraw-Hill, New York, NY, pp. 419–170.

Caldwell, W.S., Byrd, G.D., deBethizy, J.D., and Crooks, P.A. (2001). Modern Instrumental Methods for Studying Mechanisms of Toxicology. In: Hayes, A.W., Ed., *Principles and Methods of Toxicology 4th edn*., Taylor & Francis, Philadelphia, PA, pp. 1689–1757.

Carmines, E.L. (2002). Evaluation of the Potential Effects of Ingredients Added to Cigarettes. Part 1: Cigarette Design, Testing Approach, and Review of Results. *Food Chem. Toxicol*., 40, 77–91.

Carnat, A., Chossegros, A., and Lamaison, J.L. (1991). The essential oil of *Satureja grandiflora* (L.) Scheele from France. *J. Essent. Oil Res.*, 3, 361–362.

Carneiro, M.R.G., Paumgartten, F.J.R., and Felzenswalb, I. (1997). Evaluation of the mutagenic potential of monoterpenoid compounds. *Mutat. Res*., 379 (Suppl. 1), S110–S111.

Chantraine, J.-M., Laurent, D., Ballivian, C., Saavedia, G., Ibanex, R., and Vilaseca, A. (1998). Insecticidal activity of essential oils on *Aedes aegypi* larvae. *Phytotherap. Res*., 13, 350–354.

Chen, L.-J, Lebetkin, E.D., and Burka, L.T. (2001). Metabolism of (R)-(+)-pulegone in F344 Rats. *Drug Metab. Dispos*., 29, 1567–1577.

Chen, L.-J, Lebetkin, E.D., and Burka, L.T. (2003a). Metabolism of (R)-(+)-menthofuran in Fischer-344 rats: identification of sulfonic acid metabolites. *Drug Metab. Dispos*., 31, 1208–1213.

Chen, L.-J, Lebetkin, E.D., and Burka, L.T. (2003b). Comparative disposition of (R)-(+)-pulegone in B6C3F1 mice and F344 rats. *Drug Metab. Dispos*., 31, 892–899.

Chiej, R. (1984). *The MacDonald Encyclopedia of Medicinal Plants*. MacDonald & Co. Publ. Ltd., London, U.K., pp. 195.

Clark, G.S. (1988). Menthol, A Profile of an aroma chemical. *Perfum. Flav*., 13(5), 37–46.

Clark, G.S. (1998). An aroma chemical profile: Menthol. *Perfum. Flav*., 23(5), 33–46.

COE (1999). Committee of Experts on Flavoring Substances 41st meeting, RD 4–12/1–45, Datasheet on (R)-(+)-menthofuran. Council of Europe Publishing.

COE (2000). Chemically-defined Flavouring Subtances. Council of Europe Publishing, pp. 66.

Coffman, B.L., King, C.D., Rios, G.R., and Tehply, P.R. (1998). The glucuronidation of opioids, other xenobiotics, and androgens by human UGT2B7Y(268) and UGT2B7H(268). *Drug Metab. Dispos*., 26, 73–77.

Collins, N.F., Graven, E.H., van Beek, T.A., and Lelyveld, G.P. (1996). Chemotaxonomy of commercial Buchu species (*Agathosma betulina* and *A. crenulata*). *J. Essent. Oil Res.*, 8, 229–235.

Cosmetic Ingredients Review Expert Panel (CIREP) (2001). Final report on the safety assessment of *Mentha Piperita* (Peppermint) oil, *Mentha Piperita* (Peppermint) leaf extract, *Mentha Piperita* (Peppermint) leaf, and *Mentha Piperita* (Peppermint) leaf water. *Intern. J. Toxicol.*, 20(3), 61–73.

Couladis, M. and Tzakou, O. (2001). The oil of *Fumana thymifolia* (L.) Spach ex Webb from Greece. *J. Essent. Oil Res.*, 12, 434–435.

Cramer, G., Ford, R., and Hall, R. (1978). Estimation of toxic hazard-a decision tree approach. *Food Cosmetic Toxicol*., 16, 255–276.

Crebelli, R. Aquilina, G., and Carere, A. (1990). Microbial mutagenicity screening of natural flavouring substances. *Microbiologica*, 13, 115–119.

de L. Bordignon, S.A., Schenkel, E.P., and Spitzer, V. (1998). Essential oil of *Cunila menthoides* Benth. (Lamiacea). *J. Essent. Oil Res.*, 10, 317–320.

De Pooter, H., Goetghebeur, P., and Schamp, N. (1987). Variability in composition of the essential oil of *Calamintha nepeta. Phytochemistry*, 26, 3355–3356.

deBethizy, J.D. and Hayes, J.R. (2001). Metabolism: A Determinant of Toxicity. In: Hayes, A.W., Ed., *Principles and Methods of Toxicology 4th edn*., Taylor & Francis, Philadelphia, PA, pp. 77–136.

DeFeo, V., Ricciardi, A.I., Biscardi, D., and Senatore, F. (1998). Chemical composition and antimicrobial screening of the essential oil of *Mintostachys verticillata* Griseb.) Epl. (Lamiaceae). *J. Essent. Oil Res*., 10, 61–65.

Dembitskii, A.D., Yurina, R.A., Krotova, G.I., Goryaev, M.I., and Sinitsyn, G.S. (1979). Study of *Ziziphora bungeana* essential oil. *Izv. Akad. Nauk Kaz. SSR. Ser. Khim.*, 29, 63–65.

DiPasquale, L.C. and Hayes, A.W. (2001). Acute Toxicity and Eye Irritancy. In A.W. Hayes, Ed., *Principles and Methods of Toxicology 4th edn*., Taylor & Francis, Philadelphia, PA, pp. 853–916.

Dresser, G.K., Wacher, V., Wong, S., Wong, G.T., and Bailey, D.G. (2002). Evaluation of peppermint oil and ascorbyl palmitate as inhibitors of cytochrome P4503A4 activity *in vitro* and *in vivo*. *Clin. Pharmacol. Ther*., 72, 247–255.

Duke, J.A., Bogenschultz-Godwin, M.J., duCellier, J., and Duke, P-A.K. (2002). *Handbook of Medicinal Herbs 2nd edn*., CRC Press, Boca Raton, FL, pp. 562–564.

Dzhumaev, Kh. K., Zenkevich, I., Tkachenko, K.G., and Tsibul'skaya, I.A. (1990). Essential oils from inflorescences and leaves of *Ziziphora brevicalyx. Khim. Prir. Soedin*., (1), 122–123.

Eccles, R. (1994). Menthol and related cooling compounds. *J. Pharm. Pharmacol*., 46, 618–630.

Economou, D. and Nahrstedt, A. (1991). Chemical, physiological and toxicological aspects of the essential oil of some species of the genus *Bystropogon*. *Planta Med.*, 57, 347–351.

EEC (1988). Council Directive 88/388/EEC of 21 June 1988 on the approximation of the laws of the member states related to flavorings for use in foodstuffs and to source materials for their production. *Offic. J. Europ. Commun.*, L184, pp. 61–67.

Eickholt, T.H. and Box, R.H. (1965). Toxicities of peppermint and Pycnanthemum albescens oils, fam. Labiaeae. *J. Pharm. Sci*., 54, 1071–1072.

EMEA/HMPWP (2004). Draft position paper on the use of herbal medicinal products containing pulegone and menthofuran. http://www.emea.eu.int.

Engel, E. (2001). *In vivo* studies on the metabolism of the monoterpenes S-(+)- and R-(−)-carvone in humans using the metabolism of ingestion-correlated amounts (MICA) approach. *J. Agric. Food Chem*., 49, 4069–4075.

Engel, W. (2003). *In vivo* studies on the metabolism of the monoterpene pulegone in humans using the metabolism of ingestion-correlated amounts (MICA) approach: Explanation for the toxicity differences between (S)-(−)- and (R)-(+)-pulegone. *J. Agric. Food Chem*., 51, 6589–6597.

Erman, M. (2004). Progress in physiological cooling agents. *Perfum. Flav*., 29(8), 34–50.

European Commission, Health & Consumer Protection Directorate-General (ECH & CPDG) (2002). Opinion of the Scientific Committee on Food on pulegone and menthofuran. (http://europa.eu.int/comm./food/fs/sc/scf/index en.html). Accessed on Nov. 8, 2004.

European Pharmacopoeia (1997).

FDA (1993). *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food, "Redbook II."* Division of Health Effects Evaluation (HFS-225), CFSAN, FDA, 200 C Street S.W., Washington, DC (see http://www.cfsan.fda.gov for updates for this publication).

FDA, Department of Health and Human Services. (2003a). *Code of Federal Regulations*. 21CFR § 182.20 Essential Oils, Oleoresins (solvent-free), and Natural Extractives (including distillates). Office of the Federal Register, National Archives and Records Administration, Washington, DC.

FDA, Department of Health and Human Services. (2003b). *Code of Federal Regulations*. 21CFR §172.515 Synthetic Flavoring Substances and Adjuvants. Office of the Federal Register, National Archives and Records Administration, Washington, DC.

FDRL (1973). Teratologic Evaluation of FDA 71–57 (Menthol Natural, Brazilian). Report prepared for U.S. FDA under contract No. FDA 71–260. Dated June 1, 1973. PB-223 815. National Technical Information Service.

Firmage, D.H. and Irving, R.C. (1979). Effect of development on monoterpene composition of *Hedeoma drummondii. Phytochemistry*, 18, 1827–1829.

Fleisher Z. and Fleisher, A. (1991). Aromatic plants of the Holy Land and the Sinai. Part VII. The essential oil of *Micromeria fruticosa* (L.) Druce subsp. *barbata* (Boiss. et Ky.) P.H. Davis. *J. Essent. Oil Res*., 3, 477–479.

Fleming, C.J. and Forsyth, A. (1998). D5 patch test reactions to menthol and peppermint. *Contact Dermatitis*, 38, 337–365.

Florin, I., Rutberg, L., Curvall, M., and Enzell, C.R. (1980). Screening of tobacco smoke constituents for mutagenicity using the Ames test. *Toxicology*, 15, 219–232.

Flück, H. and Jaspersen-Schib, R. (1976). *Medicinal Plants and their uses*. J.M. Rowson (translator), W. Foulsham & Co. Ltd., London, U.K., pp. 135–136.

Ford, R.A., Domeyer, B., Easterday, D., Maier, K., and Middleton, J. (2000). Criteria for development of a database for safety evaluation of fragrance ingredients. Regulatory *Toxicol. Pharmcol.*, 31, 166–181.

Franzios, G., Mirotsou, M., Hatziapostolou, E., Kral, J., Scouras, Z.G., and Mavragani-Tsipidou, P. (1997). Insecticidal and genotoxic activities of mint essential oils. *J. Agric. Food Chem.*, 45, 2690–2694.

Frosch, P.J., Johansen, J.D., Menne', T., Pirker, C., Rastogi, S.C., Andersen, K.E., Bruze, M., Goossens, A., Lepoittevin, J.P., and White, I.R. (2002). Further important sensitizers in patients sensitive to fragrances II. Reactivity to essential oils. *Contact Dermatitis*, 47, 279–287.

Fujita, S-I. and Fujita, Y. (1970). Miscellaneous contributions to the essential oils of the plants from various territories. XXLV. Essential oil of *Agastache formosanum* Hay. *Yakugaku Zasshi*, 90, 1514–1516.

Furia, T. and Bellanca, W. (1975). *Fenaroli's Handbook of Flavor Ingredients 2nd edn.*, *Vol.* 1. CRC Press, Boca Raton, FL.

Garg, S.C. and Banerjee, A.K. (1997). Insect and pest control activity of essential oils. *Ind. Perfum.*, 41(2), 73–84.

Garnon, P. (1992). 10 Ans de Production en France: Bilan et Perspectives. In: Compte rendu des 3 èmes rencontres techniques et économiques plantes aromatiques et médicinales. N. Verlet., Ed., CFPPA, Nyons, France, pp. 261–231.

Gaworski, C.L., Vollmuth, T.A., Dozier, M.M., Heck, J.D., Dunn, L.T., Ratajczak, H.V., and Thomas, P.T. (1994). An immunotoxicity assessment of food flavoring ingredients. *Food Chem. Toxicol.*, 32, 409–415.

Gaworski, C.L., Dozier, M.M., Heck, J.D., Gerhart, J.M., Rajendran, N., David, R.M., Brennecke, L.H., and Morrissey, R. (1998). Toxicologic evaluation of flavor ingredients added to cigarette tobacco: 13-week inhalation exposures in rats. *Inhal. Toxicol.*, 10, 357–381.

Gaworski, C.L., Heck, J.D., Bennett, M.B., and Wenk, M.L. (1999). Toxicologic evaluation of flavor ingredients added to cigarette tobacco: Skin painting bioassay of cigarette smoke condensate in SENCAR mice. *Toxicology*, 139, 1–17.

Gelal, A., Jacob, P., Yu, L., and Benowitz, N.L. (1999). Disposition kinetics and effects of menthol. *Clin. Pharmacol. Ther.*, 66, 128–135.

Gobel, H., Schmidt, G., and Soyka, D. (1991). Effect of peppermint and eucalyptus oil preparations on neurophysiological and experimental algesimetric headache parameters. *Cephalagia*, 14, 228–234.

Goldstein, E., Cooper, A.D., and Tarkington, B. (1976). Effect of inhalation medication vapors from a colds preparation on murine pulmonary bacterial defense systems. *J. Toxicol. & Env. Health*, 2, 371–388.

Gordon, W.P., Forte, A.J., McMurtry, R.J., Gal, J. and Nelson, S.D. (1982). Hepatotoxicity and pulmonary toxicity of Pennyroyal oil and its constituent terpenes in the mouse. *Toxicol. Appl. Pharmacol.*, 65, 413–424.

Gordon, W.P., Huitrie, A.C., Seth, C.L., McClanahan, R.H., and Nelson, S.D. (1987). The metabolism of the abortifacient terpene, (R)-(+)-pulegone, to a proximate toxin, menthofuran. *Drug Metab. Dispos.*, 15, 589–594.

Green, M.D., Belanger, G., Hum, D.W., Belanger, A., and Tephly, T.R. (1997). Glucuronidation of opioids, carboxylic acid-containing drugs, and hydroxylated xenobiotics catalyzed by expressed monkey UDP-glucuronosyltransferase 2B9 protein. *Drug Metab. Dispos.*, 25, 1389–1394.

Green, M.D., King, C.D., Mojarrabi, B., Mackenzie, P.I., and Tephly, T.R. (1998). Glucuronidation of amines and other xenobiotics catalyzed by expressed human UDP-glucuronosyltransferase 1A3. *Drug Metab. Dispos.*, 26, 507–512.

Grundschober, F. (1979). Literature review of pulegone. *Perfum. Flav. Int.*, 4(1), 15–17.
Haarmann & Reimer Corporation (1985). H&R Product Menthol. Springfield, NJ.
Harmandar, M. (1988). Analysis of essential oil from leaves of *Micromeria fruticosa* (L.) Druce ssp. *serpyllifolia* (M. Bieb.). P.H. Davis. *Doga Tu. J. Chem.*, 12, 188–194.
Harwood, S.H., Moldenke, A.F., and Berry, R.E. (1990). Toxicity of peppermint monoterpenes to the variegated cutworm (*Lepidoptera: Noctuidae*). *J. Econ. Entomol.*, 83, 1761–1767.
Hayakawa, R., Yamamura, M., and Sugiura, M. (1996). Contact dermatitis from L-menthol. *Cosmetics & Toiletries Magazine*, pp. 28–29.
Hayashi, M., Kishi, M., Sofuni, T., and Ishidate, M., Jr. (1988). Micronucleus tests in mice on 39 food additives and eight miscellaneous chemicals. *Food Chem. Toxicol.*, 26, 487–500.
Hendriks, H. (1998). Pharmaceutical aspects of some Mentha herbs and their essential oils. *Perfum. Flav.*, 23(6), 15–23.
Hopp, R. (1993). Menthol: Its Origins, Chemistry, Physiology and Toxicological Properties. Haarmann & Reimer GmbH. Corporate Research. 47th Tobacco Chemists' Research Conference. *Recent Adv. Tob. Sci*, 19, 3–46.
Hori, M. (1999). Antifeeding, settling inhibitory and toxic activities of Labiatae essential oils against the green peach aphid, *Myzus persicae* (Sulzer) Homoptea: Aphididae. *Appl. Entomol. Zool.*, 34, 113–118.
Hori, M. and Harada, H. (1995). Screening plants resistant ot green peach aphid *Myzus persicae* (Sulzer) Homotera: Aphididae. *Appl. Entomol. Zool.*, 30, 246–249.
Hsu, H-Y., Chen, Y-P., Shen, S-J., Hsu, C-S., Chen, C-C., and Chang, H-C. (1986). *Oriental Materia Medica, a concise guide*. Oriental Healing Arts Institute, Long Beach, CA, pp. 79–80.
Huang, K-C. (1998). *The Pharmacology of Chinese Herbs*. CRC Press, Boca Raton, FL, pp. 218–219.
Ishidate, M., Jr., Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada, J., and Matsuoka, A. (1984). Primary mutagenicity screening of food additives currently used in Japan. *Food Chem. Toxicol.*, 22, 623–636.
Ivett, J.L., Brown, B.M., Rodgers, C., Anderson, B.E., Resnick, M.A., and Zeiger, E. (1989). Chromosomal Aberrations and Sister Chromatid Exchange Tests in Chinese Hamster Ovary Cells *In Vitro*. IV. Results with 15 Chemicals. *Environ. Molec. Mutag.*, 14, 165–187.
Jager, W., Mayer, M., Platzer, P., Reznicek, G., Deitrich, H., and Buchbauer, G. (2000). Stereoselective metabolism of the monoterpene carvone by rat and human liver microsomes. *J. Pharm. Pharmacol.*, 52, 191–197.
Jager, W., Mayer, Reznicek, G., and Buchbauer, G. (2001). Percutaneous absorption of the monoterpene carvone: implication of stereoselective metabolism on blood levels. *J. Pharm. Pharmacol.*, 53, 637–642.
JECFA/WHO (1978). 20th Report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additives Series No. 10.
JECFA/WHO (1999). Safety Evaluation of Certain Food Additives. WHO Food Additives Series No. 42. Menthol. <http://www.inchem.org/documents/jecfa/jecmono/v042je21.htm>. Accessed May 10, 2004.
JECFA/WHO (2000). Summary evaluations performed by the joint FAO/WHO expert committee on Food Additives: Pulegone http://JECFA.ILF.ORE, Accessed on March 20, 2004.
Jenner, P.M., Hagan, E.C., Taylor, J.M., Cook, E.L., and Fitzhugh, O.G. (1964). Food flavourings and compounds of related structure. I. Acute oral toxicity. *Food Cosmet. Toxicol.*, 2, 327–343.
Kaffenberger, R.M. and Doyle, M.J. (1990). Determination of menthol and menthol glucuronide in human urine by gas chromatography using an enzyme-sensitive internal standard and flame ionization detection. *J. Chromatogr.*, 527, 59–66.
Khojasteh-Bakht, S.C., Koenigs, L.L., Peter, R.M., Trager, W.F., and Nelson, S.D. (1998). (R)-(+)-Menthofuran is a potent, mechanism-based inactivator of human liver cytochrome P450 2A6. *Drug Metab. Dispos.*, 26, 701–704.
Khojasteh-Bakht, S.C., Chen, W., Koenigs, L.L., Peter, R.M., and Nelson, S.D. (1999a). Metabolism of (R)-(+)-pulegone and (R)-(+)-Menthofuran by human liver cytochrome P-450s: Evidence for formation of a furan epoxide. *Drug Metab. Dispos.*, 27, 574–580.

Khojasteh-Bakht, S.C., Nelson, S.D., and Atkins, W.M. (1999b). Glutathione S-transferase catalyzes the isomerization of (R)-2-hydroxymenthofuran to mintlactones. *Arch. Biochem. Biophys*., 370, 59–65.

Kirimer, N. (1992). The essential oil of *Micromeria fruticosa* (L.) Druce subsp. *brachyclayx*. Davis, P.H., *J. Essent. Oil Res*., 5, 79–80.

Kitic, D., Palic, R., Stojanovic, G., and Ristic, M. (2002). Composition of the essential oil of *Calamintha vardarensis* (Greuter et Burdet) Silic. *J. Essent. Oil Res.*, 14, 58–59.

Kokkalou, E. (1988). The volatile constituents of *Ziziphora tauric* subsp. *Cleonioides*. *Planta Med*., 54, 163–164

Konstantopoulou, I., Vassilopoulou, L., Mauragani-Tsipidov, P., and Scoures, Z.G. (1992). Insecticidal effects of essential oils. A study of the effects of essential oils extracted from eleven Greek aromatic plants on *Drosophila auraria*. *Experientia*, 48, 616–619.

Koroch, A.R., Juliana, Jr., A.R., Trippi, V.S., and Juliana, H.R. (1999). Chemical constituents of the essential oil of *Hedeoma multiflorum* Benth. (Lamiaceae). *J. Essent. Oil Res.*, 11, 165–166.

Landing, J.E. (1969). *American Essence, A History of the Peppermint and Spearmint Industry in the United States*. Copyright, A.M. Todd Foundation, Kalamazoo Public Museum, Kalamazoo, MI.

Larsen, W., Nakayama, H., Fischer, T., Elsner, P., Frosch, P., Burrows, D., Jordan, W., Shaw, S., Wilkinson, J., Marks, J., Jr., Sugawara, M., Nethercott, M., and Nethercott, J. (2001). Fragrance contact dermatitis: a worldwide multicenter investigation (Part II). *Contact Dermatitis*, 44, 344–346.

Laurent, D., Vilaseca, L.A., Chantaraine, J-M., Ballivian, C., Saavedra, G., and Ibanez, R. (1997). Insecticidal activity of essential oils on *Triatoma infestans*. *Phytotherap. Res*., 11, 285–290.

Lawrence, B.M. (1978). Progress in Essential Oils. *Perfum. Flav.*, 3(5), 36–41.

Lawrence, B.M. (1993). Labiatae oils—Mother Nature's chemical factory. In: Lawrence, B.M., Ed., Essential oils 1988–1991. Allured Publ. Corp., Carol Stream, IL, pp. 188–206.

Lawrence, B.M. (1999). The composition of essential oils of *Pycnanthemum*, a North American endemic genus. In: Kirimer, N. and Mab, A., Eds., *Essential Oils*. Anadalou University, Eskisehir, Turkey, pp. 164–195.

Lawrence, B.M. (2004). Unpublished information.

Lazutka, J.R., Mierauskiene, J., Slapsyte, G., and Dedonyte, V. (2001). Genotoxicity of dill (*Anethum graveolens* L.), peppermint (*Mentha piperita* L.) and pine (*Pinus sylvestris* L.) essential oils in human lymphocytes and *Drosophila melanogaster*. *Food Chem. Toxicol*., 39, 485–492.

Leicester, R.J. and Hunt, R.H. (1982). Peppermint oil to reduce colonic spasm during endoscopy. *Lancet*, 2, 989–990.

Letizia, C.S., Cocchiara, J., Lalko, J., and Api, A.M., (2003). Fragrance material review on linalool. *Food Chem. Toxicol.*, 41, 943–964.

Leung, A.Y. (1980). Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics. John Wiley & Sons, New York, NY, pp. 231–234.

Lewis, E.J. and Friedrich, E.C. (1990). The essential oil of *Poliomintha incana. Planta Med.*, 56, 224–226.

Litton Bionetics, Inc. (1975). Mutagenic Evaluation of Compound FDA 71–57, menthol. Prepared for the U.S. Food and Drug Administration under contract No. FDA 71–57. Report dated January 14, 1975. PB-245 444. National Technical Information Service.

MacDougall, J.M., Fandrick, K., Zhang, X., Serafin, S.V., and Cashman, J.R. (2003). Inhibition of human liver microsomal (S)-nicotine oxidation by (−)-menthol and analogues. *Chem. Res. Toxicol.*, 8, 988–993.

Madyastha, K.M. and Gaikwad, N.W. (1998). Metabolic fate of S-(−)-pulegone in rat. *Xenobiotica*, 28, 723–734.

Madyastha, K.M. and Gaikwad, N.W. (1999). Metabolic disposition of a monoterpene ketone, piperitenone, in rats: evidence for the formation of known toxin, *p*-cresol. *Drug Metab. Dispos*., 27, 74–80.

Madyastha, K.M. and Moorthy, B. (1989). Pulegone mediated hepatotoxicity: evidence for covalent binding of R(+)-[^{14}C]pulegone to microsomal proteins *in vitro*. *Chem. Biol. Interactions*, 72, 325–333.

Madyastha, K.M. and Moorthy, B. (1990). (+)-Pulegone medicated hepatotoxicity in rat: role of microsomal cytochrome P-450. In: Reddy, C.C., Hamilton, G.A., and Madyastha, K.M., Eds., *Biological Oxidation Systems*, *Vol. 1*, Academic Press, Inc., San Diego, USA, pp. 115–131.

Madyastha, K.M. and Raj, C.P. (1990). Biotransformation of R-(+)-pulegone and menthofuran *in vitro*: chemical basis for toxicity. *Biochem. Biophys. Res. Commun.*, 173, 1086–1092.

Madyastha, K.M. and Raj, C.P. (1991). Evidence for the formation of a known toxin, *p*-cresol, from menthofuran. *Biochem. Biophys. Res. Commun*., 177, 440–446.

Madyastha, K.M. and Raj, P. (1992). Metabolic fate of menthofuran in rats: novel oxidative pathways. *Drug Metab. Dispos*., 20, 295–301.

Madyastha, K.M. and Raj, C.P. (1993). Studies on the metabolism of a monoterpene ketone R-(+)-pulegone—a hepatotoxin in rat: Isolation and characterization of new metabolites. *Xenobiotica*, 23, 509–518.

Madyastha, K.M. and Raj, C.P. (1994). Effects of menthofuran, a monoterpene furan on rat liver microsomal enzymes, *in vivo*. *Toxicology*, 89, 119–125.

Madyastha, K.M. and Raj, C.P. (2002). Stereoselective hydroxylation of 4-methyl-2-cyclohexenone in rats: its relevance to R-(+)-pulegone-mediated hepatotoxicity. *Biochem. Biophys. Res. Commun*., 297, 202–205.

Madyastha, K.M. and Srivatsan, V. (1988). Studies on the metabolism of 1-menthol in rats. *Drug Metab. Dispos.*, 16, 765–772.

Madyastha, P., Moorthy, B., Vaidyanathan, C.S., and Madyastha, K.M. (1985). *In vivo* and *in vitro* destruction of rat liver cytochrome P450 by a monoterpene ketone, pulegone. *Biochem. Biophys. Res. Comm*., 128, 921–927.

Maftei, M. (1992). Dry culinary herbs: an overview of selected Western European Markets. In: Verlet, N., Ed., *Compete Rendu des 3 émes Rencontres Techniques et Economiques Plantes Aromatiques et Médicinales*., C.F.P.P.A., Nyons, pp. 249–292.

Mahmoud, S.S. and Croteau, R.B. (2001). Metabolic engineering of essential oil yield and composition in mint by altering expression of deoxyxylulose phosphate reductoisomerase and menthofuran synthase. *Proc. Natl. Acad. Sci. USA*, 98, 8915–8920.

Mahmoud, S.S. and Croteau, R.B. (2003). Menthofuran regulates essential oil biosynthesis in peppermint by controlling a downstream monoterpene reductase. *Proc. Natl. Acad. Sci. USA*, 100, 114481–14486.

Maibach, H. and Patrick, E. (2001). Dermatotoxicology. In: Hayes, A.W., Ed., *Principles and Methods of Toxicology 4th edn*., Taylor & Francis, Philadelphia, PA, pp. 1039–1084.

Manabe, A., Nakayama, S., and Sakamoto, K. (1987). Effects of Essential Oils on Erythrocytes and Hepatocytes from Rats and Dipalmitoylphosphatidylcholine Liposomes. *Jap. J. Pharmacol*., 44, 77–84.

Marcus, C. and Lichtenstein, E.P. (1982). Interactions of naturally occurring food plant components with insecticides and pentobarbital in rats and mice. *J. Agric. Food Chem*., 30, 563–568.

Martin, D., Valdez, J., Boren, J., and Mayersohn, M. (2004). Dermal absorption of camphor, menthol, and methyl salicylate in humans. *J. Clin. Pharmacol*., 44, 1151–1157.

McClanahan, R.H., Thomassen, D., Slattery, J.T., and Nelson, S.D. (1989). Metabolic activation of (R)-(+)-pulegone to a reactive enonal that covalently binds to mouse liver proteins. *Chem. Res. Toxicol*., 2, 349–355.

McKenzie, J. and Gallacher, M. (1989). A sweet-smelling success: use of peppermint oil in helping patients accept their colostomies. *Nursing Times*, 85, 48–49.

McKemy, D.D., Neuhausser, W.M., and Julius, D. (2002). Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature*, 416, 52–58.

Mengs, U. and Stotzem, C.D. (1989). Toxicological evaluation of peppermint oil in rodents and dogs. *Med. Sci. Res*., 57, 499–500.

Merck Index (1989). An encyclopedia of chemicals, drugs and biologicals. Budavari, S., O'Neil, M.J., Smith, A., and Heckelman, P.E., Eds., Merck & Co., Inc., Rahway, NJ p. 916, 1263.

Micklefield, G.H., Greving, I., and May, B. (2000). Effects of peppermint oil and caraway oil on gastroduodenal motility. *Phytother. Res*., 14, 20–23.

Misra, R.C. and Kumar, J. (1983). Evaluation of *Mentha piperita* L. oil as a fumigant against red flour beetle, *Tribolium castaneum* (Herbst), *Ind. Perfum*., 27(2), 73–76.

Miyazawa, M., Watanabe, H., and Kameoka, H. (1997). Inhibition of acetylcholinesterase activity by monoterpenoids with a p-menthane skeleton. *J. Agric. Food Chem*., 45, 677–679.

Mizutani, T., Nomura, H., Nakanishi, K., and Fujita, S. (1987). Effects of drug metabolism modifiers on pulegone-induced hepatotoxicity in mice. *Res., Commun. Chem. Pathol. Pharmacol*., 58, 75–83.

Molck, A.-M., Poulsen, M., Lauridsen, S.T., and Olsen, P. (1998). Lack of histological cerebellar changes in Wistar rats given pulegone for 28 days. Comparison of immersion and perfusion tissue fixation. *Tox. Lett*., 95, 117–122.

Moldenke, H.N. and Moldenke, A.L. (1952). *Plants of the Bible*. Chronica Botanica Co. Waltham, MA, pp. 139–140.

Moorthy, B., Madyastha, P., and Madyastha, K.M. (1989). Hepatotoxicity of pulegone in rats: its effects on microsomal enzymes, *in vivo. Toxicology*, 55, 327–337.

Moreira, E.A. and Krambeck, R. (1976). Chromatographic analysis of essential oil from leaves of *Cunila angustifolia* Benth. Labiatae. *Trib. Farm. Curitiba*, 44(1), 50–59.

Morton, J.F. (1977). *Major Medicinal Plants Botany, Culture and Uses*. Charles C. Thomas Publ., Sprinsfield, IL, pp. 271–275.

Muhayimana, A., Chalchat, J-C., and Garry, R.-P. (1998). Chemical composition of essential oils of some medicinal plants from Rwanda. *J. Essent. Oil Res.*, 10, 251–259.

Muñoz, S., Collin, G.J., Gagnan, M., and Soriano Ferrufino, J. (1990). The essential oils of *Hedeona mandoniana* Wedd. and of *Minthostachys andina* (Brett.) Epling. *J. Essent. Oil Res.*, 2, 61–66.

Munro, I., Ford, R., Kennepohl, E., and Sprenger, J. (1996). Correlation of structural class with No-Observed-Effect-Levels: a proposal for establishing a threshold of concern. *Food Chem. Toxicol*., 34, 829–867.

Munroe, M. (2004). Personal correspondence.

Muschietti, L., Van Baren, C., Coussio, J., Vila, R., Clos, M., Cañigueral, S., and Adzet, T. (1996). Chemical composition of the leaf oils of *Satureja adora* and *Satureja pavifolia. J. Essent. Oil Res*., 8, 681–684.

Nahrstedt. A., Economou, D., and Hammerschmidt. F-J. (1985). The essential oil of *Bystropogon plumosus. Planta Med.*, 51, 247–249.

Naldi, L. (2002). The epidemiology of fragrance allergy: questions and needs. *Dermatology*, 205, 89–97.

Nath, D.R., Das, N.G., and Mulhotra, P.R. (1986). Efficacy of certain essential oils and insect repellents against land leeches. *Defense Sci. J.*, 36, 327–330.

National Institute for Occupational Safety and Health (NIOSH) (1983). National Occupational Exposure Survey Conducted from 1981 to 1983. Last updated July 1, 1990. <http://www.cdc.gov/noes/>. Accessed May 13, 2004.

National Toxicology Program (2005). Pulegone, CAS NO: 89–82–7. Last updated May 23, 2005. <http://www.ntp.niehs.nih.gov>.

NCI (1979). Bioassay of DL-Menthol for Possible Carcinogenicity. Technical Report No. 98. U.S. Department of Health, Education, and Welfare. Publication No. PB 288 761.

NCI (1993). Menthol and Other Isomers. NCI Contract No. N01-CP-05619 (5/93). pp. 1–31.

Nelson, S.D. (1995). Mechanisms of the formation and disposition of reactive metabolites that can cause liver injury. *Drug Metab. Rev*., 27(1–2), 147–177.

Nelson, S.D. and Trager, W.F. (2003). The use of deuterium isotope effects to probe the active site properties, mechanism of cytochrome P450-catalyzed reactions, and mechanism of metabolically dependent toxicity. *Drug Metab. Dispos.*, 31, 1481–1498.

Nelson, S.D., McClanahan, R.H., Thomassen, D., Gordon, W.P., and Knebel, N. (1992). Investigations of mechanisms of reactive metabolite formation from (R)-(+)-pulegone. *Xenobiotica*, 9–10, 1157–1164.

Ody, P. (1996). *Handbook of Over-the-Counter Herbal Medicines*. Kyle Cathie Ltd., London, U.K., pp. 480.

OECD (1992). *Guidelines for Testing of Chemicals*, Section 4: Health Effects. Guideline No. 406. Organization of Economic Cooperation and Development, Paris.

Ohsumi, T., Kuroki, K., Kimura, T. Murakami, Y. (1984). A study on acute toxicities of essential oils used in endodontic treatment. *J. Kyushu Dental Soc*., 38, 1064–1071.

Olsen, P. and Thorup, I. (1983). Neurotoxicity in Rats Dosed with Peppermint Oil and Pulegone. In: Chambers, P.L., Preziosi, P., and Chambers, C.M., Eds., *Disease, Metabolism and Reproduction in the Toxic Response to Drugs and Other Chemicals*., *Arch. Toxicol.*, 7(Supp), 408–409.

Olowe, S.A. and Ransome-Kati, O. (1980). The risk of jaundice in glucose-6-phosphate dehydrogenase deficient-babies exposed to menthol. *Acta. Paediat. Scand*., 69, 341–345.

Papachristos, D.P. and Stampoulos, D.C. (2002). Repellent toxic and reproduction inhibitory effects of essential oil vapors on *Acanthoscelides obtectus* Say (Coleoptera:Burchidac). *J. Stored Prod. Res*., 38, 117–128.

Parkinson, A. (2001). Biotransformation of Xenobiotics. In: Klaassen, C.D., Ed., *Casarett & Doull's Toxicology: The Basic Science of Poisons 6th edn*., McGraw-Hill, New York, NY, pp. 133–224.

Pathak, N., Mittal, P.K., Singh, O.P., Sagar, D.V., and Vasudevan, P. (2000). Lavicidal action of essential oils from plants against vector mosquitoes *Anopheles stephensi* (Liston), *Culex quinuefasciatus* (Say) and *Aedel aegypti* (L). *Int. Pest Control*, 42(2), 53–55.

Paustenbach, D.J. (2001). The Practice of Exposure Assessment. In: Hayes, A.W., Ed., *Principles and Methods of Toxicology 4th edn*., Taylor & Francis, Philadelphia, PA, pp. 387–448.

Pavlovic, S., Kuznjetsova, G.A., Zivanovic, P., Sevarda, A.L., Jancic, R., and Vujcic, S. (1984a). Quantity and structure of essential oil and some anatomical characteristics of the plant species *Acinos suaveolens* (Sibth. et Smith) G. Don f. *Arh. Farm. (Yugoslavia)*, 34, 65–71.

Pavlovic, S., Zivanovic, P., Jancic, R., Kuznjetsova, G.C. Vujcic, S., and Sevarda, A.L. (1984b). Contribution to a better knowledge of the species of *Acinos majoranifolius* (Mill.) Silic (Lamiaceae) from the Orjen Mountain as a new essential oil resource. *Arh. Farm. (Yugoslavia)*, 34, 27–33.

Perrucci, S., Macchioni, G., Cioni, P.L., Flamini, G., and Morelli, I. (1995). Structure/activity relationships of some natural monoterpenes as acaricides against *Psoroptes cuniculi. J. Nat. Prod*., 58, 1261–1264.

Philip Morris USA (2005). PM List of Ingredients located at <http://www.philipmorrisusa.com/en/productfacts/ingredients/tobacco_ingredients.asp>. Accessed August 26, 2005.

Pittler, M.H. and Ernst, E. (1998). Peppermint oil for irritable bowel syndrome: a critical review and metaanalysis. *Am. J. Gastroenterol*., 93, 1131–1135.

Powers, K.A. and Beasley, V.R. (1985). Toxicological aspects of linalool: a review. *Vet. Hum. Toxicol.*, 27, 484–486.

Puri, H.S. and Jain, S.P. (1988). *Micromeria capitellata* Benth. A new source of pulegone. *Parfum. Kosmet.*, 69(3), 163.

Rakieten, N., Rakieten, M.L., and Boykin, M. (1954). Effects of menthol vapor on the intact animal with special reference to the upper respiratory tract. *J. Am. Pharm. Assoc*., 43, 390–392.

Regnault-Roger, C. (1997). The potential of botanical essential oils for insect pest control. *Integrat. Pest Mgmt. Rev*., 2, 25–34.

Regnault-Roger, C. and Hamraoui, A. (1995). Fumigant toxic activity and reproductive inhibition induced by monoterpenes upon *Acanthoscelides obtectus* Say (Coleoptera) burchid of kidney bean (*Phaseolus vulgaris*). *J. Stored Prod. Res*., 31, 291–299.

Reynolds, R.J., (2005). RJR List of Ingredients (2004) located at <http://www.rjrt.com/smoking/ingredientscover.aspx>. Accessed August 26, 2005.

RIFM (1972). Unpublished report.

RIFM (2003). L-Menthol. Monograph 599. Updated August 28, 2003. RIFM-FEMA online database <http://rifm.org>. Accessed May 10, 2004.

RIFM (2004). Menthol. Monograph 5425. Updated February 20, 2004. RIFM-FEMA online database <http://rifm.org>. Accessed Nov. 8, 2004.

RIFM (2004a). L-Carvone. Monograph 195. Updated November 6, 2004.

RIFM (2004b). *Mentha arvensis*. Monograph 509. Updated September 12, 2004.

RIFM (2004c). Spearmint Oil. Monograph 771. Updated November 6, 2004.

Robbers, J.E. and Tyler, V.E. (1999). *Tyler's Herbs of Choice. The Therapeutic Use of Phytomedicinals*. The Haworth Herbal Press, New York, pp. 67–68, pp. 116–117, p. 214.

Roe, F.J.C., Palmer, A.K., Worden, A.N., and VanAbbe, N.J. (1979). Safety evaluation of toothpaste containing chloroform. I. Long term studies in mice. *J. Environ. Pathol. Toxicol.*, 2, 799–819.

Roemer, E., Tewes, F.J., Meisgen, T.J., Veltel, D.J., and Carmines, E.L. (2002). Evaluation of the Potential Effects of Ingredients Added to Cigarettes. Part 3: *In vitro* Genotoxicity and Cytotoxicity. *Food Chem. Toxicol.*, 40, 105–11.

Rojas, L.B. and Usubillaga, A.N. (1995). Essential oil of *Minthostachys mollis* Grisebach from Venezuela. *J. Essent. Oil Res.*, 7, 211–213.

Rosenkranz, H.S., Zhang, Y.P., and Klopman, G. (1998). Studies on the potential for genotoxic carcinogenicity of fragrances and other chemicals. *Food Chem. Toxicol.*, 36, 697–696.

Rustaiyan, A., Feizbakhsh, A. Masoudi, S., and Ameri, N. (2004). Comparison of the volatile oils of *Satureja atropatana* Bung. and *Satureja mutica* Fisch. et., C.A. May from Iran. *J. Essent. Oil Res.*, 16, 594–596.

Sangwan, N.K., Verma, B.S., Verma, K.K., and Dhindsa, K.S. (1990). Nematicidal activity of some essential plant oils. *Pestic. Sci.*, 28, 331–335.

Saxena, B.P. and Koul, O. (1978). Utilization of essential oils for insect control. *Ind. Perfum.*, 22(3), 139–149.

Schauenberg, P. and Paris, F. (1977). *Guide to Medicinal Plants*. Translation M. Pugh Jones, Keats Publ., Inc., New Cannan, CT, p. 245.

Senatore, F. (1998). Volatile constituents of *Minthostachys setosa* (Briq.) Epl. (Lamiaceae) from Peru. *Flav. Fragr, J.*, 13, 263–265.

Seto, S. (1987). Manufacture of pet collars containing pest repellents. Daiichi Yakka Co., Ltd., Japan, JP. 87–142137–870609.

Sezik, E. and Tümen, G. (1986). Phytochemical investigation on the plants used as folk medicine and herbal tea in Turkey. Part III. *Ziziphora taurica* M. Bieb. ssp. *tauria* J.R. Edmonson, Doga. (C), pp. 59–64.

Sezik, E., Tümen, G., and Baser, K.H.C. (1991). *Ziziphora tenuior* L., a new source of pulegone. *Flav. Fragr. J.*, 6, 101–103.

Shu, C.-K., Lawrence, B.M., and Miller, K.L. (1994). Chemical composition of the essential oil of *Pycnanthemum floridanum*. E. Grant and Epling. *J. Essent. Oil Res.*, 6, 529–331.

Singh, M., Srivastava, S., Srivastava, R.P., and Chauhan, S.S. (1995). Effect of Japanese mint (*Mentha arvensis*) oil as fumigant on nutritional quality of stored sorghum. *Plant Foods Human Nutrit.*, 47, 109–114.

Smith, R.L., Cohen, S.M., Doull, J., Feron, V.J., Goodman, J.I., Marnett, L.J., Portoghese, P.S., Waddell, W.J., Wagner, B.M., Hall, R.L., Higley, N.A., Lucas-Gavin, C., and Adams, T.B. (2005a). A procedure for the safety evaluation of natural flavor complexes used as ingredients in food: Essential oils. *Food Chem. Toxicol.*, 43, 345–363.

Smith, R.L., Cohen, S.M., Doull, J., Feron, V.J., Goodman, J.I., Marnett, L.J., Portoghese, P.S., Waddell, W.J., Wagner, B.M., and Adams, T.B. (2005b). GRAS flavoring substances 22. *Food Technol.*, 59(8), 24–62.

Soulélès, C. and Katsiotis, S. (1988). Contribution à L'étude de l'huile essentielle *d'Acinos suaveolens* (Lam.) Dandy. *Plant. Med. Phytother.*, 22, 180–183.

Spindler, P., and Madsen, C. (1992). Subchronic toxicity study of peppermint oil in rats. *Toxicol. Lett.*, 62, 215–220.

Stanic, G., Kalogjera, Z., and Petricic, J. (1988). Essential content and composition of *Micromeria croatica* (Pers.) Schott and *Micromeria thymifolia* (Scop.) Fritsch. *Acta Pharm. Jugosl.*, 38, 251–254.

Steiling, W., Basketter, D., Berthold, K., Butler, M., Garrigue, J.L., Kimber, I., Lea, L., Newsome, C., Roggeband, R., Stropp, G., Waterman, S., and Wiemann, (2001). Skin sensitization testing-new perspectives and recommendations. *Food Chem. Toxicol.*, 39, 293–301.

Stojanovic, G., Palic, I., Ursic-Jankovic, J., Vajs, V., and Dokovic, D. (1999). Chemical composition of the essential oil of *Micromeria albanica* (Griseb. ex. K. Maly) Silic. *J. Essent. Oil Res.*, 11, 785–787.

Sudekum, M., Poppinga, R.H., Raju, N., and Braselton. W.E. (1992). Pennyroyal oil toxicosis in a dog. *J. Am. Vet. Med. Assoc.*, 200, 817–818.

Sullivan, J.B., Rumack, B.H., Thomas, H., Peterson, R.G., and Bryson, P. (1979). Pennyroyal oil poisoning and hepatotoxicity. *J. Am. Med. Assoc.*, 242, 2873–2874.

Sztajnkrycer, M.D., Otten, E.J., Bond, G.R., Lindsell, C.J., and Goetz, R.J. (2003). Mitigation of pennyroyal oil hepatotoxicity in the mouse. *Acad. Emergen. Med.*, 10, 1024–1028.

Tanowitz. B.D., Smith, D.M., and Junak, S.A. (1987). Terpenoid constituents of three taxa of Monardella. *Phytochemistry*, 26, 2751–2752.

Thomas, H.K. (1962). Peppermint Oils and Menthol. A Monograph on Peppermint Oils and Menthol by Dragoco. Dragoco, Holzminden, Bouveret, Switzerland, p. 11.

Thomassen, D., Slattery, J.T., and Nelson, S.D. (1988). Contribution of menthofuran to the hepatotoxicity of pulegone: assessment based on matched area under the curve and on matched time course. *J. Pharmcol. Exp. Therap.*, 244, 825–829.

Thomassen, D., Slattery, J.T., and Nelson, S.D. (1990). Menthofuran-dependent and independent aspects of pulegone hepatotoxicity: roles of glutathione. *J. Pharmacol. Exp. Therap.*, 253, 567–572.

Thomassen, D., Pearson, P.G., Slattery, J.T., and Nelson, S.D. (1991). Partial characterization of biliary metabolites of pulegone by tandem mass spectrometry. Detection of glucuronide, glutathione, and glutathionyl glucuronide conjugates. *Drug Metab. Dispos.*, 19, 997–1003.

Thomassen, D., Knebel, N., Slattery, J.T., McClanahan, R.H., and Nelson, S.D. (1992). Reactive intermediates in the oxidation of menthofuran by cytochromes P-450. *Chem. Res. Toxicol.*, 5, 123–130.

Thorup, I., Würtzen, G., Carstensen, J., and Olsen, P. (1983a). Short Term Toxicity Study in Rats Dosed with Pulegone and Menthol. *Toxicol. Lett.*, 19, 207–210.

Thorup, I., Wurtzen, G., Carstensen, J., and Olsen, P. (1983b). Shtort term toxicity study in rats dosed with peppermint oil. *Toxicol. Lett.*, 19, 211–215.

Thulasiram, H.V., Gadad, A.K., and Madyastha, M.K. (2000). Role of C-5 chiral center in *R*-(+)-pulegone-mediated hepatotoxicity: metabolic disposition and toxicity of 5,5-dimethyl-2-(1-methylethylidene)-cyclohexanone in rats. *Drug Metab. Dispos.*, 28, 833–844.

Thulasiram, H.V., Bhat, V.B., and Madyastha, M.K. (2001). Effect of ring size in *R*-(+)-pulegone-mediated hepatotoxicity: studies on the metabolism of *R*-(+)-4-methyl-2-(1-methylethylidene)-cyclopentanone and DL-camphorone in rats. *Drug Metab. Dispos.*, 29, 821–829.

Tisserand, R. and Balacs, T. (1995). *Essential Oil Safety*. Churchill Livingstone, London, U.K.

Tripathi, A.K., Prajapati, V., Aggarwal, K.K., and Kumar, S. (2000). Effect of volatile oil constituents of *Mentha* species against the stored grain pests, *Callosobruchus maculatus* and *Tribolium castaneum*. *J. Med. Aromat. Plant Sci.*, 22, 549–556.

Tucker, A.O. and Maciarello, M.J. (1991). The essential oil of *Calamintha arkansana* (Nutt.) Shinners. *J. Essent. Oil Res.*, 3, 125–126.

Tucker, A.O., Maciarello, M.J., and McCrory, D. (1992). The essential oil of *Micromeria brownei* (Swartz) Benth. var. *pilosiuscula* Gray. *J. Essent. Oil Res.*, 4, 301–302.

Tucker, A.O., Maciarello, M.J., and Libbey, L.M. (2000). Essential oil of *Satureja viminea* L. (Lamiaceae). *J. Essent. Oil Res.*, 12, 283–284.

Turgeon, D., Carrier, J.-S., Chouinard, S., and Belanger, A. (2003). Glucuronidation activity of the UGT2B17 enzyme toward xenobiotics. *Drug Metab. Dispos.*, 31, 670–676.

U.S. Pharmacopoeia (1995).

Vadiraja, B.B., Gaikwad, N.W., and Madyastha, K.M. (1998). Hepatoprotective effect of C-phycocyanin: protection for carbon tetrachloride and R-(+)-pulegone-mediated hepatotoxicity in rats. *Biochem Biophys. Res. Commun.*, 249, 428–431.

Valnet, J. (1982). *The Practice of Aromatherapy*. Tisserand, R., Ed., Healing Arts Press, Rochester, VT, p. 213, pp. 247–250.

Vanscheeuwijck, P.M., Teredesai, A., Terpstra, P.M., Verbeeck, J., Kuhl, P., Gerstenberg, B., Gebel, S., and Carmines, E.L. (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 4: Subchronic inhalation toxicity. *Food Chem. Toxicol.*, 40, 113–131.

Velasco-Neguereuela, A. and Mata Rico, M. (1986). The volatile oil of Ziziphora hispanica L. *Flav. Fragr. J.*, 1, 111–113.

Velasco-Neguereuela, A., Pérez-Alonso, M.J., and Mata Rico, M. (1987). Essential oils of Iberian Lamiaceae with pulegone as a fundamental component. *Anal. Bromatol.*, 39, 357–372.

Velasco-Negueruela, A., Pérez-Alonso, M.J., and Pérez de Paz, P.L. (1992). The volatiles of two *Bystropogon* species from the Canary Islands (Spain). *Planta Med.*, 58, 461–463.

Velasco-Negueruela, A., Pérez-Alonso, M.J., Esteban, J.L., Garcia Vallejo, M.C., Zygadlo, J.A., Guzmán, C.A., and Ariza-Espinar, L. (1996). Essential oils of *Calamintha nepeta* (L.) Savi and *Mentha* aff. *suaveolens* Ehrh., grown in Córdoba, Argentina. *J. Essent. Oil Res.*, 8, 81–84.

Viana, F., dela Peña, E., and Belmonte, C. (2002). Specificity of cold transduction is determined by differential ionic channel expression. *Nat. Neurosci.*, 5, 254–260.

Viaseca, A., Guy, I., Charles, B., Guinaudeau, H., Rojas de Arias, A., and Fournet, A. (2004). Chemical composition and insecticidal activity of *Hedeoma mandoniana* Wedd. essential oils. *J. Essent. Oil Res.*, 16, 380–383.

Vila, R., Milo, B., Labbé, C., Muñoz, O., Urrunaga Soria, E., and Urrunago Sorria, R. (1996). Chemical composition of two samples of essential oil of *Satureja boliviana* (Benth.) Briq. from Peru and Bolivia. *J. Essent. Oil Res.*, 8, 307–309.

VonSkramlik, E. (1959). On the toxicity and compatibility of essential oils. *Die Pharmazie*, 14, 435–445.

Walker, G.T. (1967). Menthol. properties, uses and some methods of manufacture. *Mfg. Chemist Aerosol News.*, (March), 51–53.

Watson, H.R., Helms, R., Rowsell, D.G., and Spring, D.J. (1978). New compounds with the menthol cooling effect. *J. Soc. Cosmet. Chem.*, 29, 185–2000.

Wichtl, M. (1994). *Herbal Drugs and Phytopharmaceuticals*. N.G. Bissett (translator), CRC Press, Boca Raton, FL, pp. 333–335.

Williams, G.M. and Iatropoulos, J.M. (2001). Principles of Testing for Carcinogenic Activity. In A.W. Hayes., Ed., *Principles and Methods of Toxicology 4th edn.*, Taylor & Francis, Philadelphia, PA, pp. 959–1000.

Wilson, N.H., Hardisty, J.F., and Hayes, J. R. (2001). Short-term, Subchronic, and Chronic Toxicology Studies. In: Hayes, A.W., Ed., *Principles and Methods of Toxicology 4th edn.*, Taylor & Francis, Philadelphia, PA, pp. 917–957.

Wren, R.C. (1989). Potter's New Cyclopaedia of Botanical Drugs and Preparations rewritten by Williamson, E.M. and Evans, F.J. C.W. Daniel Co., Ltd., Saffron Walden, U.K., p. 255.

Yamaguchi, T., Caldwell, J., and Farmer, P.B. (1994). Metabolic fate of [3H]-1-menthol in the rat. *Drug Metab. Dispos.*, 22, 616–624.

Zimmermann, F.K., Scheel, I., and Resnick, M.A. (1989). Induction of chromosome loss by mixtures of organic solvents including neurotoxins. *Mutation Res.*, 224, 287–303.

13 Antimicrobial Activity of Essential Oils and Constituents of *Mentha* Species

Stanley G. Deans

CONTENTS

13.1 INTRODUCTION

The preservative properties of aromatic and medicinal plant essential (volatile) oils and extracts have been recognized since Biblical times, while attempts to characterize these properties in the laboratory date back to the early 1900s (Martindale, 1910; Hoffman and Evans, 1911).

Traditionally, plants and their isolated extracts, including *Mentha* species, have been used to extend the shelf life of foods, beverages, and pharmaceutical–cosmetic products, and they also have a plant protection function through their antimicrobial and antioxidant properties (Ueda et al., 1982; Jay and Rivers, 1984; Shelef, 1984; Gallardo et al., 1987; Janssen et al., 1988; Shapiro et al., 1994; Cai and Wu, 1996; Baratta et al., 1998a, 1998b).

More recently, attempts have been made to identify the component(s) responsible for such bioactivities (Deans and Ritchie, 1987; Vokou et al., 1993; Lis-Balchin and Deans, 1997; Lis-Balchin et al., 1998; Dorman and Deans, 2000). The antimicrobial properties of essential oils and their constituents from a wide variety of plants have been assessed and reviewed

(Larrondo et al., 1995). Investigations into the antimicrobial activities, mode of action, and potential uses of plant volatile oils have regained momentum and there appears to be a revival in the use of traditional approaches to livestock welfare and food preservation (Franz, personal communication). The activity of the oils would be expected to relate to and reflect the respective composition of the essential oils, the structural configuration of the constituent components, and their functional groups along with potential synergistic interactions between components. In the study by Dorman and Deans (2000), a correlation of the antimicrobial activity of compounds under test, and their relative percentage composition in the oils, with their chemical structure, functional groups, and configuration, suggested a number of observations on the structure–function relationship.

Components with phenolic structures, such as carvacrol and thymol, were highly active against test bacteria, despite their low capacity to dissolve in water. The importance of the hydroxyl group in the phenolic ring was confirmed in terms of activity when carvacrol was compared with its methyl ester. The high activity of the phenolic components may be further explained in terms of the alkyl substitution into the phenolic nucleus, which is known to enhance the antimicrobial activity of phenols (Pelczar et al., 1988). The introduction of alkylation has been proposed to alter the distribution ratio between the aqueous and nonaqueous phases, including bacterial phases, by reducing the surface tension or altering the species selectivity. Alkyl-substituted phenolic compounds form phenoxyl radicals that interact with isomeric alkyl substituents (Pauli and Knobloch, 1987). This does not occur with etherified–esterified isomeric molecules, possibly explaining their relative lack of activity.

The presence of an acetate moiety in the structure appears to increase the activity of the parent compound as in the case of geraniol where geranyl acetate demonstrated an increase in activity. Aldehydes, notably formaldehyde and glutaraldehyde, are known to possess powerful antimicrobial activity. It has been suggested that an aldehyde group conjugated to a carbon=carbon double bond is a highly electronegative arrangement, which may explain their activity (Moleyar and Narasimham, 1986), suggesting that an increase in electronegativity increases the antibacterial activity (Kurita et al., 1979, 1981). Such electronegative compounds may interfere in biological processes involving electron transfer and react with vital nitrogen components such as proteins and nucleic acids, and therefore inhibit the growth of microorganisms. The citral aldehydes *cis*-(neral) and *trans*-(geranial) displayed moderate activity against test bacteria while citronellal was less active. Alcohols are known to possess bactericidal rather than bacteriostatic activity against vegetative bacterial cells. The terpenoid alcohols studied did show some activity against test bacteria, acting as protein denaturing agents, solvents, or dehydrating agents.

A number of oil components are ketones, wherein the presence of an oxygen function in the framework increases the antimicrobial properties of the terpenoids (Naigre et al., 1996). From this study, it was detected that by using the contact method, the bacteriostatic and fungistatic action of terpenoids was increased when carbonylated. Menthone was shown to have modest activity, with *Clostridium sporogenes* and *Staphylococcus aureus* being the most significantly affected (Dorman and Deans, 2000). An increase in activity dependent on the type of alkyl substituent incorporated into a nonphenolic ring structure appeared to occur in this study. An alkenyl substituent (1-methylethenyl) resulted in increased antibacterial activity, as seen in limonene (1-methyl-4-(1-methylethenyl)-cyclohexene), compared with an alkyl (1-methylethyl) substituent as in *p*-cymene (1-methyl-4-(1-methylethyl)-benzene). The inclusion of a double bond increased the activity of limonene relative to *p*-cymene, which demonstrated no activity against the test bacteria. In addition, the susceptible organisms were principally Gram-negative, which suggests that alkylation influences Gram-reaction sensitivity of the bacteria. The importance of the antimicrobial activity of alkylated phenols in relation to phenol has been previously reported

(Pelczar et al., 1988). Their data suggest that an allylic side chain seems to enhance the inhibitory effects of a component and chiefly against Gram-negative organisms.

The stereochemistry, furthermore, has an influence on bioactivity. It was observed that α-isomers are inactive relative to β-isomers, for example, α-pinene; *cis*-isomers are inactive contrary to *trans*-isomers, for example, geraniol and nerol; compounds with methyl–isopropyl cyclohexane rings are most active; or unsaturation of the cyclohexane ring further increases the antibacterial activity, for example, α-terpinene, α-terpineol, and terpinolene (Hinou et al., 1989).

Investigations into the effects of terpenoids on isolated bacterial membranes suggest that their activity is a function of the lipophilic properties of the constituent terpenes, the potency of their functional groups, and their aqueous solubility. Their site of action appeared to be at the phospholipid bilayer, caused by biochemical mechanisms catalyzed by these phospholipid bilayers of the cell. These processes include the inhibition of electron transport, protein translocation, phosphorylation steps, and other enzyme-dependent reactions. Their activity in whole cells appears more complex. Although a similar water-soluble tendency is observed, specific statements on the action of single terpenoids *in vivo* have to be assessed singularly, taking into account not only the structure of the terpenoid, but also the chemical structure of the cell wall (Knobloch et al., 1986, 1988). The plant extracts clearly demonstrate antibacterial properties, although the mechanistic processes are poorly understood.

13.2 ANTIMICROBIAL PROPERTIES OF *MENTHA*

The number of different measurements of microbial growth inhibition makes for difficult and often impossible comparisons to be drawn. Even the choice of growth medium (solid or liquid) has a profound impact on inhibitory profiles. There seems to be no concerted desire for those working in the field to adopt a simple uniform assay for measurement thereby allowing direct comparison of results (Janssen et al., 1987; Sivropoulou et al., 1995; Griffin et al., 1999; Inouye et al., 2001). Only then would it become apparent that differences in plant cultivation methods including fertilizer-application regimes, geographical location and attendant climatic factors, genetic factors, and other variables can affect chemical composition and hence bioactivity. Some of these issues will be discussed later.

13.3 MRSA AND VRE

The need to combat these serious secondary pathogens was highlighted by Nelson (1997) who showed the efficacy of peppermint oil in controlling growth (MIC and MBC) of MRSA at concentrations of 0.5% (v/v). Vancomycin-resistant *Enterococcus faecium* (VRE) required 0.5% to 1.0% to achieve MIC_{50} and MIC_{90}, while 0.5% to 2.0% was required to realize MBC_{50} and MBC_{90} values. The MBC was defined as the lowest concentration that reduced the original inoculums by $\geq$99.9% after overnight aerobic incubation at 37°C.

13.4 ENTEROHEMORRHAGIC *Escherichia coli*

Osawa et al. (1999) undertook a detailed investigation into the antibacterial activities of peppermint oil and a number of its constituents. The findings are summarized in Table 13.1 and show that the greatest inhibition was caused by the compounds neomenthol and carvacrol, both recording MIC and MBC values of 200 μg/mL.

Imai et al. (2001), in a subsequent investigation, examined the impact of cornmint, peppermint, and spearmint on the growth of *E. coli* O157:H7, *Salmonella enteritidis*, antibiotic-resistant and sensitive strains of *Helicobacter pylori* as well as methicillin-resistant and

TABLE 13.1
MIC and MBC Values (μg/mL) of Peppermint Oil and Its Constituents against *E. coli* O157:H7

Test Material	MIC Value	MBC Value
Peppermint oil	400	400
Menthol	400	400
Menthone	400	400
Neomenthol	200	200
Menthofuran	>800	>800
(+)-Limonene	>800	>800
Piperitone	>800	>800
3-Octanol	800	800
cis-Jasmone	800	800
Mint lactone	800	800
(−)-Myrtenol	800	800
Piperitol	800	800
Eugenol	800	800
Carvacrol	200	200
2-Ethylfuran	800	>800
Ocimene	>800	>800

Source: After Osawa, K., Saeki, T., Yasuda, H., Hamashima, H., Sasatsu, M., and Arai, T., *Escherichia coli Biocontrol Sci.*, 4, 1–7, 1999. With permission.

methicillin-sensitive *Staph. aureus*. The MIC of cornmint, peppermint, and spearmint were 50 to 100 μg/mL against *H. pylori*, 400 to 800 μg/mL against *S. enteritidis* and *E. coli* O157:H7, and 200 to 400 μg/mL against *Staph. aureus*, respectively. The authors stated that the antibacterial activity is bactericidal, rather than merely growth inhibitory, against all the test microorganisms. Moreover, L-limonene and myrcene, two of the main constituents of native spearmint oil, other than L-carvone, were the main cause of activity against *H. pylori*.

The MBC_{90} values were determined for *M. pulegium* and spearmint against a range of bacteria isolated from cured meat intended for human consumption. The results show a wide variation not only between the different genera, but also within species (Table 13.2).

Singh et al. (1998) demonstrated antibacterial activity by peppermint and spearmint against several Gram-positive and Gram-negative bacteria by measurement of the zone of inhibition (mm diameter) around a paper disk containing known weights of oils. Neat oil and oil diluted to 1:50, 1:100, and 1:200 were tested, and all neat oils and most 1:50 dilutions gave inhibition zones against *Corynebacterium diphtheriae* (peppermint 12, 7; spearmint 10, 0, respectively), *Staph. aureus* (31, 12; 19, 14), *Bacillus subtilis* (18, 13; 19, 12), *Strep. haemolyticus* (32, 18; 23, 18), *Ps. aeruginosa* (10, 10; 12, 10), *E. coli* (19, 8; 24, 19), *Klebsiella* sp. (16, 9; 21, 14), and *Proteus vulgaris* (12, 10; 18, 12). Peppermint was more active against the Gram-positive organisms, while spearmint, overall, was more active against the Gram-negative bacteria. Dilutions beyond 1:50 gave variable results, with no clear pattern emerging in respect of inhibition based on Gram reaction. Similar results were reported by Agarkar and Garode (2002).

Oil dilutions were also used to determine the antimicrobial efficacy of wild *Mentha* species and ecotypes from southern Turkey against *Staph. aureus*, *E. coli*, and *Enterococcus faecalis*. All oils at concentrations >10 μL exhibited strong antimicrobial activity against *E. faecalis*,

TABLE 13.2
MBC_{90} Values (mg/mL) for *M. pulegium* and *M. spicata*

	MBC_{90} Values	
Bacteria	***M. pulegium***	***M. spicata***
Enterobacter sp.	14.7	27.5
Enterobacter aerogenes	29.6	3.4
Enterobacter agglomerans	7.4	3.4
Escherichia coli I	14.7	6.9
Escherichia coli II	1.8	3.4
Escherichia coli III	3.7	3.4
Escherichia coli IV	7.4	27.5
Escherichia coli V	7.4	6.9
Citrobacter freundii I	29.6	13.8
Citrobacter freundii II	7.4	17.5
Citrobacter freundii III	7.4	6.9
Proteus vulgaris I	29.6	3.4
Proteus vulgaris II	29.6	6.9
Hafnia alvei I	3.7	13.8
Hafnia alvei II	3.7	13.8
Klebsiella pneumoniae	14.7	6.9

Source: After Montes, M.A., Wilkomirsky, T., and Bello, H., *Fitoterapia,* 69, 170–172, (1998). With permission.

except two; a similar pattern was seen against *Staph. aureus* where the same two ecotypes of *Mentha longifolia* were less effective (Özgüven et al., 1998).

One of the earlier comprehensive evaluations of antimicrobial activity in essential oil constituents was carried out by Morris et al. (1979), using *E. coli*, *Staph. aureus*, *Candida albicans*, and a diphtheroid (probably of the genus *Corynebacterium*). This study did not use multiple resistant strains of either *E. coli* or *Staph. aureus*, and therefore has less relevance to today's problem bacteria. In addition, there are considerable differences in the level of antimicrobial activity between this study and those that followed.

Measurement of impedance was chosen by Marino et al. (2001) as the most sensitive method of assessing the inhibitory effect of peppermint oil on the growth of *E. coli* O157:H7 and other bacteria. The technique detects changes in conductance caused by bacterial metabolism in the growth medium. The researchers chose the detection time (DT) as the parameter for defining and quantifying the antibacterial activity of the oil. DT is defined as the time required by the microbial population to reach the concentration, defined as the ''threshold quantity,'' causing a rapid deviation in the curve of the percentage electrical variation. For given experimental conditions, DT is a function of the initial microbial concentration, microbial generation time, and the length of the lag phase. Table 13.3 shows the impact of peppermint oil on the DT values of a number of bacteria, including *E. coli* O157:H7.

The increase in DT values for *E. coli* O157:H7 was by a factor of 4.3, while *Y. enterocolitica* was increased by a factor of >8 and *Staph. aureus* by >9. This technique highlights the variability in the susceptibility to peppermint oil, and it would be useful to repeat the experiments with the individual oil constituents to elucidate the most inhibitory compounds present in the oil.

TABLE 13.3
DT Values (h) of Bacteria in the Presence of *Mentha piperita* at a Concentration of 800 ppm

Bacterium	Control DT Values	Peppermint Oil DT Values
Escherichia coli	3.6	6.2
Escherichia coli O157:H7	4.1	18.7
Proteus mirabilis	3.2	10.7
Proteus vulgaris	3.7	22.0
Salmonella typhimurium	3.4	14.2
Serratia marcescens	3.4	7.2
Yersinia enterocolitica	5.5	45.6
Pseudomonas fluorescens	11.1	24.8
Pseudomonas putida	8.6	27.0
Micrococcus sp.	7.1	25.5
Sarcina flava	14.2	28.4
Staphylococcus aureus	4.2	38.3
Bacillus licheniformis	5.7	34.4
Bacillus thuringiensis	3.6	45.0
Lactobacillus innocua	5.1	17.4

Source: After Marino, M., Bersani, C., and Comi, G., *Int. J. Food. Microbiol.*, 67, 187–195, 2001. With permission.

13.5 FOOD-POISONING BACTERIA

The marked preservative action of numerous herbs and spices has recently received increased attention. Included in this interest is peppermint, and several research papers report on the ability of peppermint oil, oil constituents, and other extracts to inhibit the growth of food-poisoning bacteria. A study by Aktuğ and Karapinar (1986) demonstrated this effect with *Salmonella typhi*, *Staph. aureus*, and *Vibrio parahaemolyticus*. Using alcohol extracts rather than essential oil, they reported that only at concentrations >4000 ppm was there any reduction in growth. Interestingly, at concentrations <2000 ppm, there was as abundant growth as in the controls. It was not until the concentrations were raised to 6000 ppm that the growth was stopped with *V. parahaemolyticus*, and only at the maximum tested level of 8000 ppm was the growth of *S. typhi* and *Staph. aureus* halted.

This study also examined the impact on bacterial growth of the incorporation of ground peppermint into the growth medium. The results were reported as mint concentration (%), making comparison with the alcohol extracts difficult. However, converting the percentage values to ppm, it would appear that the ground mint was more effective at inhibiting all three bacteria. The results showed *Staph. aureus* to be the most susceptible (1000 ppm) followed by *V. parahaemolyticus* (5000 ppm) then *S. typhi* (20,000 ppm). That no peppermint oil was used leaves open the question as to how the current results would have compared with the inhibition profile of the oil. Tkachenko et al. (1999) reported only modest activity against *Staph. aureus* while showing allergenic reactions following skin scarification tests, while Arakawa and Osawa (2000) reported inhibition of passive cutaneous anaphylaxis by intraperitoneal injection of peppermint oil.

The foodborne pathogens *S. enteritidis* and *Listeria monocytogenes* were exposed to peppermint oil in culture media and three model food systems: tzatzik (pH 4.5), taramosalata (pH 5.0), and pâté (pH 6.8) (Tassou et al., 1995). Growth was monitored with a Malthus 2000 growth analyzer, whereby the measurement of conductance gave an indication of bacterial

metabolism. Several studies have shown a decreased level of inhibition by essential oils when evaluated *in vivo*. This has been attributed to decreases in water content (a_w) and increases in protein and fat levels, allowing a degree of protection to the bacteria that are not available in laboratory media. In these experiments, *S. enteritidis* was comprehensively inhibited in all three model systems. *Listeria monocytogenes* populations showed a decline toward the end of the storage period (8 to 9 days), but increased in pâté. The antibacterial action of peppermint oil depended on a number of factors including concentration, food pH, composition, storage temperature, and the nature of the microorganism.

In a subsequent paper, Tassou et al. (2000) reported on the effect of peppermint oil on the growth and toxin production of *Staph. aureus* and growth of *S. enteritidis*, again measuring changes in conductance. The total viable counts were reduced by 6 to 7 logs for *Staph. aureus* but only 3 log cycles for *S. enteritidis*. Enterotoxin B production was markedly reduced from a titer value of 40,000 in the control to 0.4 at oil concentrations of 0.1% to 0.3% (v/v) and was not detected at oil concentration of 1.9% (v/v). These experiments were carried out solely using nutrient broth. Attempts were made to correlate the antimicrobial effect with individual classes of compounds within the oil. It has been established that essential oils and phenolics do not have the same mechanism of action and there may be several targets leading to bacterial inhibition. Much of the studies on the mechanism of phenolic compounds center on their effects on cellular membranes (Davidson, 1997). Phenolics not only attack the cytoplasmic membrane, thereby destroying its permeability, but also cause the release of intracellular constituents and cause membrane dysfunction in respect of electron transport, nutrient uptake, nucleic acid synthesis, and ATPase activity.

In general, essential oils cause damage to biological membranes due to their lipophilic properties; however, specific functional groups as noted earlier, are also effective. Hence, the presence of menthol (an alcohol) and menthone (a ketone) resulted in reduction of the relative respiratory activities of whole cells of the Gram-negative bacterium *Rhodopseudomonas sphaeroides* by 74% (menthol) and the relative antibacterial activity by ~50% (menthol) or ~75% (menthone) (Knobloch et al., 1989). Griffin et al. (1999, 2001) showed that the outer membrane was important for the resistance of *Ps. aeruginosa* and *E. coli* to a number of monoterpenes. However, they also demonstrated that even active compounds are not completely unaffected by the protective mechanism the outer membrane provides and that the degree to which this mechanism is effective is highly susceptible to small changes in molecular structure and the resulting molecular properties. What these subtle molecular changes mean in terms of compound-cell interactions and subsequent death remains to be elucidated.

El-Kady et al. (1993) exposed several Gram-positive and Gram-negative bacteria to peppermint oil. The Gram-positive organisms were more inhibited than the Gram-negative, with *Staph. aureus* showing the greatest inhibition. *Klebsiella pneumoniae*, *Ps. pyocyanea*, and *Ps. fluorescens* showed no inhibition. Piccaglia et al. (1993) examined the activity of peppermint oil against a set of 25 test bacteria, with half (13) of the genera showing growth inhibition. In these experiments, *Aeromonas hydrophila* was the most sensitive. In a separate study dealing solely with *Aeromonas* species, Zazki et al. (2001) showed that at oil concentrations >12,500 μg/mL, all isolates were inhibited.

Three types of mint, cornmint, peppermint, and spearmint (ex. *M. spicata*) oils were investigated for antibacterial action against a collection of 10 bacteria. Cornmint was most effective against *Sarcina lutea* (18-mm diameter zone of inhibition), *E. carotovora* (10-mm zone), and *Ps. solanacearum* (10-mm zone); peppermint against *Ps. solanacearum* (9-mm zone) and *Staph. albus* (9-mm zone); spearmint against *Staph. albus* (20 mm). Even closely related mint species can have marked differences in their antibacterial properties (Rao and Nigam, 1978). The choice of test bacteria is not explained, as these are not the normal pathogen or secondary infection types usually associated with such bioassays.

Of the four species of mint studied by Diaz et al. (1988), H_2O, ethanol, and diethyl ether extracts (but no essential oil) from *M. gracilis* (ex. *M. gentilis*) were most active against *B. subtilis* and *Micrococcus luteus* but inactive against *E. coli*; *M. longifolia* was less active with only the ethanol and diethyl ether extracts showing activity; *M. suaveolens* ethanol extract showed slight activity toward *B. subtilis* while *M. pulegium* was completely inactive in all the bioassays. This was in contrast to the findings of Thoppil et al. (2001) who reported activity against *Bacillus* sp., *E. coli*, *Staph. aureus*, *Ps. aeruginosa*, *Proteus vulgaris*, and *Xanthamonas campestris*.

Aridoğan et al. (2002) stated that peppermint oil was only active against *Staph. aureus* (inhibition zone diameter of 12 mm), recording no inhibition against either *E. coli or Ps. aeruginosa*. They proposed that this discrepancy was due to species differences, although they quote ATTC references for the three bacteria. In a study contradictory to these findings, Agarkar and Garode (2002) reported greater zones of inhibition using the same test bacteria: *E. coli* (10 mm), *Ps. aeruginosa* (30 mm), *Staph. aureus* (15 mm), *S. typhi* (15 mm), and *Proteus vulgaris* (16 mm), these being more typical values for this type of assay.

Biavati et al. (1997) examined the effect of 20 essential oils on the growth of 53 microbial strains of which 45 were bacterial strains and included a number of food spoilage types. MIC values showed that peppermint oil was most active against Gram-positive organisms, namely *Bacillus brevis* (400 ppm), *B. amylolyticus* (400 ppm), *Lactobacillus lactis* (200 ppm), *Streptococcus salivarius* (400 ppm), *Clostridium sporogenes* (400 ppm), *Cl. acetobutyricum* (400 ppm), *Cl. tyrobutyricum* (400 ppm), and several yeasts (400 to 1000 ppm).

Another comprehensive investigation looked at the antimicrobial activities of 52 plant oils and extracts against 10 bacteria using an agar dilution method and a broth microdilution technique (Hammer et al., 1999). In the agar dilution experiments, both peppermint and spearmint showed MICs of $\leq$2.0% (v/v) in all but the case of *Ps. aeruginosa*. However, when the broth microdilution technique was employed, peppermint showed MIC values lower than the agar dilution method and comparison showed differences exceeding two serial dilutions.

Disease-causing and secondary infection bacteria have been shown to be susceptible to peppermint oil at concentrations as low as 0.06% (v/v) against *Listeria monocytogenes* and *Staph. aureus*, and 0.1% (v/v) for *Staph. epidermidis*, *Staph. aprophiticus*, and *Staph. xylosis*. Gram-negative bacteria were more resistant with 0.2% (v/v) required to inhibit *Klebsiella pneumoniae* and *Shigella flexneri*. *E. coli* required 2.0% (v/v) while *Pseudomonas aeruginosa* needed 4.0% (v/v) (Reichling et al., 1999).

Four different peppermint oils (A, B, C, D) from commercial sources were evaluated for their antimicrobial activity against a collection of 21 human and plant pathogens by a combination of *in vitro* techniques including microdilution, agar diffusion, and bioautography (İşcan et al., 2002). It was shown that all the peppermint oils screened strongly inhibited plant pathogenic microorganisms, whereas human pathogens were only moderately inhibited. Using the bioautography assay, (−)-menthol (M1) was found to be responsible for the antimicrobial activity of these oils while (−)-menthone (M2) had much less activity as shown in Table 13.4 .

Three species of *Bifidobacteria* have so far been found in human dental caries. *Bifidobacterium dentium* was first described in 1974, while *B. inopinatum* and *B. denticolens* were not described until 1996 (Crociani et al., 1997). A collection of 20 plant essential oils was evaluated with a view to gaining insight into the susceptibilities of the bacteria and the potential for the oils in preventative treatments. MICs were determined for peppermint oil using TPY broth in microtiter plates. The values obtained were expressed in ppm oil and were as follows: *B. dentium* 1600 to >2000; *B. inopinatum* >2000; *B. denticolens* 600 to 1000. These results are similar to those of other bacteria as detailed above, suggesting their potential in dental hygiene and other preventative measures.

TABLE 13.4
Antimicrobial Activity of Peppermint Oils, Menthol, and Menthone MIC Values Expressed as mg/mL

Microorganism	A	B	C	D	M1	M2
Bacillus cereus	1.25	1.25	1.25	1.25	1.25	1.25
Enterobacter aerogenes	1.25	1.25	1.25	2.5	1.25	5.0
Escherichia coli	2.5	1.25	1.25	1.25	1.25	5.0
Klebsiella pneumoniae	2.5	2.5	2.5	2.5	2.5	5.0
Listeria monocytogenes	0.156	0.625	2.5	0.312	0.625	1.25
Proteus vulgaris	2.5	5.0	1.25	1.25	1.25	2.5
Pseudomonas aeruginosa	2.5	2.5	5.0	2.5	2.5	5.0
Pseudomonas syringae pv. phaeseolicola	2.5	1.25	1.25	0.625	1.25	2.5
Pseudomonas syringae pv. syringae	0.312	0.156	0.312	0.312	0.156	2.5
Pseudomonas syringae pv. tomato	0.07	0.07	0.625	0.312	0.07	1.25
Salmonella typhimurium	1.25	1.25	1.25	2.5	0.625	5.0
Staphylococcus aureus	0.625	0.625	1.25	2.5	0.625	2.5
Staphylococcus epidermidis	0. 625	2.5	0.625	1.25	0.625	0.625
Xanthomonas campestris pv. campestris	0.156	0.156	0.312	0.156	0.156	1.25
Xanthomonas campestris pv. phaseoli	0.625	0.312	0.625	0.156	0.625	2.5
Yersinia enterocolitica	2.5	2.5	2.5	2.5	2.5	2.5
Candida albicans	0.625	0.625	0.312	0.625	0.625	2.5

Source: After İşcan, G., Kirimar, N., Kürkcüoğlu, M., Başer, K.H.C., and Demirici, F., *J. Agric. Food Chem.*, 50, 3943–3946, 2002. With permission.

A more detailed investigation into the impact of essential oils and oil components on the growth of oral bacteria, other than *Bifidobacterium* sp., was undertaken by Shapiro et al. (1994). Their studies focused on obligate anaerobes, facultative anaerobes, and capnophilic microaerophiles implicated in the development or progression of dental disease. Essential oils and oil components were evaluated both singly and in dyadic combinations. Thus, peppermint oil was combined, as pairs, with sage oil, eugenol, thymol, Australian tea tree oil (*Melaleuca alternifolia*), and MINTY HACKS flavor as summarized in Table 13.5.

TABLE 13.5
Relative Growth Response of Oral Anaerobes to Pairs (25% MIC Each) of Peppermint Oil and Other Botanicals: Tests for Synergism

Obligate Anaerobes, Capnophilic Microaerophiles	P + S	P + E	P + T	P + TT	P + MH
Actinobacillus actinomycetemcomitans	39	100	100	0	100
Porphyromonas gingivalis	100	100	100	33	100
Peptostreptococcus anaerobius	67	100	100	100	100
Facultative anaerobes					
Streptococcus sanguis	100	100	100	100	100
Actinomyces viscosus	67	100	100	100	100
Streptococcus sobrinus	83	75	100	85	100

Source: After Shapiro, S., Meier, A., and Guggenheim, B., *Oral Microbiol. Immunol.*, 9, 202–208, 1994. With permission.
P, peppermint oil; S, sage oil; E, eugenol; T, thymol; TT, tea tree oil; MH, minty hacks.

Under these circumstances, growth of two-thirds of the obligate anaerobes and capnophilic microaerophiles was substantially inhibited by peppermint oil and Australian tea tree oil. An attempt to define the contribution of each component to the purported synergy was performed by reciprocally varying the relative concentrations of each of the components of a pair (10% MIC of substance A + 10%, 30%, or 90% of substance B) and comparing the extent of growth with that obtained for the same percentage MIC of pure substances. These data suggest that peppermint oil may slightly enhance the bacteriostatic potency of Australian tea tree oil toward *Porphyromonas gingivalis*. The authors stress, however, that the magnitude of synergism found was of no practical importance. There was some indication that peppermint oil with thymol and MINTY HACKS flavor with thymol may be antagonistic combinations, but the degree of interaction between the components was slight and, again, of no practical significance.

The essential oils obtained from two mint species, *M. pulegium* and *M. spicata*, exhibited antimicrobial properties against eight strains of Gram-negative and Gram-positive bacteria (Table 13.6). These data highlight the variable degree of antimicrobial activity not only between different bacterial strains, but also between different strains of the same bacteria. In general, the three *M. pulegium* oils showed slightly higher levels of antimicrobial activity than that of *M. spicata*.

Mimica-Dukić et al. (2003) studied the antimicrobial and antioxidant activity of three *Mentha* species: *M. piperita*, *M. aquatica*, and *M. longifolia* against five Gram-positive and eight Gram-negative bacteria. In general, Gram-positive types were more susceptible than Gram-negative organisms, but the multiresistant Gram-negative test strains also exhibited a notable susceptibility to the test oils. As in other experiments, the results showed variable levels of activity against all bacterial strains tested, with the exception of both strains of *Ps. aeruginosa*. It was of interest to note that all oils manifested similar activity against all strains of *E. coli* and *S. typhi*, two organisms known to be highly resistant to synthetic antibiotics. Significant antibacterial activity of essential oils of *M. piperita* and *M. longifolia* was recorded against *S. enteritidis* (28 and 30 mm, respectively), *Sarcina lutea* (16 mm, 23.2 mm), and *Staph. aureus* (39.8, 40.2 mm). The highest sensitivity was expressed by *Shigella sonnei* (47.4 mm) and *Micrococcus flavus* (60 mm) against the oil of peppermint. In contrast to these findings, Özcan and Erkmen (2001) reported zero activity for spearmint oil against *B. cereus*,

TABLE 13.6
Antimicrobial Activity of Mint Oils*

Bacteria	M1	M2	M3	M4
Escherichia coli I	15	11	13	13
Escherichia coli II	16	14	19	16
Salmonella typhimurium	16	13	13	12
Pseudomonas aeruginosa	9	6	5	5
Rhizobium leguminosarum	10	11	12	10
Staphylococcus aureus I	21	17	16	12
Staphylococcus aureus II	15	15	17	16
Bacillus subtilis	18	18	15	11

Source: After Sivropoulou, A., Kokkini, S., Lanaras, T., and Arsenakis, M., *J. Agric. Food Chem.*, 43, 2384–2388, 1995. With permission.

M1, *M. pulegium* strain 1; M2, *M. pulegium* strain 2; M3, *M. pulegium* strain 3; M4, *M. spicata*.
*Inhibition zone includes the diameter of the paper disk (5 mm).

Enterococcus faecalis, *E. coli*, *S. typhimurium*, *Staph. aureus*, and *Yersinia enterocolitica*, even when used at 15% v/v.

To evaluate the growth-inhibiting properties of spearmint and peppermint oils, both agar disk diffusion and broth assays were used, with *Streptococcus pneumoniae* as the test bacterium. This organism is a major cause of illness in young children, and is responsible for illness and mortality in the elderly and immunocompromised persons. An estimated 40,000 deaths per year (U.S.) alone are attributed to pneumococcal disease, and in some areas, up to 35% of isolates are resistant to penicillin as well as other antibiotics. In the agar diffusion assay, peppermint was ranked fourth out of the 73 oils tested, with a zone of inhibition of 11 mm; spearmint had a zone of 9 mm. In the broth assay, both oils caused bacterial lysis (Horne et al., 2001).

In an effort to evaluate the contributions of the main components of the mint essential oils to the antimicrobial properties, the activity of individual compounds, from commercial sources, was determined using the disk diffusion assay. The results, summarized in Table 13.7, show that all the 12 compounds exhibited a variable degree of antimicrobial activity with the exception of isomenthol, whose activity was negligible. Pulegone showed the highest activity against *S. typhimurium* while it was inactive against *Staph. aureus* I. On the contrary, isomenthone showed the highest activity against the same strain of *Staph. aureus*, while it was inactive against *S. typhimurium*. Notably, *Ps. aeruginosa* showed resistance to all test compounds with the exception of pulegone, isopulegol, and piperitone. Pulegone was also shown to be inhibitory against three species of *Salmonella* at concentrations as low as 1 μL: *S. paratyphi* B, 9.3 mm diameter of inhibition zone; *S. typhimurium*, 8 mm; and *S. venziana*, 10.6 mm (Flamini et al., 1999). Similar findings were reported by Hinou et al. (1989) and Fanaki and El-Nakeeb (1997) where *Staph. aureus* was found to be very susceptible to

TABLE 13.7
Antimicrobial Activity of Mint Oil Constituents*

	Bacteria							
Oil Constituent	**EC I**	**EC II**	**ST**	**PA**	**RL**	**SA I**	**SA II**	**BS**
Carvone	9	5	11	5	9	10	6	10
Dihydrocarvone**	8.5	9	7	5	9	9	7	8
Carveol	12	18	10	5	12	7	7	14
Dihydrocarveol	12	18	13	5	11	9	10	11
Menthol	5	6	5	5	8	7	7	7
Isomenthol	5	5	5	5	6	5	5	5
Neomenthol	9	10	10	5	10	8	8	12
Menthone	8	8	8	5	9	11	11	9
Isomenthone	6	8	5	5	8	9	7	7
Pulegone	7	10	14	11	10	5	9	10
Isopulegol	9	10	15	15	16	7	8	10
Piperitone	10	12	10	10	12	9	5	12

Source: After Sivropoulou, A., Kokkini, S., Lanaras, T., and Arsenakis, M., *J. Agric. Food Chem.*, 43, 2384–2388, 1995. With permission.

EC I, *E. coli* strain I; EC II, *E. coli* strain II; ST, *Salmonella typhinurium*; PA, *Ps. aeruginisa*; RL, *Rhizobium legunosarum*; SA I, *Staph. aureus* strain I; SA II, *staph. aureus* strain II; BS, *Bacillus subtilis*.

*Inhibition zone includes the diameter of the paper disk (5 mm).

**Consisting of *cis*- and *trans*-isomers.

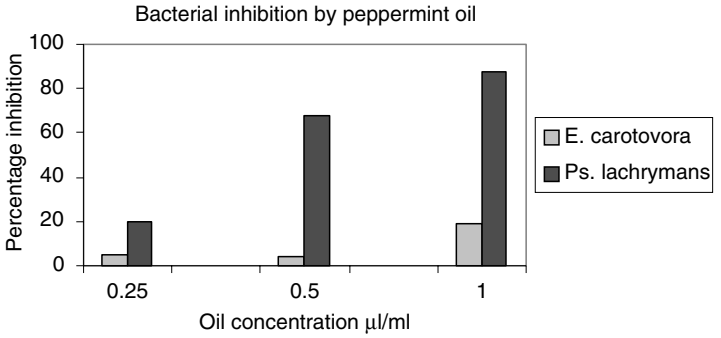


FIGURE 13.1 Levels of growth inhibition to *Erwinia carotovora* subsp. *atroseptica* and *Pseudomonas lachrymans* by peppermint oil.

piperitone and carvacrol (inhibition zones >16 mm), *E. coli* to carvacrol (>16 mm), and *Ps. aeruginosa* to menthone (>16 mm).

Peppermint oil, 1,8-cineole, and menthol were tested for antibacterial activity against a collection of clinical isolates, including several strains of *Vibrio cholerae*, against which the peppermint oil showed the greatest growth inhibition with zones up to 36 mm in diameter (Pattnaik et al., 1997).

Four major bacteria causing respiratory tract infections were treated with peppermint oil. *Haemophilus influenzae* was the most susceptible with an MIC value of 0.08% (w/v), followed by *Streptococcus pyogenes* and *Staph. aureus* with MICs of 0.16% (w/v), and lastly, the least susceptible were *Strep. pneumoniae* strain IP-692 (penicillin-sensitive), *Strep. pneumoniae* strain PRC-53 (penicillin-resistant), and *E. coli* (used as a control), all with MICs of 0.32% (w/v). Experimental trials of essential oils for the treatment of respiratory tract infections have revealed reduced frequency of relapse, augmentation of the output of demulcent respiratory fluid, maintenance of permanent ventilation and drainage of sinuses, and the detoxification of bacterial toxins (Inouye et al., 2001).

Three *Mentha* species, including peppermint, were used to determine the antimicrobial effect on the vaginal flora. *C. albicans* was inhibited by the mints at 800 to 900 ppm, while pathogens such as *Neisseria gonorrhoeae* and *Gardnerella vaginalis* were unaffected by concentrations of 3000 ppm. The normal flora, including *Staph. epidermidis*, *Lactobacillus gasseri*, *L. plantarum*, *Corynebacterium asperum*, and *Peptostreptococcus anaerobius* were equally unaffected by these concentrations, suggesting that these bacteria are somewhat resistant to the oils. No details were given as to the chemical composition or source of the oils (Viollon et al., 1993).

Plants are as susceptible to bacterial infections as humans and animals, albeit different genera and species. Peppermint oil has been shown to strongly inhibit the growth of *Erwinia carotovora* subsp. *atroseptica*, and to a lesser extent *Pseudomonas lachrymans*. Figure 13.1 shows the level of bacterial inhibition on exposure to 0.25%, 0.5%, and 1.0% (v/v) peppermint oil (Klimach and Wieczorek, 1996).

13.6 ANTIFUNGAL ACTIVITY OF *MENTHA*

Peppermint oil was also effective at inhibiting the growth of the pathogenic fungi *Pythium* sp. and *Fusarium sulphureum* and, in greenhouse experiments, the oil (at concentrations of 0.5% and 1.0%) proved efficient at limiting the spread of cucumber damping off (Klimach and Wieczorek, 1996). What is not clear, however, is whether the effect is due to the prevention of hyphal extension or that of dormancy-breaking events in fungal spore germination: were it to

prove to be the latter, the usefulness of this feature would be significant. Hyphal extension depends on the balance between synthesis enzymes such as chitin synthase, and the lytic elements such as α- and β-glucanase and chitinase. Any imbalance leads to lysis (lytic > synthetic activity) or hyphal wall thickening (synthetic > lytic activity), both conditions resulting in inhibition of growth (Deans, 1978).

An earlier study into the antimycotic properties of peppermint oil (Conner and Beuchat, 1984) demonstrated activity against a number of food spoilage yeasts. Inhibition zones around a 5-cm disk were quoted for *Geotrichum candidum* (so called ''dairy yeast'' due to its high incidence in dairy plants), 7 cm; *Metchnikowia pulcherima*, 9 cm; *Rhodotorula rubra*, 8 cm; and *Torulopsis glabrata*, 8 cm. Of these species, only *R. rubra* exhibited pseudomycelium production. Pseudomycelium is generally believed to form as a result of impairment of cell division mechanisms. This can be caused by elevation of temperatures to 37°C and increased levels of CO_2. Peppermint oil caused a delay in the appearance of pseudomycelium from the normal appearance at ~18 days.

In a study of the effects of peppermint oil on micromycetes (filamentous fungi), Motiejūnaite and Pečiulyte (2001) reported that the response by the test fungi differed by species and composition of the nutrient medium. *Trichoderma viride* and *Cladosporium resinae* were most resistant, while *Penicillium chrysogenum*, *Fusarium solanum*, and *Mucor ramasissimus* were most sensitive to the oil.

In an earlier trial, Ahmed et al. (1994) illustrated the *in vitro* antimycotic effect of peppermint oil on several pathogenic fungi isolated from dead chickens. One of the major problems within the poultry industry is the increase in mortality rate due to fungal infections, with members of the genus *Aspergillus* featuring prominently. Peppermint oil showed the greatest inhibitory effect against *A. fumigatus* (50-mm zone of inhibition on a Petri plate of Sabouraud agar), followed by *A. nidulans* (27-mm zone), *Penicillium* sp. (25 mm), and *A. flavus* (24 mm), while the other isolated fungi gave a low response. No precise volumes of oil used were stated; rather a sterile 5-mm filter paper disk was completely moistened in the oil or oil dilutions before placing on the inoculated agar surface.

Singh (1996), in a comprehensive survey of fungicidal activities in essential oils, lists cornmint oil as possessing inhibitory properties against the plant pathogenic genus *Helminthosporium*, *Penicillium italicum*, and *Pen. notatum*. This author also mentions that an unspecified *Mentha* sp. is active against the mycotoxigenic *Aspergillus parasiticus*. Pandey et al. (1998) tested cornmint and Scotch spearmint oils against *A. niger* at concentrations of 500 and 1000 ppm. Scotch spearmint oil showed strong activity at both concentrations, while cornmint showed such activity only at the higher concentration. Scotch spearmint oil was most potent antifungal oil in the tests, also exhibiting strong activity as an antibacterial agent.

Singh et al., (1992), in a short report on the bioactivity of cornmint oil, showed that the Gram-positive bacteria were significantly more inhibited than the Gram-negative organisms. The Gram-positive species had inhibition zones of 10 (*Streptococcus thermophilus*) to 30 mm diameter (*Bacillus brevis*), while the Gram-negative species were only in the range 10 (*Salmonella* sp.) to 15 mm diameter (*Xanthomonas* sp.). The cornmint oil was also active against a number of plant pathogens including *Alternaria* (10-mm diameter zone of inhibition), *Fusarium* (10.5 mm), *Sclerotium* (12 mm), and *Aspergillus parasiticus* (15 mm), an aflotoxigenic fungus. Hence, cornmint oil can be used in postharvest protection of grain crops, especially when the pH is changed from the normal value ~pH 5 to either pH 4 or pH 9. At the normal pH, the minimal effective concentration (MEC) was 2000 ppm against the test fungus *Helminthosporium oryzae*; at pH 4, this was reduced to 500 ppm, and at pH 9, reduced by 50% to 1000 ppm (Dikshit et al., 1982).

Cornmint oil was used in a series of experiments to demonstrate antifungal action against *Aspergillus* sp. (30-mm diameter zone of inhibition), *Fusarium* sp. (31 mm), and *Cladosporium* sp.

TABLE 13.8
MICs and MFCs of Cornmint Oil against Four Filamentous Fungi

Fungus	MIC 2d	MIC 8d	MFC
Aspergillus niger	1:80	1:20	1:20
Mucor sp.	1:160	1:80	1:40
Penicillium chrysogenum	1:5120	1:320	1:320
Rhizopus sp.	1:160	1:80	1:40

Source: After Akgül, A. and Kivanç, M., *J. Sci. Food Agric*., 47, 129–132, 1989. With permission.

(33 mm) (Mehmood et al., 1997). The antimycotic properties of cornmint oil were also tested using *A. niger*, *Mucor* sp., *Pen. Chrysogenum*, and a *Rhizopus* sp. Two measurements were recorded: MIC and minimal fungicidal concentration (MFC) (Table 13.8). Cornmint oil had a great effect on *Pen. chrysogenum*, making it the most sensitive of the four test fungi.

L-Menthol at concentrations of 0.5 m*M* and 1.0 m*M* resulted in several days of growth inhibition, when combined with ethanol (3%) and NaCl (3%), of seven filamentous fungi. These were *Aspergillus oryzae* (2 days at 0.5 mM, 3 days at 1.0 mM, respectively), *Aspergillus niger* (3 days, 5 days), *Penicillium citrinum* (3 days, 6 days), *Pen. viridicatum* (7 days, 11 days), *Fusarium graminearum* (3 days, 5 days), *Aureobasidium pullulans* (11 days, >20 days), and *Paecilomyces lilacinus* (6 days, 9 days) (Kurita and Koike, 1983).

Fusarium moniliforme is an important postharvest fungal pathogen of cereal crops. A similar technique to that used by Ahmed et al. (1994), though using PDA media, revealed a modest degree of inhibition by peppermint oil: 8.8-mm zone at a concentration of 25% (v/v) (Baruah et al., 1996).

In a detailed study into the structure–fungitoxicity relationships of peppermint oil and its primary monoterpene constituents, Nidiry (1998) grew *Colletotrichum gloeosporioides* on agar impregnated with the whole oil, menthol, neomenthol, linalool, menthyl acetate, and menthone. Whole peppermint oil at a maximum concentration of 1000 ppm resulted in 55% mycelial growth inhibition. Menthol and neomenthol at concentrations of 700 ppm resulted in 75.6% and 70.3% inhibition, respectively; menthyl acetate and menthone at concentrations of 3000 ppm resulted in 77.8% and 75.7% inhibition, respectively, while linalool was used at a maximum concentration of 1500 ppm, resulting in 64.9% inhibition.

Human dermatophytic fungi have been shown to be susceptible to mint essential oils (Janssen et al., 1988; Müller-Riebau et al., 1995). Three frequently occurring dermatophytes, *Epidermophyton floccosum*, *Trichophyton mentagrophytes* var. *interdigitale*, and *T. rubrum*, were exposed to oils from peppermint, spearmint, and *M. pulegium*. Against *E. floccosum*, all three oils had a minimum inhibitory dilution (MID) of 1:3200 to 1:1600; against *T. mentagrophytes*, the ID values were all 1:1600 to 1:800; and against *T. rubrum*, *M spicata* had the greatest effect, being active at dilutions >1:3200, the other two with values of 1:3200 to 1:1600. In a similar screening program, Dikshit and Husain (1984) studied the antimycotic activity of essential oils from 28 plants, including three mint species, against *Microsporum gypseum*, *Trichophyton equinum*, and *T. rubrum*. However, the measurement of inhibition was different from that described by Janssen et al. (1988), in that percentage mycelial inhibition was indicated. In overall activity, the percentage for Mentha citrata (Bergamot mint) was greater than that for peppermint, which was greater than that for spearmint.

Japanese mint, another popular name for cornmint, was found to be an effective antimycotic agent when used with the human pathogens *C. albicans*, *Cryptococcus neoformans*,

Microsporum gypseum, *Trichophyton rubrum*, and *Sporothrix schenckii*. In accurately timed experiments, the length of time taken to kill the fungi was determined: all fungi were killed within a 15-min contact, although some were killed off faster (*C. albicans* and *S. schenckii* within 5 min) (Rath et al., 2001). Japanese mint was also investigated, at concentrations of 2.5, 5, and 10 μL per disk, for bactericidal activity against five microorganisms of significance to human health. The results were presented as zones of growth inhibition including the 5-mm paper disk used to apply the oil. The zones were *E. coli* (12, 17, and 23 mm, respectively), *S. typhimurium* (15, 16, and >40 mm), *Shigella boydii* (15, 35, and >40 mm), *Pseudomonas aeruginosa* (12, 15, and 17 mm), and *Bacillus cereus* (15, 19, and 27 mm). The impact of the oil is most marked against the serious enteric pathogens *S. typhimurium* and *Sh. boydii*, suggesting their control could be effectively realized using cornmint oil (Rath et al., 2002).

Using a collection of seven fungi, Aggarwal et al. (2002) determined the zones of growth inhibition on Sabouraud dextrose agar and the MIC by the broth dilution method in the presence of spearmint oil and its major components *S*-(−)-carvone (>56%) and *S*-(−)-limonene (>27%). Comparison was made with the enantiomers of these compounds, isolated from *Anethum sowa* (Indian dill), where *R*-(+)-carvone (>50%) and *R*-(+)-limonene (>21%) are the major constituents of the oil. *In vitro* bioactivity evaluation revealed that both the optical isomers of carvone, but only the *R*-(+)-limonene, were active against a wide spectrum of human pathogenic fungi and bacteria (Table 13.9). Oil from spearmint, at levels of <2 μL/mL,

TABLE 13.9
Antimicrobial Activity of Spearmint Oil (SEO), S-(−)-Carvone (S C), R-(+)-Carvone (R C), and *R*-(+)-Limonene (R L)*

Microorganism	SEO	S -C	R -C	R -L
Bacillus subtilis	14	14	14	18
Enterobacter aerogenes	9	14	15	ND
Enterococcus faecalis	10	14	17	15
Escherichia coli	9	5	5	17
Klebsiella pneumoniae	9	15	15	ND
Mycobacterium smegmatis	13	14	23	17
Pseudomonas aeruginosa	5	11	16	ND
Salmonella typhi	5	14	14	17
Salmonella typhimurium	9	14	15	ND
Staphylococcus aureus	15	5	17	18
Staphylococcus epidermidis	16	5	15	ND
Streptococcus mutans	13	15	18	28
Yersinia enterocolitica	11	17	16	ND
Aspergillus flavus	10	5	5	30
Aspergillus niger	11	17	22	38
Candida albicans I	13	34	30	22
Candida albicans II	16	35	34	31
Microsporum gypseum	>40	25	29	35
Sporothrix schenckii	15	28	36	28
Trichophyton rubrum	21	18	36	20

Source: After Aggarwal, K.K., Khanuja, S.P.S., Ahmad, A., Kumar, T.R.S., Gupta, V.K., and Kumar, S., *Flav. Fragr. J.*, 7, 59–63, 2002. With permission.
ND: Not determined.
*Diameter of inhibition zone in mm (includes 5-mm diameter disk).

was found to result in 100% inhibition of mycelial growth in *Epidermophyton floccosum*, *Microsporum gypseum*, and *Microsporum nanum*, three human dermatophytes (Pandey et al., 2002). Lis-Balchin et al. (1996) reported similar activities with the enantiomers of limonene.

13.7 DETOXIFICATION STUDIES

In contrast to the antifungal properties, two fungi, *Aspergillus niger* and *Rhizopus stolonifer*, have been shown to detoxify citral and menthol (Moleyar and Narasimham, 1987). Citral and menthol were, however, both fungistatic and fungicidal depending on their concentration in the growth medium. Citral up to 100 μg/mL and menthol up to 200 μg/mL were only fungistatic to *A. niger* and *R. stolonifer*, respectively, whereas the same compounds at 200 and 400 μg/mL, respectively, were fungicidal after 48-h treatment. Susceptibility or resistance of a fungus to the antifungal activity of an essential oil component depends on the capacity of the fungus to detoxify the compound by either degrading or converting to another substance. Thus, *A. niger* was able to detoxify menthol faster than citral, while in contrast, *R. stolonifer* could detoxify citral faster than menthol, these abilities reflecting differences in enzyme profiles.

Similar studies with a *Corynebacterium* species were undertaken by Williams and Trudgill (1994) who grew the bacterium in the presence of (−)-menthol, (−)-menthone, and other cyclic monoterpenes as sole carbon sources. Growth on menthol was very slow, with a doubling time of >24 h, and was not rapid with menthone (doubling time 12 h). (−)-Menthone-grown cultures transiently accumulated 3,7-dimethyl-6-hydroxyoctanoate during growth, while (−)-menthol-grown cells oxidized (−)-menthol, (−)-menthone, 3,7-dimethyl-6-octanolide, and 3,7-dimethyl-6-hydroxyoctanoate. Although neither a menthol oxidase nor a menthol dehydrogenase could be detected in extracts of either cells, an induced NADPH-linked monooxygenase with activity toward (−)-menthone was readily detected. With crude cell extracts, only 3,7-dimethyl-6-hydroxyoctanoate was detected as the reaction product. When the (−)-menthone monooxygenase was separated from an induced 3,7-dimethyl-6-octanolide hydrolase by chromatography, the lactone 3,7-dimethyl-6-octanolide was shown to be the product of oxygenation.

13.8 PROTOZOA

Trypanosoma and *Leishmania* are closely related parasitic protozoa afflicting millions of people worldwide. African trypanosomes cause sleeping sickness in humans, which, if not treated, is fatal. *Leishmania* parasites manifest a wide spectrum of disease severity depending on the species and the immunological status of the human host. At present, only a few drugs with serious side effects are available for the treatment of *Trypanosoma* and *Leishmania* infections. At a time when the efficacy of currently available drugs is on the decline, it is timeous to search for novel, safe, and effective alternatives. Peppermint has been shown to be effective against *T. brucei* with an ED_{50} of 4.2 μg/mL. It is, however, less effective against *L. major* where the ED_{50} value is 227.5 μg/mL (Mikus et al., 2000).

13.9 ANTIVIRAL ACTIVITY

Antiviral properties have been claimed, both *in vivo* and *in vitro*, for peppermint oil against potato virus Y at concentrations between 500 and 2000 ppm (Ismail, 1994). *Datura metel* was the test host, chosen for its ease of cultivation and susceptibility to the virus. The results were promising, with the 100% infection being reduced to 0% under certain circumstances,

this level of reduction being realized immediately after spraying infected plants with the 1000 ppm oil in a Tween 80 emulsion. Concerns have been voiced regarding the use of emulsifiers such as Tween 80 by reducing the bioactivity of essential oils, probably because of the formation of micelles, inhibiting adequate contact between oil and test organism (Inouye et al., 2001). These authors recommended the use of agar as a chemically and microbiologically inert stabilizer for agar dilution assays.

13.10 POTATO SPROUTING INHIBITION

The major problems encountered during potato storage are sprouting and rotting due to phytopathogens. Different approaches have been adopted to prolong the period of tuber dormancy and several chemicals are used commercially, although their side effects include residues left in the tubers. *Mentha pulegium* and spearmint were evaluated as sprout suppressants and antimicrobial agents. Unfortunately, the mint essential oils appeared to promote sprout emergence, although they did control the bacterial pathogen *Erwinia carotovora* (Vokou et al., 1993). To date, only *S*-(+)-carvone, a monoterpene produced from caraway seeds, has been developed commercially as a competitive alternative to the synthetic pesticides. Coleman et al. (2001) examined the sprout-inhibiting properties of menthone and neomenthol and found them to be five to ten times more effective than *S*-(+)-carvone when applied together at 0.5 μL/L each. An examination of changes in CO_2 output, glucose, or sucrose levels over a 4-week period at 10°C indicated that the effects of menthone and *S*-(+)-carvone were similar.

13.11 INSECT DISEASE CONTROL

American Foulbrood, caused by the spore-forming bacterium *Paenibacillus larvae*, is the most serious disease of bacterial origin affecting the larvae and pupae stages of honeybees. Currently, the alternative to burning the infected hives for control of this disease is treatment with antibiotics. This approach, however, has the downside that the honey may become contaminated with antibiotic residues, affecting the quality of the product. Peppermint oil was investigated as an alternative control measure, and was found to have an MIC of 600 to 650 μL/L of media, depending on the strain of *P. larvae* (Alippi et al., 1996).

13.12 TOXICITY

The question of toxicity in *Mentha* species has been controversial, and in a study of peppermint oil, shown to contain 38.1% menthol, 33.7% menthone, and 1.7% pulegone, the mutagenic effects were evaluated by the *S. typhimurium*–mammalian-microsome test of Ames et al. (1975). Andersen and Jensen (1984) concluded that peppermint oil, menthol, and pulegone, at concentrations of 800, 160, 32, and 6.4 μg per plate, did not demonstrate any mutagenic properties. Menthone, at the same concentrations, proved mutagenic.

13.13 CONCLUSIONS

The genus *Mentha* clearly has marked antimicrobial characteristics across the spectrum from fungi and parasites, through bacteria, to viruses. There is some difficulty in comparing the different results obtained by research groups across the world since so many variables exist. However, there is some thread of similarity in that the microorganisms against which the various members of the genus have been evaluated can be grouped together as important

disease-causing bacteria, including *E. coli*, with strain O157:H7, *Salmonella*, and *Shigella* sp., *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Streptococci*, and *Staph. aureus*. It is significant that MRSA and VRE organisms are susceptible to mint oils since no development of resistance appears to occur.

Fungi are another problem group of microbes from both the human pathogenic and mycotoxigenic types to the phytopathogens and their costly effect on stored postharvest crops. It is encouraging to see that several research groups are addressing the issue of applying mint and other plant essential oils to alleviate or even eliminate these problems.

Finally, the use of mint oils to control *Trypanosoma* and *Leishmania* parasites is worthy of note since there are very few drugs with efficacy against them, and these have undesirable side effects. The inhibition of potato sprouting by mint oil constituents is a promising solution to an old problem.

REFERENCES

Agarkar, S.V. and Garode, A.M. (2002). Antibacterial screening of *Mentha piperita* oil. *Asian J. Microbiol. Biotech. Environ. Sci.*, **4**, 427–428.

Aggarwal, K.K., Khanuja, S.P.S., Ahmad, A., Kumar, T.R.S., Gupta, V.K., and Kumar, S. (2002). Antimicrobial activity profiles of the two enantiomers of limonene and carvone isolated from the oils of *Mentha spicata* and *Anethum sowa*. *Flav. Fragr. J.*, **7**, 59–63.

Ahmed, F.H., El-Badri, A.A., Ibrahim, M.M.K., El-Shahed, A.S., and El-Khalafawy, H.M.M. (1994). Comparative studies of antifungal potentialities for some natural plant oils against different fungi isolated from poultry. *Grasas Aceites (Seville)*, **45**, 260–264.

Akgül, A. and Kivanç, M. (1989). Sensitivity of four foodborne moulds to essential oils from Turkish spices, herbs and citrus peel. *J. Sci. Food Agric.*, **47**, 129–132.

Aktuğ, Ş.E. and Karapinar, M. (1986). Sensitivity of some common food-poisoning bacteria to thyme, mint and bay leaves. *Int. J. Food Microbiol.*, **3**, 349–354.

Alippi, A.M., Ringuelet, J.A., Cerimele, E.L., Re, M.S., and Henning, C.P. (1996). Antimicrobial activity of some essential oils against *Paenibacillus larvae*, the causal agent of American foulbrood disease. *J. Herbs, Spices Med. Plants*, **4**(2), 9–16.

Ames, B.N., McCann, J., and Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. *Mutat. Res.*, **31,** 347–364.

Andersen, P.H. and Jensen, N.J. (1984). Mutagenic investigation of peppermint oil in the *Salmonella*/mammalian-microsome test. *Mutat. Res.*, **138**, 17–20.

Arakawa, T. and Osawa, K. (2000). Pharmacological study and application to food of mint-flavour-antibacterial and antiallergic principles. *Arom. Res.*, **1**(1), 20–23.

Aridoğan, B.C., Baydar, H., Kaya, S., Demirci, M., Özbaşar, D., and Mumcu, E. (2002). Antimicrobial activity and chemical composition of some essential oils. *Arch. Pharm. Res.*, **25,** 860–864.

Baratta, M.T., Dorman, H.J.D., Deans, S.G., Biondi, D.M., and Ruberto, G. (1998a). Chemical composition, antibacterial and antioxidative activity of laurel, sage, rosemary, oregano and coriander essential oils. *J. Essent. Oil Res.*, **10**, 618–627.

Baratta, M.T., Dorman, H.J.D., Deans, S.G., Figueiredo, A.C., Barroso, J.G., and Ruberto, G. (1998b). Antimicrobial and antioxidant properties of some commercial essential oils. *Flav. Fragr. J.*, **13,** 235–244.

Baruah, P., Sharma, R.K., Singh, R.S., and Ghosh, A.C. (1996). Fungicidal activity of some naturally occurring essential oils against *Fusarium moniliforme*. *J. Essent. Oil Res.*, **8**, 411–412.

Biavati, B., Franzoni, S., Ghazvinizadeh, H., and Piccaglia, R. (1997). Antimicrobial and antioxidant properties of plant essential oils. In: *Proceedings of 27th International Symposium on Essential Oils, Vienna, 1996*. Franz, Ch., Mathé, A., and Buchbauer, G., Eds., Allured Publ., Carol Stream, IL, pp. 326–331.

Cai, L. and Wu, C.D. (1996). Compounds from *Syzygium aromaticum* possessing growth inhibitory activity against oral pathogens. *J. Nat. Prod.*, **59**, 987–990.

Coleman, W.K., Lonergan, G., and Silk, P. (2001). Potato sprout growth suppression by menthone, neomenthol, volatile oil components of *Minthostachys, Satureja, Bystropogon* and *Mentha* species. *Am. J. Potato Res.*, **78**, 345–354.

Conner, D.E. and Beuchat, L.R. (1984). Effects of essential oils from plants on growth of food spoilage yeasts. *J. Food Sci.*, **49**, 429–434.

Crociani, F., Biavati, B., Alessandrini, A., and Zani, G. (1997). Growth inhibition of essential oils and other antimicrobial agents towards bifidobacteria from dental caries. In: *Proceedings of 27th International Symposium on Essential Oils, Vienna, 1996*. Franz, Ch., Mathé, A., and Buchbauer, G., Eds., Allured Publ., Carol Stream, IL, pp. 40–44.

Davidson, P.M. (1997). Chemical preservatives and natural antimicrobial compounds. In: *Food Microbiology Fundamentals and Frontiers*, Doyle, M.P., Beuchat, L.R., and Montville, T.J., Eds., ASM Press, pp. 520–556.

Deans, S.G. (1978). Cell wall ontogeny during microcycle conidiation in *Aspergillus niger*. Ph.D. Thesis, University of Strathclyde, Glasgow, Scotland.

Deans, S.G. and Ritchie, G. (1987). Antibacterial properties of plant essential oils. *Int. J. Food Microbiol.*, **5**, 165–180.

Diaz, R., Quevedo-Sarmiento, J., Ramos-Cormenzana, A., Cabo, P., and Cabo, J. (1988). Phytochemical and antibacterial screening of some species of Spanish *Lamiaceae. Fitoterapia*, **59**, 329–332.

Dikshit, A. and Husain, A. (1984). Antifungal action of some essential oils against animal pathogens. *Fitoterapia*, **55**, 171–176.

Dikshit, A., Singh, A.K., and Dixit, S.N. (1982). Effect of pH on fungitoxic activity of some essential oils. *J. Antibact. Antifung. Agents*, **9**(11), 9–10.

Dorman, H.J.D. and Deans, S.G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Microbiol.*, **88**, 308–316.

El-Kady, I.A., Mohamed El-Maraghy, S.S., and Eman Mostafa, M. (1993). Antibacterial and antidermatophyte activities of some essential oils from spices. *Qatar Univ. Sci. J.*, **13**(1), 63–69.

Fanaki, N.H. and El-Nakeeb, M.A. (1997). Antimicrobial activity of some essential oil components against multi-resistant clinical isolates. *Alex. J. Pharm. Sci.*, **11**(3), 149–153.

Flamini, G., Cioni, P.L., Puleio, R., Morelli, I., and Panizzi, L. (1999). Antimicrobial activity of the essential oil of *Calamintha nepeta* and its constituent pulegone against bacteria and fungi. *Phytother. Res.*, **13**, 349–351.

Gallardo, P.P.R., Salinas, R.J., and Villar, L.M.P. (1987). The antimicrobial activity of some spices on microorganisms of great interest to health. IV: seeds, leaves and others. *Microbiol. Aliments Nutr.*, **5**, 77–82.

Griffin, S.G., Wyllie, S.G., Markham, J.L., and Leach, D.N. (1999). The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. *Flav. Fragr. J.*, **14**, 322–332.

Griffin, S.G., Wyllie, S.G., and Markham, J.L. (2001). Role of the outer membrane of *Escherichia coli* AG100 and *Pseudomonas aeruginosa* NCTC 6749 and resistance/susceptibility to monoterpenes of similar chemical structure. *J. Essent. Oil Res.*, **13**, 380–386.

Hammer, K.A., Carson, C.F., and Riley, T.V. (1999). Antimicrobial activity of essential oils and other plant extracts. *J. Appl. Bact.*, **86**, 985–990.

Hinou, J.B., Harvala, C.E., and Hinou, E.B. (1989). Antimicrobial activity screening of 32 common constituents of essential oils. *Pharmazie*, **44**, 302–303.

Hoffman, C. and Evans, A.C. (1911). The use of spices as preservatives. *J. Ind. Eng. Chem.*, **3**, 835–838.

Horne, D., Holm, M., Oberg, C., Chao, S., and Young, D.G. (2001). Antimicrobial effects of essential oils on *Streptococcus pneumoniae. J. Essent.Oil Res.*, **13,** 387–392.

Imai, H., Osawa, K., Yasuda, H., Hamashima, H., Arai, T., and Sasatsu, M. (2001). Inhibition by the essential oils of peppermint and spearmint of the growth of pathogenic bacteria. *Microbios*, **106**, 31–39.

Inouye, S., Yamaguchi, H., and Takizawa, T. (2001). Screening of the antibacterial effects of a variety of essential oils on respiratory tract pathogens, using a modified dilution assay method. *J. Infect. Chemother.*, **7**, 251–254.

İşcan, G., Kirimer, N., Kürkcüoğlu, M., Başer, K.H.C., and Demirci, F. (2002). Antimicrobial screening of *Mentha piperita* essential oils. *J. Agric. Food Chem.*, **50**, 3943–3946.

Ismail, M.H. (1994). Essential oils as inhibitors of potato virus Y. *Egypt J. Bot*., **34**(2), 167–176.
Janssen, A.M., Scheffer, J.J.C., and Baerheim-Svendsen, A. (1987). Antimicrobial activity of essential oils: a 1976–86 literature review. Aspects of test methods. *Planta Med*., **53**, 395–398.
Janssen, A.M., Scheffer, J.J.C., Parhan-van Atten, A.W., and Baerheim Svendsen, A. (1988). Screening of some essential oils for their activities on dermatophytes. *Pharm. Weekbl*., **10**, 277–280.
Jay, J.M. and Rivers, G.M. (1984). Antimicrobial activity of some food flavouring compounds. *J. Food Safety*, **6**, 129–139.
Klimach, A. and Wieczorek, W. (1996). Influence of etheral oils on incidence limitation of some fungus and bacterial diseases of plants. *Pestycydy*, **1**, 45–54.
Knobloch, K., Weigand, H., Weis, N., and Vigenschow, H. (1986). Action of terpenoids on energy metabolism. In: *Progress Essential Oil Research*, Brunke, E.J., Ed., Walter de Gruyter, Berlin, pp. 429–445.
Knobloch, K., Pauli, A., Iberl, N., Weis, N., and Weigand, H. (1988). Modes of action of essential oil components on whole cells of bacteria and fungi in plate tests. In: *Bioflavour*, Schreier, P., Ed., Walter de Gruyter, Berlin, pp. 287–299.
Knobloch, K., Pauli, A., Iberl, B., Weigand, H., and Weis, N. (1989). Antibacterial and antifungal properties of essential oil components. *J. Essent. Oil Res.*, **1**, 119–128.
Kurita, N. and Koike, S. (1983). Synergistic antimicrobial effect of ethanol, sodium chloride, acetic acid and essential oil components. *Agric. Biol. Chem.*, **47**, 67–75.
Kurita, N., Miyaji, M., Kurane, R., Takahara, Y., and Ichimura, K. (1979). Antifungal activity of molecular orbital energies of aldehyde compounds from oils of higher plants. *Agric. Biol. Chem*., **43**, 2365–2371.
Kurita, N., Miyaji, M., Kurana, R., and Takahara, Y. (1981). Antifungal activity of components of essential oils. *Agric. Biol. Chem.*, **45**, 945–952.
Larrondo, J.V., Agut, M., and Calvo-Torras, M.A. (1995). Antimicrobial activity of essences from *Labiatae. Microbios*, **82**, 171–172.
Lis-Balchin, M. and Deans, S.G. (1997). Bioactivity of selected plant essential oils against *Listeria monocytogenes*. *J. Appl. Microbiol*., **82**, 759–762.
Lis-Balchin, M., Ochoka, R.J., Deans, S.G., and Hart, S. (1996). Bioactivity of the enantiomers of limonene. *Med. Sci. Res*., **24**, 309–310.
Lis-Balchin, M., Deans, S.G., and Eaglesham, E. (1998). Relationship between bioactivity and chemical composition of commercial essential oils. *Flav. Fragr. J*., **13**, 98–104.
Marino, M., Bersani, C., and Comi, G. (2001). Impedance measurements to study the antimicrobial activity of essential oils from *Lamiaceae* and *Compositae*. *Int. J. Food. Microbiol*., **67**, 187–195.
Martindale, W.H. (1910). Essential oils in relation to their antiseptic powers as determined by their carbolic coefficients. *Perfum. Essent. Oil Res*., **1**, 266–296.
Mehmood, Z., Ahmad, S., and Mohammad, F. (1997). Antifungal activity of some essential oils and their major constituents. *Ind. J. Nat. Prod*., **13**(2), 10–13.
Mikus, J., Harkenthal, M., Steverding, D., and Reichling, J. (2000). *In vitro* effect of essential oils and isolated mono- and sesquiterpenes on *Leishmania major* and *Trypanosoma brucei*. *Planta Med.*, **66**, 366–368.
Mimica-Dukić, N., Božin, B., Soković, M., and Mihajlović, M. (2003). Antimicrobial and antioxidant activities of three *Mentha* species essential oils. *Planta Med.*, **69**, 413–419.
Moleyar, V. and Narasimham, P. (1986). Antifungal activity of some essential oil components. *Food Microbiol*., **3**, 331–336.
Moleyar, V. and Narasimham, P. (1987). Detoxification of essential oil components (citral and menthol) by *Aspergillus niger* and *Rhizopus stolonifer*. *J. Sci. Food Agric.*, **39**, 239–246.
Montes, M.A., Wilkomirsky, T., and Bello, H. (1998). Antibacterial activity of essential oils from aromatic plants growing in Chile. *Fitoterapia*, **69**, 170–172.
Morris, J.A., Khettry, A., and Seitz, E.W. (1979). Antimicrobial activity of aroma chemicals in essential oils. *J. Am. Oil Chem. Soc.*, **56**, 595–603.
Motiejūnaitė, O. and Pečiulytė, D. (2001). Response of micromycetes to the effect of mint volatile oil. *Biologija*, **3**, 38–41.

Müller-Riebau, F., Berger, B., and Yegen, O. (1995). Chemical composition and fungitoxic properties to phytopathogenic fungi of essential oils of selected aromatic plants growing wild in Turkey. *J. Agric. Food Chem.*, **43**, 2262–2266.

Naigre, R., Kalck, P., Roques, C., Roux, I., and Michel, G. (1996). Comparison of antimicrobial properties of monoterpenes and their carbonylated products. *Planta Med.*, **62**, 275–277.

Nelson, R.R.S. (1997). *In vitro* activities of five plant essential oils against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium*. *J. Antimicrob. Chemother.*, **40**, 305–306.

Nidiry, E.S.J. (1998). Structure–fungitoxicity relationships of the monoterpenoids of the essential oils of peppermint (*Mentha piperita*) and scented geranium (*Pelargonium graveolens*). *J. Essent. Oil Res.*, **10**, 628–631.

Osawa, K., Saeki, T., Yasuda, H., Hamashima, H., Sasatsu, M., and Arai, T. (1999). The antibacterial activities of peppermint oil and green tea polyphenols, alone and in combination, against enterohaemorrhagic *Escherichia coli*. *Biocontrol Sci.*, **4**(1), 1–7.

Özcan, M. and Erkmen, O. (2001). Antimicrobial activity of the essential oils of Turkish plant spices. *Eur. Food Res. Technol.*, **212**, 658–660.

Özgüven, M., Kirici, S., Yaman, A., Aksungur, P., and Gür, A. (1998). Antimicrobial activity of essential oil of wild *Mentha* species growing in Southern Turkey. *Pharm. Pharmacol. Lett.*, **8**, 164–167.

Pandey, R., Mehrotra, N., Gaur, R., and Darmwal, N.S. (1998). Effect of some essential oils on microorganisms. *Hind. Antibiot. Bull.*, **40**, 59–61.

Pandey, K.P., Shahi, S.K., Singh, R., Dutta, S., and Dikshit, A. (2002). Antifungal efficacy of *Taxodium* and *Mentha* oils against some human pathogenic fungi. *Flav. Fragr. J.*, **17**, 443–444.

Pattnaik, S., Subramanyam, V.R., Bapaji, M., and Kole, C.R. (1997). Antibacterial and antifungal activity of aromatic constituents of essential oils. *Microbios*, **89**, 39–46.

Pauli, A. and Knobloch, K. (1987). Inhibitory effects of essential oil components on growth of food-contaminating fungi. *Zeitsch. Lebensm. Untersuch. Forsch.*, **185**, 10–13.

Pelczar, M.J., Chan, E.C.S., and Krieg, N.R. (1988). Control of microorganisms by physical agents. In: *Microbiology*, McGraw-Hill International, New York, pp. 469–509.

Piccaglia, R., Marotti, M., Giovanelli, E., Deans, S.G., and Eaglesham, E. (1993). Antibacterial and antioxidant properties of Mediterranean aromatic plants. *Ind. Crops Prod.*, **2**, 47–50.

Rao, T.S.S. and Nigam, S.S. (1978). Antibacterial study of some Indian essential oils. *Ind. Perfum.*, **22**, 118–119.

Rath, C.C., Dash, S.K., and Mishra, R.K. (2001). *In vitro* susceptibility of Japanese mint (*Mentha arvensis* L.) essential oil against five human pathogens. *Ind. Perfum.*, **45**(1), 57–61.

Rath, C.C., Dash, S.K., and Mishra, R.K. (2002). Antibacterial efficacy of six Indian essential oils individually and in combination. *J. Essent. Oil-Bear. Plants*, **5**, 99–107.

Reichling, J., Harkenthal, M., and Saller, R. (1999). In-nitro-untersuchungen zur antimikrobiellen Wirkung ausgeählter äthesischer Öle. Erfahrungsheilkunde, **6**, 357–366.

Shapiro, S., Meier, A., and Guggenheim, B. (1994). The antimicrobial activity of essential oils and essential oil components towards oral bacteria. *Oral Microbiol. Immunol.*, **9**, 202–208.

Shelef, L.A. (1984). Antimicrobial effects of spices. *J. Food Safety*, **6**, 29–44.

Singh, G. (1996). Studies on fungicidal activity of essential oils. *Euro Cosmetics*, **4**, 29–32.

Singh, S.P., Negi, S., Chand, L., and Singh, A.K. (1992). Antibacterial and antifungal activities of *Mentha arvensis* essential oil. *Fitoterapia*, **63**, 76–78.

Singh, G., Kapoor, I.P.S., Panday, S.K., Singh, U.K., and Singh, R.K. (1998). Studies on essential oils Part II: antibacterial investigations of volatile oils of some higher plants. *Ind. Drugs*, **35,** 591–593.

Sivropoulou, A., Kokkini, S., Lanaras, T., and Arsenakis, M. (1995). Antimicrobial activity of mint essential oils. *J. Agric. Food Chem.*, **43**, 2384–2388.

Tassou, C.C., Drosinos, E.H., and Nychas, G.J.E. (1995). Effects of essential oil from mint (*Mentha piperita*) on *Salmonella enteritidis* and *Listeria monocytogenes* in model food systems at 4°C and 10°C. *J. Appl. Bact.*, **78**, 593–600.

Tassou, C., Koutsoumanis, K., and Nychas, G.J.-E. (2000). Inhibition of *Salmonella enteritidis* and *Staphylococcus aureus* in nutrient broth by mint essential oil. *Food Res. Int.*, **33**, 273–280.

Thoppil, J.E., Tajo, A., Deena, M.J., and Sreeranjini, K. (2001). Microbicidal properties of mint essential oils. *Ind. Perfum.*, **45**(3), 143–145.

Tkachenko, K.G., Kazarinova, N.V., Muzychenko, L.M., Shurgaya, A.M., Pavlova, O.V., and Safonova, N.G. (1999). Sanation properties of essential oils of some plant species. *Rast. Res.*, **35**(3), 11–24.

Ueda, S., Yamashita, H., Nakajima, M., and Kumabara, Y. (1982). Inhibition of microorganisms by spice extracts and flavouring compounds. *Nippon Shokuhin Kogyo Gakkaishi*, **29**, 111–116.

Viollon, C., Leger, D., and Chaumont, J.P. (1993). Activités antagonistes, *in vitro* de certains composes volatils naturels *vis-à-vis* de germes de la flore vaginale. *Plant. Med. Phytother.*, **26**, 17–22.

Vokou, D., Vareltzidou, S., and Katinakis, P. (1993). Effects of aromatic plants on potato storage: sprout suppression and antimicrobial activity. *Agric. Ecosyst. Environ.*, **47**, 223–235.

Williams, D.R. and Trudgill, P.W. (1994). Ring cleavage reactions in the metabolism of (−)-menthol and (−)-menthone by a *Corynebacterium* sp. *Microbiology*, **140**, 611–616.

Zazki, H.M., Mohamed, S.R., Salman, A.M., and Seleim, R.S. (2001). Exotoxigenicity of *Aeromonas* species isolated from different sources: inhibitory effect of essential oils on their growth. *Ver. Med. J. Giza*, **49**(1), 111–121.

14 *Mentha*: Economic Uses

Arthur O. Tucker

CONTENTS

14.1 INTRODUCTION

Mints of the genus *Mentha* have been traditionally used in flavors, fragrances, and medicines. Recent scientific studies have supported these uses with both raw extracts, essential oils or their isolates; however, cautions on some uses have been reported. This review only covers reprints received until mid-2003.

Of all the 18 species of mint, the most economically important ones are three species, *M. aquatica, M. canadensis,* and *M. spicata*, and their three hybrids, *M.* ×*gracilis*, *M.* ×*piperita*, and *M.* ×*villosonervata*. To a lesser extent, *M. pulegium* is sometimes cultivated or harvested in the wild for oil (Hendriks, 1998).

While "peppermint" and "spearmint" may designate species, in popular parlance, these names are often applied to the flavor instead. The concept of peppermint flavor is primarily based on menthol and its isomers (e.g., isomenthol, neomenthol, neoisomenthol), menthone and its isomers, and menthyl esters; piperitone also contributes to the overall impact. In peppermint oils, menthofuran adds a distinctive mustiness, described as "sweet, haylike-minty odor, sometimes referred to as 'lactone'-odor." The concept of spearmint flavor is primarily based on (*S*)-(−)-carvone [*l*-carvone; *d*-carvone, or (*R*)-(+)-carvone, smells of caraway] and dihydrocarvone, carveol and dihydrocarveol, and carvyl and dihydrocarvyl esters, and, to a lesser extent limonene. The concept of a pennyroyal odor is based on pulegone and its isomers and alcohols, while a bergamot or orange mint odor is based on linalool and linalyl acetate (Arctander, 1969).

14.2 PEPPERMINT AND MENTHOL

Peppermint has a long history of use in flavoring and medicine (Bowen and Cubbin, 1992; Briggs, 1993), and its chemistry is well known (Güntert, et al., 2001). Peppermint oil, extracted from *M.* ×*piperita* is considered to be generally recognized as safe (GRAS) (21CFR182.20, 582.20) at 8–8300 ppm (FEMA No. 2848) (Hall and Oser, 1965; Burdock, 1995). Peppermint oil is antibacterial, antifungal (Piccaglia et al., 1993; Shapiro, et al., 1994; Müller-Riebau et al., 1995; Sivropoulou et al., 1995; Tassou et al., 1995, 2000; Özgüven et al.,

1998; Imai et al., 2001; Inouye et al., 2001; Motlejūnaite and Pečiulyte, 2001), antiallergenic, anti-inflammatory (Arakawa et al., 1992; Juergens et al., 1998; Shin and Kim, 1998), and antispasmodic (Forster et al., 1980). Peppermint also enhances athletic performance (Raudenbush et al., 2001). The usefulness of postprandial peppermint is demonstrated not only by its choleretic activity, but also by its use in relaxing gastrointestinal smooth muscle by reducing calcium influx, thereby relieving irritable bowel syndrome and intestinal cramps in humans (Sigmund and McNally, 1969; Rees et al., 1979; Duthie, 1981; Taylor et al., 1983, 1985; Dew et al., 1984; Somerville et al., 1984; White et al., 1987; Hawthorn et al., 1988; Schneider and Otten, 1990; Dalvi et al., 1991; Hills and Aarson, 1991; Shaw et al., 1991; Trabace et al., 1994; Kingham, 1995; Holt et al., 1996; Liu et al., 1997; White et al., 1997; Koch, 1998; Pittler and Ernst, 1998; May et al., 1996, 2000). However, the action of peppermint oil may be by stimulation of smooth muscle (Rogers et al., 1988; Micklefield et al., 2000), and not all studies have found pain relief (Nash et al., 1986; Lawson et al., 1988). Peppermint oil may also be used to reduce colonic spasm during endoscopy (Leicester and Hunt, 1982; Sparks et al., 1995; Asao et al., 2001) and perhaps improve the manometric features of diffuse esophageal spasm (Massey, 2001; Pimentel et al., 2001). Peppermint oil reduces cholesterol synthesis in rabbits (White et al., 1986) and inhibits enterocyte glucose uptake (Beesley et al., 1996). It also returns Oddi's sphincter (the muscle fibers around the opening of the common bile duct into the duodenum) to normal after contraction by morphine hydrochloride (Giachetti et al., 1988). A combination of peppermint oil and ethanol has a significant analgesic effect with a reduction in sensitivity to headache (Göbel et al., 1995). Peppermint oil also affects behavior, as shown by its promotion of ambulation in mice (Umezu et al., 2001), and it reduces stress (Ogawa et al., 1989).

Natural menthol is primarily produced from Japanese cornmint oil, *M. canadensis* (Clark, 1998). Menthol produces a calcium-controlled outward conductance at the level of the cell membrane in sensory nerves and smooth muscle similar to that of physical cold (Schäfer et al., 1986). Menthol vapor is a significant antitussive (Laude et al., 1994; Morice *et al.,* 1994). While (*S*)-(–)-menthol (*l*-menthol) produces a marked change in nasal sensation of airflow, with a subjective sensation of nasal decongestion, (*R*)-(+)-isomenthol (*d*-isomenthol) and (*R*)-(+)-neomenthol (*d*-neomenthol) have no effect on nasal sensation of airflow (Eccles, 1990, 1994, 1995; Eccles et al., 1987, 1988, 1990a, 1990b; Eccles and Mygind 1985; Wright et al., 1998). Menthol attenuates both capsaicin and neurokinin A-induced bronchoconstriction *in vivo* and relaxes KCl and acetylcholine preconstricted bronchi *in vitro* (Wright et al., 1997). In foodstuffs, the perception of intensity of peppermint is closely linked to sweetness (Linforth and Taylor, 1998). Menthol may also aid in gall stone dissolution (Bell and Doran, 1979; Ellis et al., 1984; Leuschner et al., 1988). Menthone and neomenthol suppress potato tuber sprouting (Coleman et al., 2001).

Menthol hypersensitivity with chronic itchy skin rash, flushing, anal burning, and headaches has been associated with peppermint in some individuals (Tanaka et al., 1948; Papa and Shelley, 1964; Weston, 1987; Wilkinson and Beck, 1994; Morton et al., 1995; Fleming and Forsyth, 1998). Excessive peppermint consumption has been reported to induce stomatitis (Rogers and Pahor, 1995). Addiction to mentholated cigarettes has been reported to produce toxic exhaustive psychosis (Luke, 1962), while excessive peppermint consumption may lead to irregular heartbeat (Thomas, 1962). Nephropathy has been observed in rats for 90 days at 100 mg/kg body weight (Spindler and Madsen, 1992), while encephalopathy was observed in rats dosed with peppermint oil for 28 days at 100 mg/kg body weight (Olsen and Thorup, 1984).

Aqueous extracts of peppermint leaves protect mice against gamma irradiation (Samarth et al., 2001a–c Yasmeen and Kumar, 2001). Luteolin, a yellow pigment found in peppermint leaves, has been shown to be strongly antimutagenic (Samejima et al., 1995). The flavonoid content of peppermint may have additional therapeutic effects (Guédon and Pasquier, 1994) and is antiallergenic (Inoue et al., 2001, 2002), while the phenolic complement of *M. longifolia*

exhibits significant spasmodic, choleretic, and CNS stimulative effects as well as hepatoprotective activity (Mimica-Dukić et al., 1996, 1999); the flavonoid complement of *M. spicata* is antihistaminic (Yamamura et al., 1998).

14.3 SPEARMINT AND RELATED SPECIES

Spearmint has primarily been used historically in food, but it also has medicinal applications (Bowen and Cubbin, 1992). Spearmint oil is considered to be GRAS (21CFR182.20, 582.20) at 90–66,668 ppm (FEMA No. 3032) (Hall and Oser, 1965; Burdock, 1995). Spearmint oil is antibacterial and antifungal (Sivropoulou et al., 1995; Adam et al., 1998; Özgüven et al., 1998; Imai et al., 2001; Aggarwal et al., 2002), but the (*R*)-(+)-isomers of carvone and limonene are more bioactive (Aggarwal et al., 2002). A hexane extract of *M. spicata* is antihelminthic (Villaseñor et al., 1995), while a chloroform extract is anticarcinogenic and antiteratogenic (Villaseñor et al., 1995, 1997, 2002), although spearmint has been reported to cause contact dermatitis (Bonamonte et al., 2001). The essential oil of *M. spicata* is both insecticidal and mutagenic (Franzios et al., 1997).

The oil of *M. aquatica* inhibits acetylcholinesterase activity; to a lesser extent, so do the oils of *M. arvensis* and *M.* ×*gracilis* (Miyazawa et al., 1998). The toluene extract of *M. spicata* repels flies (Thorsell et al., 1989). The methanol extract of *M. suaveolens* reduces the mean arterial blood pressure and heart rate in induced hypertensive rats (Bello et al., 2001). The compound *d*-8-acetoxycarvotanacetone extracted from the oil of *M. canadensis* acts as a repellent against mosquitoes, gnats, and gadflies (Ding and Sun, 1983).

14.4 PENNYROYAL

European pennyroyal, *M. pulegium*, derives its Latin name from *pulex,* or flea, alluding to its traditional use as a strewing herb to repel fleas, and the essential oil is arrestant and insecticidal (Franzios et al., 1997; Landolt et al., 1999). European pennyroyal also has a long history of usage in flavoring foods and as a therapeutic agent (Briggs, 1989). The essential oil is considered to be GRAS by the U.S. Food and Drug Administration for usage in food (21CFR172.510) at 0.59–22.43 ppm (FEMA No. 2839) (Hall and Oser, 1965; Burdock, 1995). The International Organization of the Flavor Industry has recommended a maximum of 22 ppm pulegone in foods (Grundschober, 1979); currently, *The Flavourings in Food Regulations 1992*, as amended in 1994, sets a statutory limit of pulegone at 350 mg/kg in confectionary products and 250 mg/kg in beverages (Davies, 1996). The oil of pennyroyal is antibacterial and antifungal (Müller-Riebau et al., 1995; Sivropoulou et al., 1995; Özgüven et al., 1998).

Moderate to severe toxicity in humans results from exposure to at least 10 ml of the essential oil, and at least three cases of death have occurred from ingesting pennyroyal oil to induce abortions (Vallance, 1955; Gunby, 1979; Sullivan et al., 1979; Anderson et al., 1996). The LD_{50} of European pennyroyal oil in rats is 0.4 g/kg (0.22–0.58 g/kg) body weight, while the acute dermal LD_{50} value in rabbits is 4.2 g/kg (1.9–6.5 g/kg) (Opdyke, 1974). Mice dosed with 400 mg/kg body weight of pennyroyal oil exhibit both hepatotoxicity and lung toxicity; moreover, pulegone, isopulegone, and menthofuran are all toxic (Gordon, 1982). Rats and mice metabolize pulegone to hydroxylated pulegone and menthofuran (Gordon et al., 1987; Thomassen et al., 1991). Rats dosed with pulegone exhibit degeneration of the brain in 28 days with 160 mg pulegone/kg body weight (Olsen and Thorup, 1984). An oil of *M.* ×*villosa* reportedly high in 1,2-epoxypulegone induces muscle contraction in toads (Fogaça et al., 1997). Topical application of pennyroyal oil can cause toxicosis and death in dogs (Sudekum et al., 1992).

ACKNOWLEDGMENTS

Analytical research was supported by the State of Delaware and the Cooperative State Research, Education, and Extension Service (#801-15-01C).

REFERENCES

Adam, K., Sivropoulou, A., Kokkini, S., Lanaras, T., and Arsenakis, M. (1998). Antifungal activities of *Origanum vulgare* subsp. *hirtum*, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa* essential oils against human pathogenic fungi. *J. Agric. Food Chem.*, **46**, 1739–1745.

Aggarwal, K.K., Khanuja, S.P.S., Ahmad, A., Kumar, T.R.S., Gupta, V.K., and Kumar, S. (2002). Antimicrobial activity profiles of the two enantiomers of limonene and carvone isolated from the oils of *Mentha spicata* and *Anethum sowa*. *Flav. Fragr. J.*, **17**, 59–63.

Anderson, I.B., Mullen, W.H., Meeker, J.E., Khojasteh-Bakht, S.C., Oishi, S., Nelson, S.D., and Blanc, P.D. (1996). Pennyroyal toxicity: Measurement of toxic metabolite levels in two cases and review of the literature. *Ann. Int. Med.*, **124**, 726–734.

Arakawa, T., Shibata, M., Hosomi, K., Watanabe, T., Honma, Y., Kawasumi, K., and Takeuchi, Y. (1992). Anti-allergic effects of peppermint oil, chicle and jelutong. *J. Food Hygienic Soc. Jpn.*, **33**, 569–575.

Arctander, S. (1969). *Perfume and Flavor Chemicals (Aroma Chemicals)*. S. Arctander, Montclair, New Jersey.

Asao, T., Mochiki, E., Suzuki, H., Nakamura, J.-Ii., Hirayama, I., Morinaga, N., Shoji, H., Shitara, Y., and Kuwano, H. (2001). An easy method for the intraluminal administration of peppermint oil before colonscopy and its effectiveness in reducing colonic spasm. *Gastrointest. Endosc.*, **53**, 172–177.

Beesley, A., Hardcastle, J., Hardcastle, P.T., and Taylor, C.J. (1996). Influence of peppermint oil on absorptive and secretory processes in rat small intestine. *Gut*, **39**, 214–219.

Bell, G.D. and Doran, J. (1979). Gall stone dissolution in man using an essential oil preparation. *Br. Med. J.*, **278**, 24.

Bello, R., Calatayud, S., Beltrán, B., Primo-Yúfera, E., and Explugues, J. (2001). Cardiovascular effects of the methanol and dichloromethanol extracts from *Mentha suaveolens* Ehrh. *Phytother. Res.*, **15**, 447–448.

Bonamonte, D., Mundo, L., Daddabbo, M., and Foti, C. (2001). Allergic contact dermatitis from *Mentha spicata* (spearmint). *Contact Derm.*, **45**, 298.

Bowen, I.H. and Cubbin, I.J. (1992). *Mentha piperita* and *Mentha spicata*. In: *Adverse Effects of Herbal Drugs*, De Smet, P.A.G.M., Keller, K., Hänsel, R., and Chandler, R.F., Eds., Adverse effects of herbal drugs 1. Springer-Verlag, Berlin, pp. 171–178.

Briggs, C.J. (1989). Pennyroyal: A traditional medicinal herb with toxic potential. *Can. Pharm. J.*, **122**, 369, 371–372.

Briggs, C.J. (1993). Peppermint: Medicinal herb and flavoring agent. *Canad. Pharm. J.*, **126**, 89–92.

Burdock, G.A. (1995). *Fenaroli's Handbook of Flavor ingredients*. 3rd edn. CRC Press, Boca Raton, FL.

Clark, G.S. (1998). Menthol. *Perfum. Flav.*, **23**(5), 33–34, 36, 38, 40, 42–46.

Coleman, W.K., Lonergan, G., and Silk, P. (2001). Potato sprout growth suppression by menthone and neomenthol, volatile oil components of *Minthostachys, Satureja, Bystropogon*, and *Mentha* species. *Am. J. Potato Res.*, **78**, 345–354.

Dalvi, S.S., Nadkarni, P.M., Pardesi, R., and Gupta, K.C. (1991). Effect of peppermint oil on gastric emptying in man: A preliminary study using a radiolabelled solid test meal. *Indian J. Physiol. Pharmacol.*, **35**, 212–214.

Davies, A.M. (1996). Survey of biologically active principles in mint products and herbal teas. *Food Surv. Inform. Sheet*, **99**, 1–8.

Dew, M.J., Evans, B.K., and Rhodes, J. (1984). Peppermint oil for the irritable bowel syndrome: A multicentre trial. *Br. J. Clin. Pract.*, **38**, 394, 398.

Ding, D. and Sun, H. (1983). Structural determination of a repellent principle in the essential oil of *Mentha haplocalyx* Briq. *Zhiwu Xuebao*, **25**, 62–66.

Duthie, H.L. (1981). The effect of peppermint oil on colonic motility in man. *Br. J. Surg.*, **68**, 820.
Eccles, R. (1990). Effects of menthol on nasal sensation of airflow. In: *Chemical Senses*, Green, B.G., Mason, J.R., and Kare, M.R., Eds., Chemical senses. Vol. 2. Irritation. Marcel Dekker, New York, pp. 275–295.
Eccles, R. (1994). Menthol and related cooling compounds. *J. Pharm. Pharmacol.*, **46**, 618–630.
Eccles, R. (1995). Menthol: From folklore to phemacology. *Int. Pharm. J.*, **6**, 23–24.
Eccles, R. and Mygind, N. (1985). Physiology of the upper airways in allergic disease. *Clin. Rev. Allergy*, **3**, 501–516.
Eccles, R., Lancashire, B., and Tolley, N.S. (1987). The effect of aromatics on inspiratory and expiratory nasal resistance to airflow. *Clin. Otolaryngol.*, **12**, 11–14.
Eccles, R., Griffiths, D.H., Newton, C.G., and Tolley, N.S. (1988). The effect of menthol isomers on nasal sensation of airflow. *Clin. Otolarynol.*, **13**, 25–29.
Eccles, R., Morris, S., and Tolley, N.S. (1988). The effects of nasal anaesthesia upon nasal sensation of airflow. *Acta Otolaryngol.*, **106**, 151–155.
Eccles, R., Jawad, M.S., and Morris, S. (1990a). The effects of oral administration of (−)-menthol on nasal resistance to airflow and nasal sensation of airflow in subjects suffering from nasal congestion associated with the common cold. *J. Pharm. Pharmacol.*, **42**, 652–654.
Eccles, R., Morris, S., and Jawad, M.S.M. (1990b). The effects of menthol on reaction time and nasal sensation of airflow in subjects suffering from the common cold. *Clin. Otolaryngol.*, **15**, 39–42.
Ellis, W.R., Somerville, K.W., Whitten, B.H., and Bell, G.D. (1984). Pilot study of combination treatment for gall stones with medium dose chenodeoxycholic acid and a terpene preparation. *Br. Med. J.*, **289**, 153–156.
Fleming, C.J. and Forsyth, A. (1998). D5 patch test reactions to menthol and peppermint. *Contact Derm.*, **38**, 337.
Fogaça, R.T.H., Cavalcante, A.D.A., Serpa, A.K.L., Sousa, P.J.C., Coelho-de-Souza, A.N., and Leal-Cardoso, J.H. (1997). The effects of essential oil of *Mentha* ×*villosa* on skeletal muscle of toad. *Phytother. Res.*, **11**, 552–557.
Forster, H.B., Niklas, H., and Lutz, S. (1980). Antispasmodic effects of some medicinal plants. *Planta Med.*, **40**, 309–319.
Franzios, G., Mirotsou, M., Hatziapostolou, E., Kral, J., Scouras, Z.G., and Mavragani-Tsipidou, M. (1997). Insecticidal and genotoxic activities of mint essential oils. *J. Agric. Food Chem.*, **45**, 2690–2694.
Giachetti, D., Taddei, E., and Taddei, I. (1988). Pharmacological activity of essential oils on Oddi's sphincter. *Planta Med.*, **54**, 389–392.
Göbel, H., Schmidt, G., Dqorschak, M., Stolze, H., and Heuss, D. (1995). Essential plant oils and headache mechanisms. *Phytomedicine*, **2**, 93–102.
Gordon, W.P., Forte, A.J., McMurtry, R.J., Gal, J., and Nelson, S.D. (1982). Hepatotoxicity and pulmonary toxicity of pennyroyal oil and its constituent terpenes in the mouse. *Toxic. Appl. Pharmacol.*, **65**, 413–424.
Gordon, W.R., Huitric, A.C., Seth, C.L., McClanahan, R.H., and Nelson, S.D. (1987). The metabolism of the abortifacient terpene, (*R*)-(+)-pulegone, to a proximate toxin, menthofuran. *Drug. Metab. Dispos.*, **15**, 589–594.
Grundschober, F. (1979). Literature review of pulegone. *Perfum. Flavos.*, **4**(1), 15–17.
Guédon, D.J. and Pasquier, B.P. (1994). Analysis and distribution of flavonoid glycosides and rosmarinic acid in 40 *Mentha* ×*piperita* clones. *J. Agric. Food Chem.*, **42**, 670–684.
Gunby, P. (1979). Plant known for centuries still causes problems today. *J. Am. Med. Assoc.*, **241**, 2246–2247.
Güntert, M., Krammer, G., Lambrecht, S., Sommer, H., Surburg, H., and Werkhoff, P. (2001). Flavor chemistry of peppermint oil (*Mentha piperita* L.). In: *Aroma Active Compounds in Foods*. Takeoka, G., Güntert, M., and Hengel, K.-H., Eds., Am. Chem. Soc., Washington, DC, pp. 119–137.
Hall, R.L. and Oser, B.L. (1965). Recent progress in the consideration of flavoring ingredients under the Food Additives Amendment 3, GRAS substances. *Food Technol.*, **19**(2), 151–197.

Hawthorn, M., Ferrante, J., Luchowski, E., Rutledge, A., Wei, X.Y., and Triggle, D.J. (1988). The actions of peppermint oil and menthol on calcium channel dependent processes in intestinal, neuronal and cardiac preparations. *Aliment. Pharmacol. Therap.*, **2**, 101–118.

Hendriks, H. (1998). Pharmaceutical aspects of some *Mentha* herbs and their essential oils. *Perfum. Flav.*, **23**(6), 15–23.

Hills, J.M. and Aaronson, P.I. (1991). The mechanism of actin of peppermint oil on gastrointestinal smooth muscle. *Gastroenterology*, **101**, 55–65.

Holt, S., Muntyan, I., and Likver, L. (1996). Essential oils smooth gastric functioning. *Altern. Complement Ther.*, January/February 1996, 46–50.

Imai, H., Osawa, K., Yasuda, H., Hamashima, H., Arai, T., and Sasatsu, M. (2001). Inhibition by the essential oils of peppermint and spearmint of the growth of pathogenic bacteria. *Microbios*, **106**(S1), 31–39.

Inoue, T., Sugimoto, Y., Masuda, H., and Kamei, C. (2001). Effects of peppermint (*Mentha piperita* L.) extracts on experimental allergic rhinitis in rats. *Biol. Pharm. Bull.*, **24**, 92–95.

Inoue, T., Sugimoto, Y., Masuda, H., and Kamei, C. (2002). Antiallergic effect of flavonoid glycosides obtained from *Mentha piperita* L. *Biol. Pharm. Bull.*, **25**, 256–259.

Inouye, S., Yamagushi, H., and Takizawa, T. (2001). Screening of the antibacterial effects of a variety of essential oils on respiratory tract pathogens, using a modified dilution assay method. *J. Infect. Chemother.*, **7**, 251–254.

Juergens, U.R., Stöber, M., and Vetter, H. (1998). The anti-inflammatory activity of L-menthol compared to mint oil in human monocytes *in vitro*: a novel perspective for its therapeutic use in inflammatory diseases. *Eur. J. Med. Res.*, **3**, 539–545.

Kingham, J.G.C. (1995). Peppermint oil and colon spasm. *Lancet*, **1995**(2), 986.

Koch, T.R. (1998). Peppermint oil and irritable bowel syndrome. *Am. J. Gastroenterol.*, **93**, 2304–2305.

Landolt, P.J., Hofstetter, R.W., and Biddick, L.L. (1999). Plant essential oils as arrestants and repellents for neonate larvae of the codling moth (Leidoptera: Torticidae). *Physiol. Chem. Ecol.*, **28**, 954–960.

Laude, E.A., Morice, A.H., and Grattan, T.J. (1994). The antitussive effects of menthol, camphor and cineole in conscious guinea-pigs. *Pulm. Pharmacol.*, **7**, 179–184.

Lawson, M.J., Knight, R.E., Tran, K., Walker, G., and Roberts-Thomson, I.C. (1988). Failure of enteric-coated peppermint oil in the irritable bowel syndrome: A randomized, double-blind crossover study. *J. Gastroenterol. Hepatol.*, **3**, 235–238.

Leicester, R.J. and Hunt, R.H. (1982). Peppermint oil to reduce colonic spasm during endoscopy. *Lancet*, **1982**(2), 989.

Leuschner, M., Leuschner, U., Lazarovici, D., Kurtz, W., and Hellstern, A. (1988). Dissolution of gall stones with an ursodeoxycholic acid menthol preparation: A controlled prospective double blind trial. *Gut.*, **29**, 428–432.

Linforth, R. and Taylor, A. (1998). Volatile release from mint-flavored sweets. *Perfum. Flav.*, **23**(3), 47–48, 50, 52–53.

Liu, J.-H., Chen, G.-H., Yeh, H.-Z., Huang, C.-K., and Poon, S.-K. (1997). Enteric-coated peppermint-oil capsules in the treatment of irritable bowel syndrome: A prospective, randomized trial. *J. Gastroenterol.*, **32**, 765–768.

Luke, E. (1962). Addiction to mentholated cigarettes. *Lancet*, **1962**(1), 110–111.

Massey, B.T. (2001). Diffuse esophageal spasm: A case for carminatives? *J. Clin. Gastroenterol.*, **33**, 8–10.

May, B., Kuntz, H.-D., Kieser, M., and Köhler, S. (1996). Efficacy of a fixed peppermint oil/caraway oil combination in non-ulcer dyspepsia. *Arzneimittel-Forschung*, **46**, 1149–1153.

May, B., Köhler, S., and Schneider, B. (2000). Efficacy and tolerability of a fixed combination of peppermint oil and caraway oil in patients suffering from functional dyspepsia. *Aliment. Pharmacol. Ther.*, **14**, 1671–1677.

Micklefield, G.H., Greving, I., and May, B. (2000). Effects of peppermint oil and caraway oil on gastroduodenal motility. *Phytother. Res.*, **14**, 20–21.

Mimica-Dukić, N., Jakovljević, V., Mira, P., Gašić, O., and Szabo, A. (1996). Pharmacological study of *Mentha longifolia* phenolic extracts. *Int. J. Pharmacogn.*, **34**, 359–364.

Mimica-Dukic, N., Popović, M., Jakovljević, V., Szabo, A., and Gašić O. (1999). Pharmacological studies of *Mentha longifolia* phenolic extracts. II. Hepatoprotective activity. *Pharm. Biol.*, **37**, 221–224.

Miyazawa, M., Watanabe, H., Umemoto, K., and Kameoka, H. (1998). Inhibition of acetylcholinesterase activity by essential oils of *Mentha* species. *J. Agric. Food Chem.*, **46**, 3431–3434.

Morice, A.H., Marshall, A.E., Higgins, K.S., and Grattan, T.J. (1994). Effect of inhaled menthol on citric acid induced cough in normal subjects. *Thorax*, **49**, 1024–1026.

Motlejūnaite, O. and Pečiulyte, D. (2001). Response of micromyctes to the effect of mint volatile oil. *Biologija*, **2001(3)**, 38–41.

Morton, C.A., Garioch, J., Todd, P., Lamey, P.J., and Forsyth, A. (1995). Contact sensitivity to menthol and peppermint in patients with intra-oral symptoms. *Contact Derm.*, **32**, 281–284.

Müller-Riebau, F., Berger, B., and Yegen, O. (1995). Chemical composition and fungitoxic properties to phytopathogenic fungi of essential oils of selected aromatic plants growing wild in Turkey. *J. Agric. Food Chem.*, **43**, 2262–2266.

Nash, P., Gould, S.R., and Barnardo, D.E. (1986). Peppermint oil does not relieve the pain of irritable bowel syndrome. *Br. J. Clin. Pract.*, **40**, 292–293.

Ogawa, T., Nakata, S., Konishi, H., Kishi, M., Nakai, C., Mizutani, H., Kawaguchi, T., and Jijiwa, H. (1989). Effects of fragrance on stress. *J. Jpn. Cosmet. Sci. Soc.*, **13(3)**, 140–143.

Olsen, P. and Thorup, I. (1984). Neurotoxicity in rats dosed with peppermint oil and pulegone. *Arch. Toxicol. Suppl.*, **7**, 408–409.

Opdyke, D.L.J. (1974). Monographs on fragrance raw materials. *Food Cosmet. Toxicol.*, **12**, 807–1016.

Özgüven, M., Kirici, S., Yaman, A., Aksungur, P., and Gür, A. (1998). Antimicrobial activity of essential oil of wild *Mentha* species growing in Southern-Turkey. *Pharm. Pharmacol. Lett.*, **8**, 164–167.

Papa, C.M. and Shelley, W.B. (1964). Menthol hypersensitivity. *J. Am. Med. Assoc.*, **189**, 546–548.

Piccaglia, R., Marotti, M., Giovanelli, E., Deans, S.G., and Eaglesham, E. (1993). Antibacterial and antioxidant properties of Mediterranean aromatic plants. *Ind. Crops Prod.*, **2**, 47–50.

Pimentel, M., Bonorris, G.G., Chow, E.J., and Lin, H.C. (2001). Peppermint oil improves the manometric findings in diffuse esophageal spasm. *J. Clin. Gastroenterol.*, **33**, 27–31.

Pittler, M.H. and Ernst, E. (1998). Peppermint oil for irritable bowel syndrome: A critical review and metaanalysis. *Am. J. Gastroenterol.*, **93**, 1131–1135.

Raudenbush, B., Corley, N., and Eppich, W. (2001). Enhancing athletic performance through the administration of peppermint odor. *J. Sport Exerc. Psychol.*, **23**, 156–160.

Rees, W.D., Evans, B.K., and Rhodes, J. (1979). Treating irritable bowel syndrome with peppermint oil. *Br. Med. J.*, **2**(6194), 835–836.

Rogers, J., Tay, H.H., and Misievicz, J.J. (1988). Peppermint oil. *Lancet*, **1988**(2), 98–99.

Rogers, S.N. and Pahor, A.L. (1995). A form of stomatitis induced by excessive peppermint consumption. *Dent. Update*, **22**, 36–37.

Samarth, R.M., Goyal, P.K., and Kumar, A. (2001a). Radio protective activity of *Mentha piperita* (Linn) against radiation induced alterations in peripheral blood of Swiss albino mice. *Radiat Prot. Environ.*, **24**, 31–35.

Samarth, R.M., Goyal, P.K., and Kumar, A. (2001b). Modulatory effect of *Mentha piperita* (Linn.) on serum phosphatases activity in Swiss albino mice against gamma irradiation. *Ind. J. Exp. Biol.*, **39**, 479–482.

Samarth, R.M., Goyal, P.K., and Kumar, A. (2001c). Radio protective effects of *Mentha Piperita*. *J. Med. Arom. Pl. Sci.*, **22**/**23**(4A/1A), 91–97.

Samejima, K., Kanazawa, K., Ashida, H., and Danno, G.-I. (1995). Luteolin: A strong antibutagen against dietary carcinogen, Trp-P-2, in peppermint, sage, and thyme. *J. Agric. Food Chem.*, **43**, 410–414.

Schäfer, K., Braun, H.A., and Isenberg, C. (1986). Effect of menthol on cold receptor activity. *J. Gen. Physiol.*, **88**, 757–776.

Schneider, M.M.E. and Otten, M.H. (1990). Efficacy of colpermin in the treatment of patients with the irritable bowel syndrome. *Gastroenterology*, **98**, A389.

Shapiro, S., Meier, A., and Guggenheim, B. (1994). The antimicrobial activity of essential oils and essential oil components toward oral bacteria. *Oral Microbiol. Immunol.*, **9**, 202–208.

Shaw, G., Srivastava, E.D., Sadier, M., Swann, P., James, J.Y., and Rhodes, J. (1991). Stress management for irritable bowel syndrome: A controlled trial. *Digestion*, **50**, 36–42.

Shin, T.-Y. and Kim, D.-K. (1998). Antiallergic activity of menthae herba. *Kor. J. Pharmacogn.*, **29**, 248–253.

Sigmund, C.J. and McNally, E.F. (1969). The action of a carminative on the lower esophageal sphincter. *Gastroenterology*, **56**, 13–18.

Sivropoulou, A., Kokkini, S., Lanaras, T., and Arsenakis, M. (1995). Antimicrobial activity of mint essential oils. *J. Agric. Food Chem.*, **43**, 2384–2388.

Somerville, K.W., Richmond, C.R., and Bell, G.D. (1984). Delayed release peppermint oil capsules (colpermin) for the spastic colon syndrome: A pharmacokinetic study. *Br. J. Clin. Pharmacol.*, **18**, 638–640.

Sparks, M.J.W., O'Sullivan, P., Herrington, A.A., and Marcos, S.K. (1995). Does peppermint oil relieve spasm during barium enema? *Br. J. Radiol.*, **68**, 841–843.

Spindler, P. and Madesen, C. (1992). Subchronic toxicity study of peppermint oil in rats. *Toxicol. Lett.*, **62**, 215–220.

Sudekum, M., Poppenga, R.H., Raju, N., and Braselton, W.E. (1992). Pennyroyal oil toxicosis in a dog. *J. Am. Vet. Med. Assoc.*, **200**, 817–818.

Sullivan, J.B., Rumack, B.H., Thomas, H., Peterson, R.G., and Brywon, P. 1979. Pennyroyal oil poisoning and hepatotoxicity. *J. Am. Med. Assoc.*, **242**, 2873–2874.

Tanaka, T., Tanabe, T., and Tanaka, M. (1948). On the skin-irritant action of the principle of peppermint oil. *Folia Pharmacol. Jpn.*, **43**, 80–84.

Tassou, C.C., Drosinos, E.H., and Nychas, G.J. E. (1995). Effects of essential oil from mint (*Mentha piperita*) on *Salmonella enteritidis* and *Listeria monocytogenes* in model food systems at 4°C and 10°C. *J. Appl. Bacteriol.*, **78**, 593–600.

Tasou, C.C., Koutsoumanis, K., and Nychas, G.-J.E. (2000). Inhibition of *Salmonella enteridis* and *Staphylococcus aureus* in nutrient broth by mint essential oil. *Food Res. Int.*, **33**, 273–280.

Taylor, B.A., Luscombe, D.K., and Duthie, H.L. (1983). Inhibitory effect of peppermint oil on gastrointestinal smooth muscle. *Gut*, **24**, 992.

Taylor, B.A., Duthie, H.L., and Luscombe, D.K. (1985). Mechanism by which peppermint oil exerts its relaxant effect on gastrointestinal smooth muscle. *J. Pharm. Pharmacol.*, **37**, 104P.

Thomas, J.G. (1962). Peppermint fibrillation. *Lancet*, **1962**(1), 222.

Thomassen, D., Pearson, P.G., Slattery, J.T., and Nelson, S.D. (1991). Partial characterization of biliary metabolites of pulegone by tandem mass spectrometry. Detection of glucuronide, glutathione, and glutathionyl glucuronide conjugates. *Drug Metab. Dispos.*, **19**, 997–1003.

Thorsell, W., Mikiover, A., Main, E., and Mikiver, M. (1989). Flugrepellenter ur extrakt från *Mentha spicata* × *crispata*–studier med *Protophormia terraenovae. Entomol. Tidskr.*, **110**, 109–112.

Trabace, L., Avato, P., Mazzoccoli, M., and Siro-Brigani, G. (1994). Choleretic activity of Thapsia Chem. I, II, and II in rats: Comparison with terpenoid constituents and peppermint oil. *Phytother. Res.*, **8**, 305–307.

Umezu, T., Sakata, A., and Ito, H. (2001). Ambulation-promoting effect of peppermint oil and identification of its active constituents. *Pharmacol. Biochem. Behav.*, **69**, 383–390.

Vallance, W.B. (1955). Pennyroyal oil poisoning: A fatal case. *Lancet*, **2**, 850–851.

Villaseñirm I., Catlon, M.A.L.N.D., Chu, C.C.S., Edu, D.A., and Nakar, J.P. (1995). Preliminary bioactivity studies on *Mentha cordifolia* Opiz. leaf extracts. *Philip. J. Sci.*, **124**, 333–343.

Villaseñirm I., Echegoyen, D.E., and Angelada, J.S. (2002). A new antimutagen from *Mentha cordifolia* Opiz. *Mutation Res. Genet. Toxicol. Environ. Mutagen.*, **515**, 141–146.

Villaseñor, I., Aberion, D.P.S., and Angelada, J.S. (1997). Anticarcinogenicity and antiteratogenicity potential of the antimutagenic chloroform leaf extract from *Mentha cordifolia* Opiz. *Philip. J. Sci.*, **126**, 207–213.

Weston, C.F.M. (1987). Anal burning and peppermint oil. *Postgrad. Med. J.*, **63**(642), 717.

White, D.A., Bell, G.D., and Middleton, B. (1986). Effects of terpenes and terpene analogues on cholesterol metabolism. In: *Pharmacological Control of Hyperlipidaemia*, Miller, N., Prous, J.R., Eds., Rome, 389–399.

White, D.A., Thompson, S.P., Wilson, C.G., and Bell, G.D. (1987). A pharmacokinetic comparison of two delayed-release peppermint oil preparations, Colpermin and Mintec, for treatment of the irritable bowel syndrome. *Int. J. Pharm.*, **40**, 151–155.

White, C.E., Laude, E.A., Grattan, T.J., and Morice, A.H. (1997). Capsaicin and neurokinin A-induced bronchoconstriction in the anaesthetized guinea-pig: evidence for a direct action of menthol on isolated bronchial smooth muscle. *Br. J. Pharmacol.*, **121**, 1645–1650.

Wilkinson, S.M. and Beck, M.H. (1994). Allergic contact dermatitis from menthol in peppermint. *Contact Derm.*, **30**, 42–43.

Wright, C.E., Bowen, W.P., Grattan, T.J., and Morice, A.H. (1998). Identification of the L-menthol binding site in guinea-pig lung membranes. *Br. J. Pharmacol.*, **123**, 481–486.

Yamamura, S., Ozawa, K., Ohtani, K., Kasai, O., and Yamasaki, K. (1998). Antihistaminic flavones and aliphatic glycosides from *Mentha spicata. Phytochemistry*, **48**, 131–136.

Yasmeen, A. and Kumar, A. (2001). Evaluation of chemoprevention of skin papilloma by *Mentha Piperita. J. Med. Arom. Pl. Sci.*, **22**/**23**(4A/1A), 84–88.

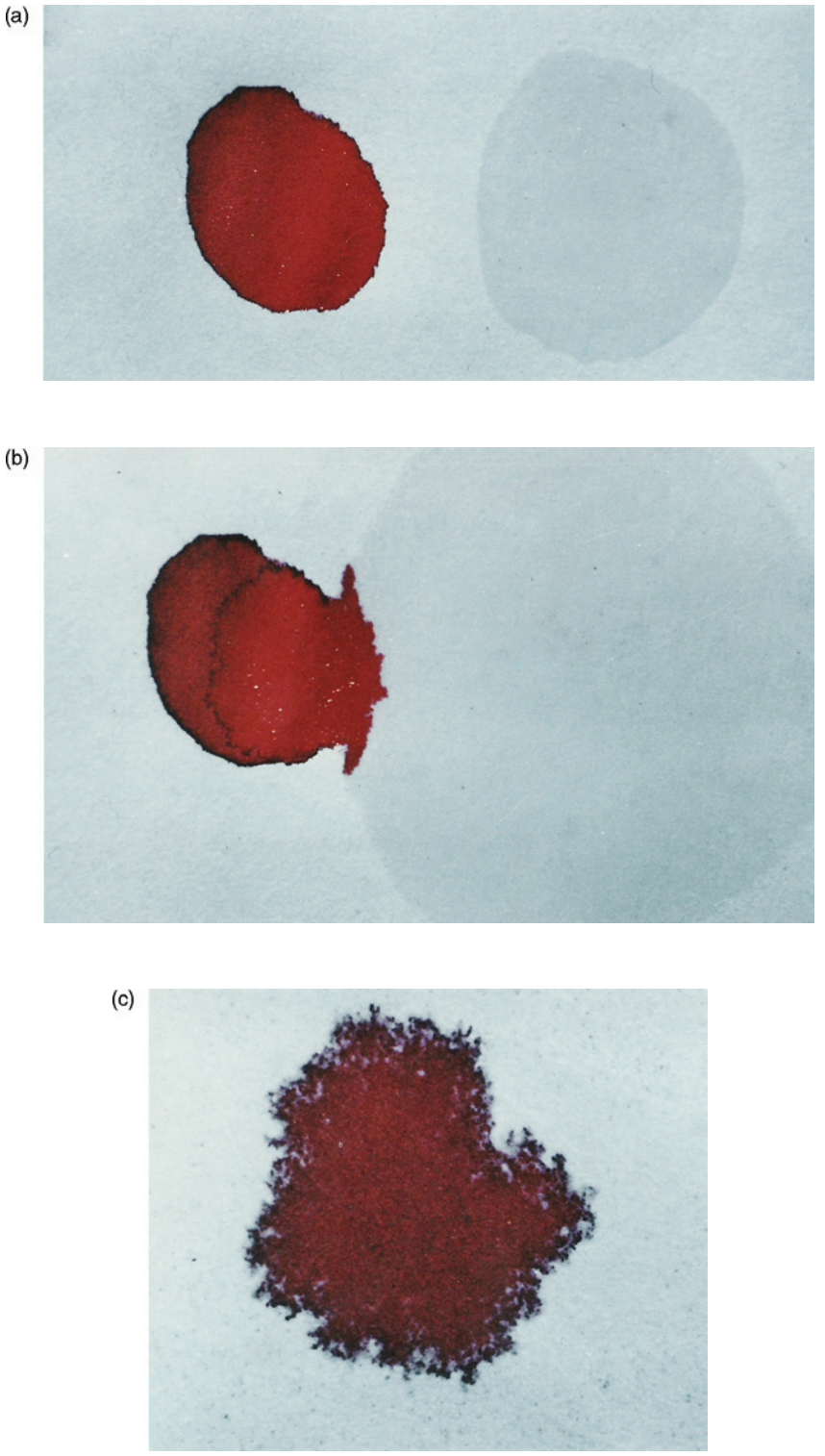


FIGURE 6.11 Oil and water on an absorptive surface. (a) Drops of oil and water on absorptive paper (water that is not oil soluble is dyed red). (b) The spreading areas of oil and water meet and intermingle. (c) The intermingling of oil and water at the interface.

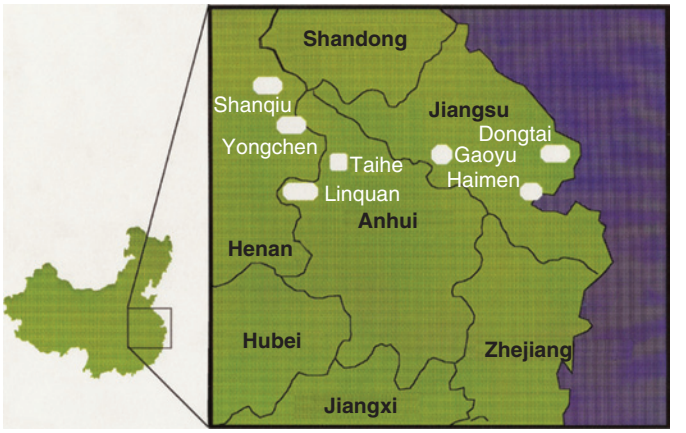


FIGURE 10.3 Chinese cornmint growing areas.

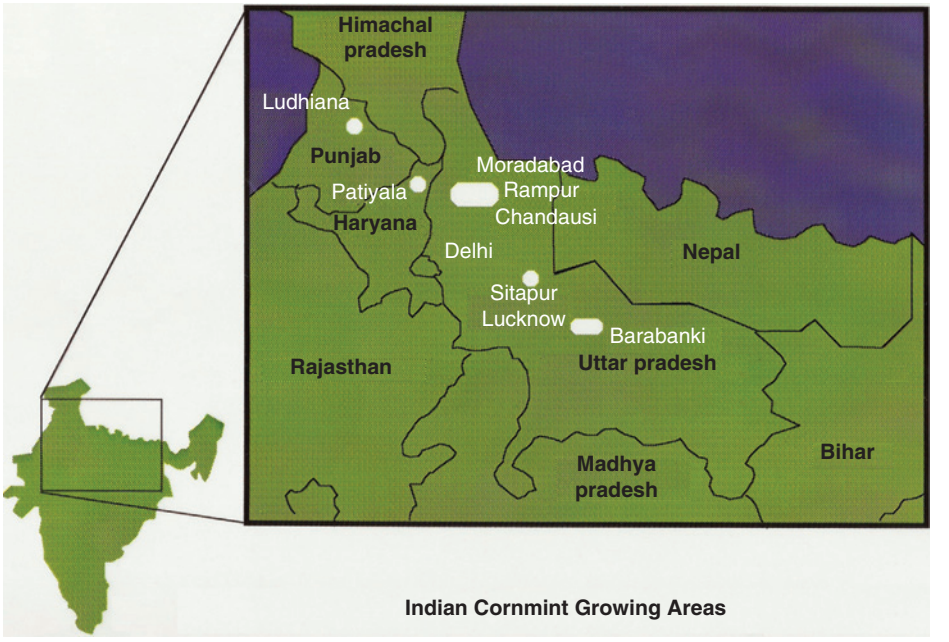


FIGURE 10.4 Indian cornmint growing areas.