THE YIELD AND ESSENTIAL OIL CONTENT OF MINT (*MENTHA SSP.*) IN NORTHERN OSTROBOTHNIA

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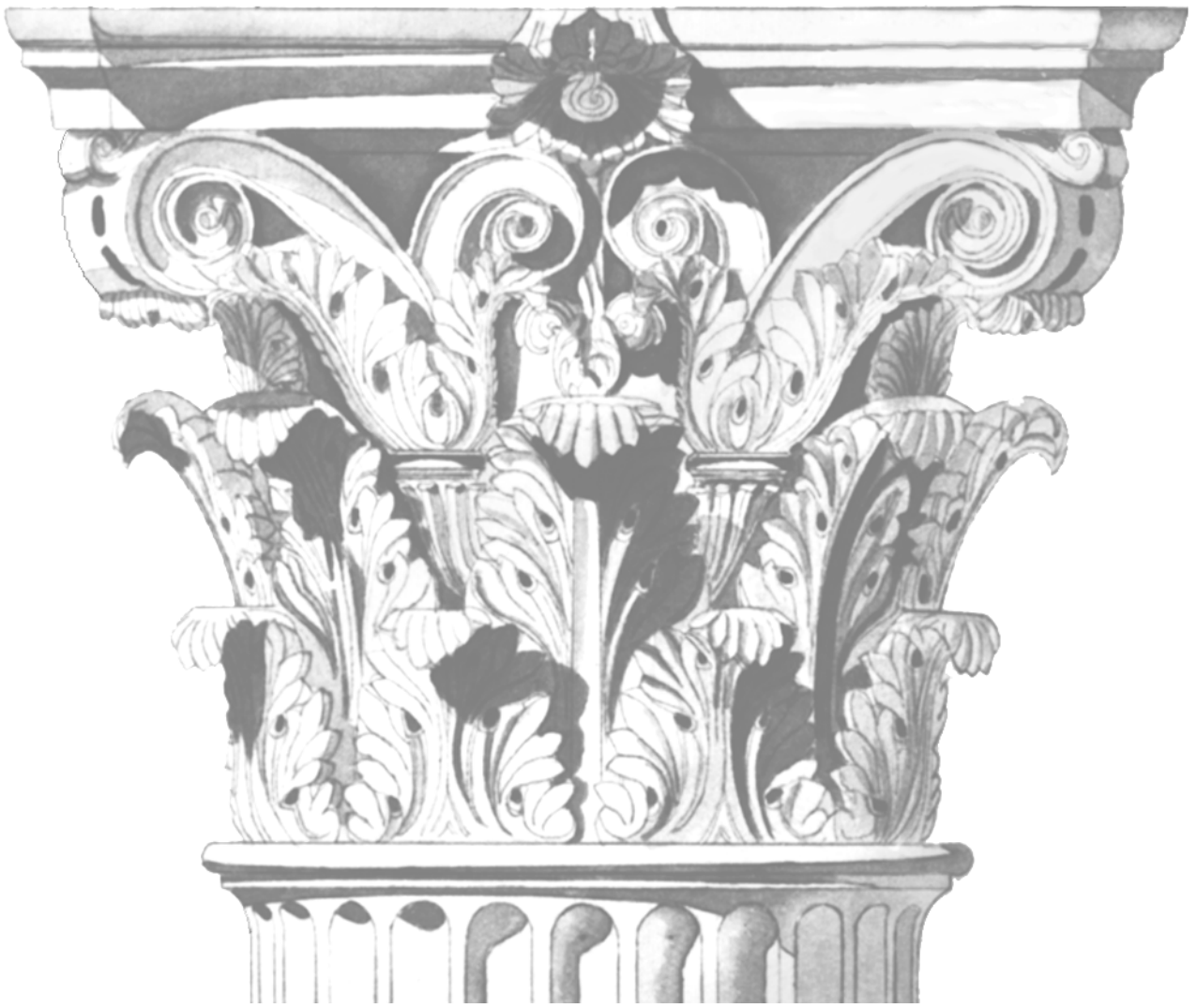

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Abstract

Peppermint (*Mentha* x *piperita* L.) oil is one of the most popular and widely used essential oils, mostly because of its main components menthol and menthone. Peppermint oil is used for flavouring pharmaceuticals and oral preparations. Corn mint is the richest source of natural menthol. Carvone-scented mint plants, such as spearmint (*M. spicata*), are rich in carvone and are widely used as spices, and they are cultivated in several countries. Studies were made into the yield and essential oil content of several mint species and the original. The general aim of the work was to examine the optimal conditions for cultivating mint in Northern Finland. The specific aims of the study were (first) to investigate the differences in the oil content for several mint species and (secondly) to compare the effect of various factors such as plant spacing (10, 20 and 30 × 50 cm), liming (0, 4, 8, 12 and 16 tons ha^{-1}), propagation methods (micropropagated and conventionally propagated plants) and harvest date (once at the end of August in comparison with first cut at the beginning of August and second cut in mid September) on the cultivation success, quality and quantity of the plants. The constituents of the essential oil were analysed from leaf samples using GC-MS.

Among the peppermints of different origins studied, peppermint of USA and Egypt origin ('Black Mitcham') contain the highest menthol and optimum oil yield. Corn mint and Sachalin mints both had high menthol content. Due to several reasons, such as no significant differences between the different densities and oil composition, markedly higher amount of weeds at 30 × 50 cm than at 10 × 50 and 20 × 50 cm spacing and the high seedling costs and the danger of fungi and disease at a 10 × 50 cm spacing, a plant optimum of 20 × 50 cm spacing is recommended for Northern Ostrobothnia. If the pH value is lower than 6, or levels of Mg and Ca are low, liming at a rate of 4–8 t ha^{-1} for sandy soils in Finland is recommended in order to achieve higher fresh and oil yields. In the first year, there were no differences in the dry leaf yield of micropropagated and conventionally propagated plants, but the menthol content was significantly higher in conventionally than in micropropagated plants. In the second year, only the dry leaf yield of micropropagated plants was higher than that of their conventionally propagated counterparts. Cutting peppermint only once during full bloom (the end of August) gives the maximum oil yield of good quality. In conclusion, it is possible to achieve as high as or even higher oil quality and dry yield in North Ostrobothnia than it is in central Europe or south Asia. However, this requires observing certain cultivation factors such as having the right type of mint, soil pH, planting density, harvesting time and propagation method In addition, mints must be cultivated in the same place for only two and a maximum for three years.

Keywords: harvest time, in vitro propagation, Lamiaceae, lime, Mentha arvensis var. piperascens, Mentha arvensis var. sachalinensis, Mentha canadensis, Mentha x piperita, menthol, menthone, pH, plant density, propagation methods, roots, stolons

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List of original papers

This thesis is based on the following publications. They are referred to in the text by their Roman numerals:

I Aflatuni A, Heikkinen K, Tomperi P, Jalonen J & Laine K (2000) Variation in the extract composition of mints of different origin cultivated in Finland. Journal of Essential Oil Research 12: 462–466.

II Rissanen KS, Aflatuni A, Tomperi PH, Jalonen JE & Laine K (2002) Herbage and essential oil yield and composition of *Mentha piperita* L. in different plant densities in Northern latitudes. Journal of Essential Oil Research 14: 243–246.

III Aflatuni A, Uusitalo J, Ek S & Hohtola A (2005) Variation in the amount of yield and in the extract composition between conventionally produced and micropropagated peppermint and spearmint. Journal of Essential Oil Research 15: 66–70.

IV Aflatuni A, Uusitalo J, Ek S & Hohtola A (2003) Effect of liming on yield and quality of peppermint and Sachalin mint in fine sand soil of Northern Finland. Agricultural and Food Science in Finland 12: 107–115.

V Aflatuni A, Uusitalo J, Ek S & Hohtola A (2005) Optimum harvesting time of four *Mentha* species in northern Finland (accepted for publication in Journal of Essential Oil Research).

Contents

1 Introduction

1.1 Secondary metabolites in plants

Secondary metabolites are present in all higher plants, usually in high structural diversity. Many metabolites have been found to protect plants against viruses, bacteria, fungi, and most importantly against herbivores. Many secondary metabolities such as cyanogenic glycosides, glucosinolates, terpenes, saponins, tannins, anthraquinones, and polyacetylenes also act as allelochemicals, influencing the growth and development of neighbouring plants (Wink 2003). For example, monoterpene limonene has shown deterrent and insecticide properties and carvone is used as sprouting inhibitors (Ibrahim *et al.* 2001, Aflatuni 2003).

Essential oils are complex and highly variable mixtures of constituents that belong to two groups: terpenoids and aromatic compounds. Hydrocarbons are almost always present in monoterpenes (Bruneton 1995). Essential oils accumulate in all types of vegetative organs: flowers (bergamot tree), leaves (mint, eucalyptus), barks (cinnamon), woods (sandalwood), roots (vetiver), rhizomes (ginger), fruits (anise), and seeds (nutmeg). Essential oils are usually associated with specialized storage in plants (Bruneton 1995).

Although essential oils are comprised of many types of compounds, the major ones are monoterpenes (Seigler 1998). The synthesis and accumulation of essential oil structures are located near the surface, glandular trichomes, secretory cavities or secretory canals of the plants (Bruneton 1995).

The impact of environmental factors such as temperature, relative humidity, irradiance, photoperiod and cultivation practices influence the composition of essential oils. The influence of the method of extraction on oil composition and the labiality of the constituents of essential oil explains why the composition of the product obtained by steam distillation is most often different from that which is initially present in the secretary organs of the vegetable (Bruneton 1995).

Lamiaceae is one of the large plant families used as a framework to evaluate the occurrence of some typical secondary metabolites (Wink 2003). The typical secondary metabolism of Lamiaceae includes various terpenoids and phenolic compounds

(Hegnauer 1989). Lamiaceae is subdivided in to two major groupings: The Lamioideae and Nepetoideae (Bremer *et al.* 1998). In addition to essential oil, Nepetoideae produce a special "tannin", mainly represented by the phenolic compound, rosmarinic acid. *Mentha lingifolia* is one of the members of the subfamily Nepetoideae (Wink 2003).

1.2 Mint family

According to flower structure, the Labiates (Lamiaceae) form one of the largest and most highly devolved plant families with worldwide distribution, excluding the Arctic and Antarctic. The family contains around 200 genera and between 2000 and 5000 species of aromatic herbs and low shrubs (Good 1974; Heywood 1978, Hedge 1992).

Most labiates accumulate terpenes and a range of other components, mainly in the epidermal glands of leaves, stems and reproductive structures. Adaptations that facilitate insect pollination (flower shape, pigments, secretion of nectar) are also common (Valdeyron *et al.* 1977).

The genus *Mentha* includes 25 to 30 species that grow in the temperate regions of Eurasia, Australia and South Africa (Dorman *et al.* 2003). Some of mint species with commercial and medicinal uses or, considered as weeds, are listed in Table 1.

Table 1. Mint species and commercial and medicinal uses or weed. (Wiersema & Leon 1999).

Species	Usage or weed
M. aquatica L. (water mint)	Flavouring
M. arvensis L. (corn mint; field mint)	weed
M. canadensis L.	
syn: *M. arvensis* L. var. *piperascens* Malinv. ex L. H. Bailey	
(corn mint; Japanese mint; Chinese mint)	Essential oil
M. x gracilis Sole	
syn: *M. x cardiaca* J. Gerard ex Baker, *M. x gentilis* auct.	
(ginger mint; red mint; Scotch mint; Scotch spearmint)	Essential oil
M. longifolia L. Huds. (horsemint)	Poison, medicinal
M. x piperita L. nothosubsp. citrate (Ehrh.) Briq.	
syn: *M. citrate* Ehrh. (bergament mint)	Essential oils
M. x piperita L. nothosubsp. *piperita* (peppermint)	Flavouring and essential oils
M. pulegium L. (European pennyroyal)	Ornamental, essential oils or medicine
M. requienii Benth. (Corsican mint)	Ornamental
M. suaveolens Ehrh. syn: *M. rotundifolia* auct (apple min)	Ornamental
M. spicata L. syn: *M. viridis* (L.) L. (spearmint)	Essential oil or medicine

Spearmint is derived from a range of species, but the two most important ones are native spearmint (*Mentha spicata* L.) and Scotch spearmint (*M.* x *gracilis* Sole). In Linné's taxonomy (http://www.economicexpert.com/a/Carolus:Linnaeus.html), *M.* spicata is an origin species. *Mentha* x gracilis is a hybrid derived from *M. arvensis* and *M. spicata*.

The essential oil of the two species differs slightly in chemical composition (Lawrence 1992). The main components of spearmint are carvone and limonene (Tucker 1992).

Peppermint, *M.* x *piperita* L., which is tetraploid (2n = 72), is a sterile natural hybrid of *M. aquatica* L. (2n = 96) and *M. spicata* (2n = 48) and produces typical peppermint cyclic monoterpenes, menthol and menthone. Due to sterility, it is not amenable to improvement by sexual crosses. Spearmint, *M. spicata*, is either tetraploid (2n = 48) or triploid (2n = 36) and produces monoterpene carvone as the major oil component (Tucker 1992)

1.3 Cultivation region

The genus *Mentha* (Laminaceae) is composed of 19 geographically widespread species and 13 named hybrids (Chambers & Hummer 1994). Peppermint, *M. x piperita*, is native to Europe and it has become both cultivated and naturalised in the USA, India, China, the former USSR, Italy, France and Hungary. Its essential oil is considered industrially important (Lawrence 1985, Tucker 1992).

There are no new current statistics on the amount of mint oil production in different countries. China produced about 1700 and the USA 3000 tons of mint oil in 1984 (Lawrence 1985). The USA is one of the largest mint cultivation areas. The cultivation area was 43 6600 ha in 1994 (Small 1997) and about 49000 ha in 2003 (Unknown online, Pest management Strategic Plan for Pacific Northwest mint Production http://pestdata.nesu.edu/pmsp/pdf/pvwmintpmsp.pdf). India is also one of the largest mint oil producers. It exported about 8500 tons of oil in 2002–2003 (online, Indian spices sector. An overview is available at http://www.indiaonestop.com).

At present, cultivation of mint in Finland is dispersed in different parts of country and mint plants are cultivated only in small parts of fields and mostly as small projects such as Finn mint in North Karelia, or POHERIKA II in North Finland. There are no statistics of cultivation areas currently available (Salo 1999).

Although several of the commercially important species did originate in xeric Mediterranean environments, their developments display market plasticity, to such a degree that their phenologies can adapt to the growing season in a range of other environments. Thus without intensive selection and breeding, a similar range of (non-chill or frost sensitive) culinary herbs can be grown profitably in Finland (Galambosi 1989). *M.* x *piperita* has been found to be cold tolerant and thus suitable for Finnish climate conditions (Järvi *et al.* 1994).

1.4 Importance of mint species

Commercially, the most important mint species are peppermint (*M. x piperita*), spearmint (*M. spicata*), and corn mint *(M. canadensis.*). From these species, corn mint is cultivated only because of oil production (Small 1997, Oudhia 2003).

Peppermint (*M.* x *piperita.*) oil is one of the most popular and widely used essential oils, mostly because of its main components menthol and menthone (Gul 1994). Corn

mint is the richest source of natural menthol (Sharma & Tyagi 1991, Shasany *et al.* 2000). Carvone-scented mint plants, such as spearmint (*M. spicata*), are rich in carvone and are widely used as spices and cultivated in several countries (Kokkini *et al.* 1995). Peppermint oil is used for flavouring pharmaceuticals and oral preparations, such as toothpastes, dental creams, and mouth washes. It is also used as a flavouring agent in cough drops, chewing gums, confectionery and alcoholic liqueurs. It is used in medicines for internal use. Its pleasant taste makes it an excellent gastric stimulant (Budavari *et al.* 1989, Gupta 1991).

Plant-derived natural products are extensively used as biologically active compounds. Among them, essential oils were the first preservatives used by man (Thompson 1989). Many of these crude mixtures have been found to have antifungal, antimicrobial, cytostatic and insecticidal activities (Sivropoulu *et al.* 1995). The essential oils extracted from the mint species (*M. pulegium* and *M. spicata)*, containing mainly, pulegone, menthone, and carvone, were tested for insecticidal and genotoxic activities on *Drosophila melanogaster*. The essential oil of both these aromatic plants showed strong insecticidal activity, while only the oil of *M. spicata* exhibited a mutagenic one. Among the constituents studied, the most effective insecticide was found to be pulegone, whereas the most effective for genotoxic activity was menthone. The strong toxicity of pulegone is suppressed in the presence of menthone (Franzios *et al.* 1997).

In 1984, the total world mint production came to: peppermint 2.2 Mtons, corn mint 2.1 Mtons and spearmint 1.4 Mtons (Lawrence 1985). There are some reports concerning world menthol production but new statistics concerning mint as a crude material are not available. Clark (1998) estimated the world production of menthol at 11.8 Mtons. The producing exporting sources are estimated to be India, China and others with 5630 Mtons, 2500 Mtons, 3670 Mtons respectively. Most of the production (9400 Mtons) is from the crude oil of *M. arvensis*; the vast majority of this oil comes from India.

The average value of imported peppermint oil in Finland was 0.5 million € per year in 1992 and the same average was for menthol. Most peppermint oil is imported from Germany, the United States, Switzerland, France and the United Kingdom (Galambosi 1992).

According to Patra and co-authors (2000), India is the leading producer and exporter of corn mint oil and its products include menthol crystals, dementholised oil, mint terpenes, etc. The cultivation of corn mint or menthol mint (*M. arvensis* f. *piperascens*) is largely confined to northern and northwestern India. Finland imported about 8 tons of dry mint leaves, 10–20 tons of peppermint oil, and 110 tons of pure menthol in 1996 (Finnish National Board of Customs, Tullihallitus 1996). There has been some decrease in the import of mint in the last few years because of increasing mint cultivation in Finland. There are no reliable statistics on the mint cultivation area in Finland, but recently some farmers have started mint cultivation and even some distillation with a 2000–4000 litre capacity have been built.

1.5 Factors affecting volatile oil accumulation

Most Labiates accumulate terpenes and a range of other components in the epidermal glands of leaves, stems and reproductive structures (Gershenzon *et al.* 2000). The quantitative composition of the essential oils of many aromatic plants is greatly influenced by the genotype and agronomic conditions, such as harvesting time, plant age and crop density (Marotti *et al.* 1994).

Peppermint has been developed as a model system for the study of monoterpene metabolism. Peppermint oil is chemically complex, and the biosynthetic pathway leading to the major monoterpene component menthol (Fig. 1) involves a broad range of representative reaction types of terpenoid metabolism (Mahmoud & Croteau 2001).

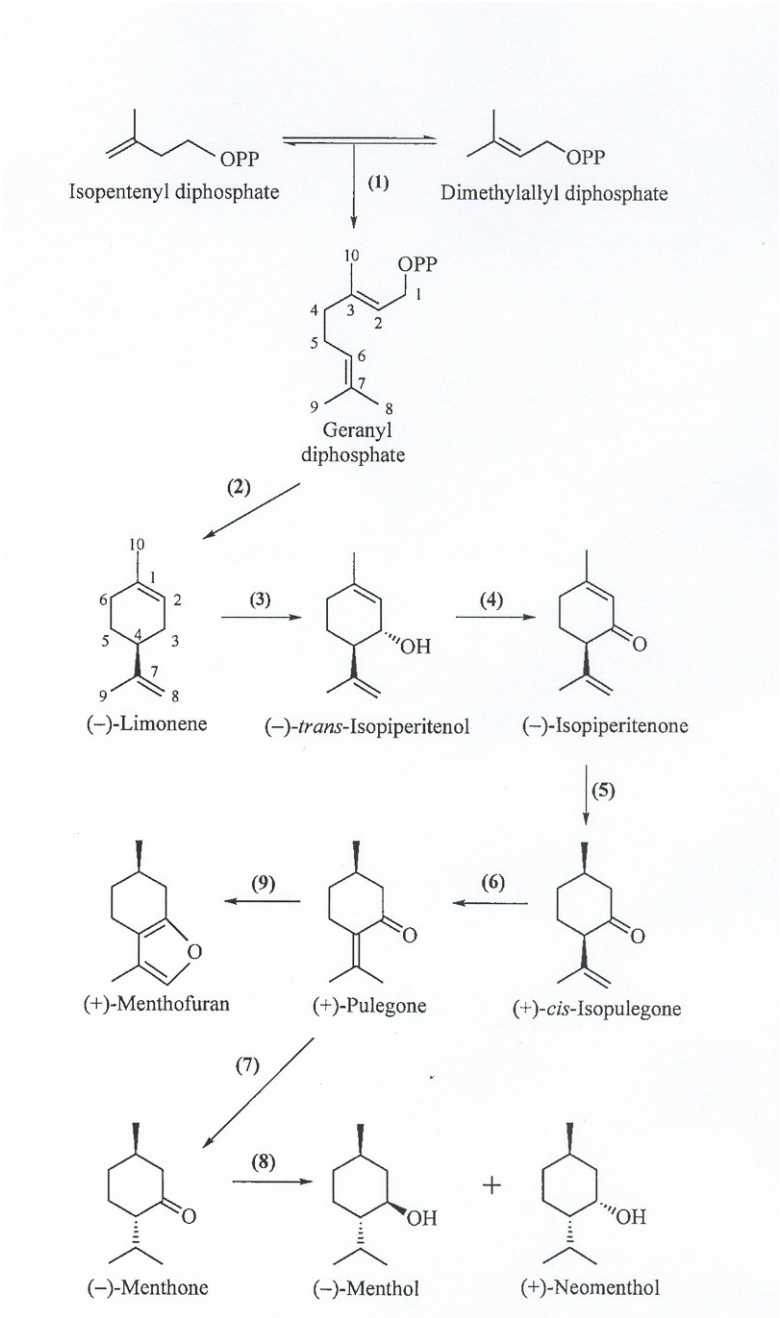

Fig. 1. The principal pathway for monoterpene biosynthesis in peppermint (Mahmoud & Croteau 2001).

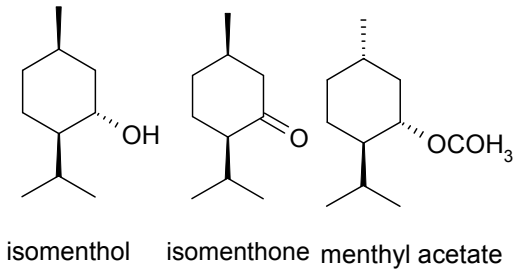

Fig. 2. **The structure of isomenthone , isomenthol and menthyl acetate.**

The chemical composition of peppermint oil is very complicated and has been the subject of numerous studies. Over 200 different constituents have been identified in peppermint oil (Lawrence & Shu 1989, Chialva *et al.* 1993, Court *et al.* 1993). The most important compounds in peppermint are menthol, menthyl acetate, menthone, menthofuran, and pulegone (ESCOP 1992) as well as limonene, eucalyptol, isomenthol, and isomenthone (European Pharmacopeias 1994).

The monoterpenes diverge from primary metabolism by conversion of isopentenyl diphosphate and dimethylallyl diphosphate, via action of the prenyltransferase geranyl diphosphate synthase, to geranyl diphosphate (Burke *et al.* 1999), which undergoes subsequent cyclization by limonene synthase to (4 S)-(–)-limonene (Alonso *et al.* 1992). Limonene serves as the common olefinic precursor of the essential oil terpenes of both peppermint and spearmint (Kjonaas and Croteau 1983) by way of a series of secondary, largely redox, transformations (Croteau and Gershenzon 1994). In peppermint, a microsomal cytochrome (Cyt) P450 limonene-3-hydroxylase introduces an oxygen atom in an allylic position to produce (–)-transisopiperitenol and thereby establishes the oxygenation pattern of all subsequent derivatives (McConkey *et al.* 2000).

A soluble NADP-dependent dehydrogenase oxidizes the alcohol to a ketone, (–)-isopiperitenone, thereby activating the adjacent double bond for reduction by a soluble, NADPH-dependent, regiospecific reductase to afford (+)-cis-isopulegone. An isomerase next moves the remaining double bound into conjugation with the carbonyl group, yielding (+)-pulegone. A NADPH-dependent reductase then converts (+)-pulegone to (+)-isomenthone (Fig 2) and (–)-menthone, which predominates. Finally, two stereoselective NADPH-dependent reductases convert (–)-menthone and (+).isomenthone to (–)-menthol and (+)-neoisomenthol, respectively, and (–)-menthone and (–)-isomenthone to (+)-neomenthol and (+)-isomenthol (Fig 2.), respectively (McConkey *et al.* 2000).

Grahle and Hoeltzel (1963) found that the leaves of *M. piperita* grown at 20^{o} C constant temperature subjected to long days (18:6) contained relatively small amounts of menthofuran and large amounts of menthol and menthone. Plants subjected to short days (12:12) contained very small quantities of menthone and menthol and large amounts of menthofuran.

A fundamental prerequisite for a good peppermint oil quality is a content of at least 45% of menthol and 15–18% of menthone and isomenthone, while the amount of the undesirable menthofuran should be lower than 1.5%. Menthol, menthone and

isomenthone occur mainly in leaves, while menthofuran decreases in leaves at the flowering stage and increases rapidly in the flowers. In terms of productivity, an average yield of 65–80 kg oil/ha is needed (Maffei *et al.* 1994). Clark and Menary (1980 a) concluded that the long day treatment resulted in reduced levels of menthofuran, pulegone, menthyl acetate (Fig. 2) and limonene as well as in increased levels of menthone, menthol, neomenthol acetate (+ unknown), trans-sabenine hydrate, cineole, and β-pinene (+)sabinene. Under a short photoperiod, menthofuran was the main monoterpene in all the leaves, while menthone and menthol were present at low percentages (Voirin *et al.* 1990).

Either short nights or cool nights, combined with full light intensity during the day, enhance the formation of menthone and depress the accumulation of menthofuran and pulegone in the leaves. It is possible that the oxidation-reduction level of the monoterpenes reflects the general oxidation-reduction state of the respiratory coenzymes of the terpene-producing cells, and that this depends on the balance between daytime photosynthesis and nighttime utilization of photosynthate (Burbott & Loomis 1967). Cool or cold night temperatures accelerate the conversion of menthone (Murray *et al.* 1988). On the other hand, cool or cold nights accelerate the maturity of herbage and it is known that the upper, less mature parts of plants has more menthone than the more mature parts of (lower half) the plant (Murray *et al.* 1988).

According to Nelson and co-authors (1971), evaporative cooling of peppermint by sprinkler irrigation resulted in lower concentrations of menthofuran, when the ambient temperature exceeded 30^{o} C. They suggested that the evaporative cooling had the same effect as cool nights. Clark and Menary (1980 c) concluded that evaporative cooling would increase CO_2 fixation of peppermint by decreasing both photorespiration and dark respiration, whereas cool nights would decrease only dark respiration.

For some species, such as peppermint, a proportion of the oil yield must be sacrificed to ensure the required oil quality (Clark and Menary 1979). Yield improvement can be achieved by adjusting elements of the growing system such as planting date, plant density, fertilizer application, water supply, harvest date and crop protection (Hay & Waterman 1993). High menthol content is the main criterion in peppermint oil quality. According to European Scientific Organization for Phytotherapy (ESCOP), the menthol content should be 44% (ESCOP 1992).

The specific chemical composition of peppermint oil produced in a particular geographical location is the result of a combination of factors such as genotype, ontogeny, light, temperature, water, and nutrients. Values ranging from 14 to 18 hours of light per day are quoted as the critical day length requirements of peppermint. *M.* x *piperita* has a distinct daylight threshold of around 14 h (Burbott and Loomis 1967), below which reproduction of oil is suppressed and both the quantity and quality of oil are law. The different compounds require different light conditions. According to Guenther (1961), the production of peppermint oil requires a day length of 15 to 16 hours.

Burbott & Loomis (1967) reported that both flowering and vegetative growth in peppermint are promoted by long periods of light or the interruption of periods of dark. They suggested that by long days, high light intensities, or low night temperatures peppermint may produce an excess of sucrose, or equivalent products of photosynthesis, which maintain reducing conditions in the oil glands. Leaf, stem ratio and herb yield are positively correlated with oil content and oil yield, whereas plant height is negatively

correlated with oil content (Sharma & Tyagi 1991). When the quality of the yield of three different mint species were compared under different Finnish and Hungarian growing conditions (light and temperature), the yield and oil percentage were higher in Hungary than in Finland, but the menthol percentage of volatile oil was higher in Finland than in Hungary (Aflatuni *et al.* 1999 b).

Typical peppermint oil contains about 20% of menthone, 50% of menthol and 8% of ester (Murray *et al.* 1988). Flower oil has much more pulegone and menthofuran than leaves oil and more than 50% of the flower oil may consist of pulegone and menthofuran, with less than half of the oil composed of menthone, menthol (Murray *et al.* 1988).

In nature or under a long photoperiodic treatment, oil high in menthyl acetate was only produced in the oldest leaves after the accumulation of menthol (Voirin *et al.* 1990). Peppermint plant grown under long day conditions produces large leaves and flowers, but the critical day length is greatly influenced by temperature (Burbott & Loomis 1967).

Clark and Menary (1979) studies showed that photoperiodic treatment itself is an important determinant of monoterpene composition. With regard to terpene composition, the shading of peppermint and changes in the day length has been found to affect the chemical composition of oil (Clark & Menary 1980 a). According to Fahlen and co-authors (1999), a majority of the *Mentha* species exposed to a 21 h photoperiod, simulating conditions typical of the July environment in northern Sweden, produced significantly higher concentrations of menthol than treatments with shorter photoperiods.

Glucose and CO_2 have been reported to be the most efficient precursors for monoterpenoid synthesis in *M. piperita*. Therefore, the rate of oil biosynthesis could be limited by the availability of assimilated carbon (Croteau *et al.* 1972). Also the UV-A radiation prompts a significant effect on photomorphogenesis and secondary metabolite production in *M. piperita*. When UV-A is given during the day, significant increases are found in the total leaf area, chlorophyll, total phenols and the total essential oil content (Maffei *et al.* 1999).

Because oil biosynthesis occurs in the leaves, their growth and photosynthetic capacity are important factors for oil production (Burbott & Loomis 1967, Duriyaprapan & Britten 1982, Srivastava & Luthra 1994). The rate of biosynthesis is the chief process that determines monoterpene accumulation in peppermint. Therefore, efforts to improve production in this species should focus on the genes, enzymes, and cell differentiation processes that regulate monoterpene biosynthesis (Gershenzon *et al.* 2000).

1.6 Cultivation

1.6.1 Conventional propagation

Mints are mainly propagated vegetatively rather than by seeds. Only some mints, such as *M. arvensis* L., *M. pulegonium* L. and *M. spicata* L., are propagated by seed (Galambosi 1995). There are many reasons for the vegetative propagation of mint. According to Hornok (1992), the peppermint as a hybrid seldom produces seeds able to germinate. For

this reason, propagation is used exclusively with vegetative parts such as green shoots, underground stolons and rooty turions.

In cutting propagation, 10–15 cm long shoots are cut from the mother plant and lower leaves are removed. The shoots are planted in a growth medium consisting of a mixture of sterilized sand and peat. Critical factors are humidity and relative humidity of 95–98% for 2–4 weeks. The temperature should be kept at 25–30^{o} C. When the plants are rooted, they are ready for transfer outdoors (Galambosi 1995). The expenses and labour involved in, the cutting method of propagation make it unpopular for extensive cultivation (Hornok 1992). In cutting propagation during spring, the yield was 20–30% less than that in traditional propagation by underground stolons, though cutting propagation was more economical (Földesi & Havas 1979). When the apical stem (the apical 5 nodes of aerial stem), basal stem (the basal 5 nodes of aerial stem), sprouted stem (midstem carrying new auxiliary buds), green stolon (growing parallel to the soil surface) and etiolated rhizome (growing below the soil surface) were employed as the source of single-node cuttings, maximum rooting percentage was obtained using etiolated rhizome, followed by green stolons (EL-Keltawi & Croteau 1986). Propagation with green cuttings cannot be utilised in the case of large-scale cultivation due to high costs and a high demand for manual labour (Hornok 1992).

The propagation material, mostly used in the multiplication of peppermint, consists of stolons grown in the soil (white) or on the surface (green) or from 8–10-cm high turions developed from underground stolons (Hornok 1992).

The satisfactory development of stolons during intensive cultivation is hindered by several known and unknown factors. The mass of roots increases with the age of the plant but the quantity of the liveable stolons decreases (Zambori & Tetenyi 1988).

Propagation by stolons is the most popular method of mint propagation because it is the most economic. The mother plants are raised for the propagation material and then they are chopped, spread and covered with soil. In practice, this method succeeds in areas with long summers and in soil free of weeds, meaning that the use of herbicides is necessary. If propagation is carried out at the beginning of summer, only surface stolons (white) are used. The best size is 10–15 cm and best growth takes place in rows at a width and depth of 10 cm (1999 a). However, Pank (1974) noticed that chopping stolons before sowing significantly reduced the yield in comparison to 10–20 cm long stolons. The best result was obtained when the stolons were separated and divided in to pieces by hand from the roots.

Nemeth & Pham (1995) studied the growth and development of the vegetative propagation organs (the stolons), and concluded that the stolon number is depended on the species and in all examined species the main of stolon branching occurs at the end of flowering. In the climate conditions of northern Finland, the best method for the conventional propagation of mints is to cut stolons from the mother plant or lift the whole plant before frost in the autumn and store them in a cool storage in moist peat during winter. Later in mid or at the end of April, the stolons are transferred to a greenhouse for preliminary growth in pots or containers. The plants are transplanted into the field as early as possible in spring after the frost is over (Aflatuni 1999 a).

1.6.2 Micropropagation

Micropropagation or plant tissue culture is a very effective method for plant mass propagation. Tissue culture techniques are being adapted to extend the propagation of commercial plants, too. Peppermint is micropropagated due to many reasons, such as breeding and the rapid multiplication of elite plants (Chaput *et al.* 1996). Several papers (Jain *et al.* 1991; Calvo & Sanchez-Gras 1993) have reported the influence of *in vitro* growth conditions on the secondary metabolite biosynthesis of terpenes.

Several media has been screened for their suitability for shoot and root induction and the development and rapid propagation of mint *in vitro*. The media used were usually based on MS-salts (Murashige & Skoog 1962) with varying hormones. Holm and co-authors (1989) studied the effect of MS-salts with various hormones and growth regulators on the growth of peppermint. Benzylaminopurine (BAP) and kinetin were used as cytokinins, and indole-3-acetic acid (IAA), naphthaleneacetic acid (NAA) were tested as auxins. According to this study, it is unlikely that an increase in the concentration of BAP would improve the auxiliary type of the growth of *M. x piperita.* The abnormal cell enlargement with BAP might indicate the beginning of adventitious shoot development.

According to Phatak and Heble (2002), leaf disc explants of *M. arvensis,* produced the highest number of shoots in MS medium with BAP (5 mg l^{-1}) and kinetin (Kin) (5 mg l^{-1}). The isolated shoots rooted well at low concentrations of cytokinin, such as BAP, Kin and thidiazuron (TDZ) at 0.5 mg l^{-1} while higher concentrations (5 mg l^{-1}) inhibited the rooting. Ravishankar and Venkatarman (1988) reported the rapid propagation of buds, shoots and roots in *M. x piperita.* Auxiliary buds were soaked in water for 3–4 hrs and cultured on Murashige and Skoog's (1962) medium containing activated charcoal, 3% sucrose and a range of phytohormones. The addition of IAA, 2.4-D and NAA to the MS medium induced plantlet formation. Indole butyric acid (IBA) gave vigorous growth and rise to multiple shoots, which formed roots upon transferring to NAA-supplemented medium.

In general, plants produced by cuttings cost approximately from half to one third the price of plantlets from tissue culture (George & Sherrington 1984).

1.6.2.1 Essential oil of micropropagated plants

According to Holm and co-authors (1989), the micropropagated peppermints had a higher content of menthol during the first year than the conventionally propagated plants, but in the second year, the content of menthol was about the same as that in conventionally propagated plants.

According to Phatak & Heble (2002), multiple shoots regenerated on cytokinin and auxin containing medium and rooted shoots accumulated pulegone as the main constituent (8%) instead of menthol followed by isopulegone, menthone and isomenthone. In contrast, callus cultures showed traces of pulegone and isopulegone with similar concentrations of compounds such as menthol, menthone and isomenthone. The oil content ranged from 1.1% to 2.6% in the vegetatively propagated plants and from 0.9% to 1.9% in the micropropagated plants.

Randhawa and co-authors (1988) reported that the application of growth regulators gibberellic acid (GA) did not increase herb and oil yields in *M. x piperita* but in the case of *M. spicata,* a lower concentration of indole acetic acid gave a significantly higher total yield than that of the control.

Only few reports that compare the quality of *in vitro* and conventionally propagated plants are available. Cameron and co-authors (1989) found that a sharp decrease in average fruit size and weight is observed in strawberry when micropropagated plants are used directly for fruit production and in many cases, no differences in total fruit production or fruit quality have been found between the progeny of the micropropagated plants and those obtained conventionally.

1.6.2.2 Mint breeding

In addition to cultivation techniques, we can influence mint oil quality and quantity by breeding**.** Because of the high value of peppermint as an essential oil crop, the improvement of peppermint has been performed by clonal selection, principally from *M.* x *piperita* cv. Mitcham (Banthorpe 1996). As a result, many cultivars with increased oil productivity have been released but none with superior flavour to Mitcham peppermint. Plant tissue culture has potential to introduce genetic variability in peppermint genotypes through somaclonal variants, somatic hybrids or transgenic plants. However, a prerequisite to applied plant biotechnology is the development of suitable and reproducible plant regeneration system (Rech & Pires 1986, Caissard *et al.* 1996).

Due to the sterility of many mint species, conventional breeding methods are often difficult. Thus, in vitro techniques such as protoplast culture or protoplast fusion are expected to provide a new possibility for increasing genetic variability and a means to transfer desirable traits to plants (Chaput *et al.* 1996).

Several hybridizations by protoplast fusion were carried out between two species of mints (Green, 1975, Murray *et al.* 1988). Diseases resistance hybrid plants are one example of *in vitro* techniques used for mint. Diseases limit mint production and *Verticillium* wilt disease is the most detrimental problem in the production of both peppermint and spearmints, wherever these crops are grown. Genetic variation for wilt resistance exists in peppermint and spearmint (Green 1963).'Black Mitcham' peppermint is most susceptible, Scotch spearmint (*M. cardiaca*) is less affected and USA or native spearmint (*M. spicata*) being least susceptible (Lacy & Horner, 1966; Brandt *et al.* 1984).

Somatic hybrid plants of 'Black Mitcham' and *M. spicata* cv. native spearmint were obtained after protoplast fusion (Krasnyanski *et al.* 1998). Hybrid plants exhibited an intermediate level of *Verticillium* resistance but oil profile resembles that of spearmint, a high level of carvone and almost no menthone or menthol (Krasnyanski *et al.* 1998). Another example of hybridization in mint plants is protoplasts isolated from leaf-mesophyll cells of *M. piperita* 'Blackmint' which were fused with those of a garden variety mint called 'gingermint' (*M. gentilis* L. cv. variegata) by electrofusion method (Sato *et al.* 1996).

Genetic transformation of *Mentha* has been achieved using *Agrobacterium tumefaciens*-meditated DNA delivery (Niu *et al.* 1998, Diemer *et al.* 1999). The stable

integration of GUS (β-glucuronidase) and NPTII (antibiotic resistance) genes in *M. arvensis* and *M. spicata* has been achieved. Krasnyanski and co-authors (1999) reported transformation of the limonene synthase gene into peppermint. Essential oil profiles of transgenic plants showed a high menthone, low menthol, high menthofuran and pulegone content in comparison to typical mid-west peppermint.

An efficient protocol for plant regeneration from protoplast of peppermint 'Mitcham Digne 38', 'Mitcham Ribecourt 19' and 'Todd's' was developed by stepwise optimization of first cell division, microcalli formation and shoot differentiation (Jullien *et al.* 1998). The rate of first cell divisions was strongly dependent on the addition of 2.4-D to callus induction medium. The best result were obtained with 1 µM 2.4-D in combination with NAA (2.5 µM) and BA (4 µM) (Jullien *et al.* 1998).

1.6.3 Soil pH and soil nutrients

Cultivation techniques have a great influence on plant growth (biomass production) and the quality and quantity of secondary metabolites. Soil pH is an important chemical property that profoundly affects the growth and nutrient uptake by crops (Fageria & Baligar 1999).

According to Van der Wat and co-authors (1991), high acidity and low calcium severely impair plant-root development. A lower availability of P and Ca in the subsoil are likely to be major limitations to the growth of maize, allowing only limited root penetration (Matabwa & Rowell 1997).

One of the factors limiting plant production in Finland appears to be soil acidity. The average pH value of agricultural soils in Finland in 1981–1987 was 5.84 (Kähäri *et al.* 1987). The pH range in the fine sand soil of northern Finland is usually 5.5–6.5 and liming is commonly needed.

Lime can raise the pH of acidic soils (Tan 1994). Liming can have many other effects on the biochemical processes in the soil and on the yield and composition of different plant species and varieties (Stevens and Laughlin 1996).

Soil pH affects the availability of several mineral nutrients, such as nitrogen, phosphorus, potassium, sulphur, calcium, magnesium, iron, manganese, boron, copper, zinc and molybdenum, and all of these nutrients are available in the pH range of 5.5 to 6.5 (Taiz & Zeiger, 1998). Loss of plant available phosphate in soils occurs by phosphate fixation, which is especially strong in acid mineral soils. Such losses can be dramatically reduced by liming soils to a pH of 6–7 (Konrad 1997). Different levels of soil pH influenced the fresh and dry weight and the essential oil yield of peppermint (Shukla *et al.* 1997, 1998).

Most authors report that significantly higher dry matter and essential oil yields for corn mint, peppermint and spearmint were obtained with higher nitrogen application but that the application of nitrogen did not affect the chemical composition of the essential oil (Atanasov *et al.* 1979, Clark & Menary 1980 b, Slavov 1985, Singh *et al.* 1989, Saxena & Singh 1995). Other authors have reported that a decrease in menthol and an increase in menthone and menthyl acetate in peppermint and corn mint has been found with increased application of N (Duhan *et al.* 1977, Duhan 1979, Hornok 1983).

According to Kothari & Singh (1995), carvone and limonene concentrations in Scotch spearmint (*M. gracilis*) were affected by levels of N, and in general, an increase of N decreased carvone but increased limonene in oil. This is not quite in agreement with Singh & Singh (1986) who reported that the application of N increased carvone in spearmint oil.

Marotti and co-authors (1994) used different levels of nitrogen and phosphorus in peppermint trials. They reported that mineral fertilization seemed to increase the menthol content of essential oil compared with untreated plants. Hornok (1974), in an experiment with peppermint fertilization, stated that the most effect on yield was achieved first by the level of nitrogen and second by the level of calcium. Phosphorus fertilization had no effect on yield. However, a high quality of peppermint oil in any region requires the optimum use of fertilizers and water to maintain herbage growth and delay maturity as long as possible so that the herbage may be harvested with a minimum of flowers (Murray *et al.* 1988).

1.6.4 Row spacing

Row spacing is an important factor in determining the microenvironment in the mint field. The optimization of this factor can lead to a higher yield in the crop by favourably affecting the absorption of nutrients and exposure of the shoot canopy to the light (Myers 1981, Gowan *et al.* 1991).

The effect of spacing on growth and development is largely due to change in the interception of radiant energy (Yao & Shaw 1964). Narrow row spacing leads to higher interception and consequently to the greater growth of peppermint (Prasad & Saxena 1980). As row spacing is reduced below a certain level, mutual shading and poor aeration may reduce the leaf-stem ratio, and consequently oil concentration, in the green herb (Nijjar 1990).

M. arvensis had better smothering effect of crop on weeds in 40 cm and highest oil concentration in 80 cm, when 40, 60 and 80 cm row spacing were compared (Singh *et al.* 1986). Different age groups and the mutual shading of leaves produce a markedly different type of oil (Kothari & Singh 1995).

Ram & Kumar (1999) concluded that plant row spacing and taking either one or two harvests were related to regenerability of plants after the first harvest. Narrow row spacing (50 cm) was the optimum for short stature varieties and wider row spacing (70 cm) was best for varieties that regenerated well.

The essential oil content of *M.* x *piperita* and *M. arvensis* did not change much, where as oil content was much influenced by planting density (Kalyan *et al.* 1989, Zheljazkov & Topalov 1996). Yields of fresh material and essential oil were enhanced by planting density (Zheljazkov & Topalov 1996).

1.6.5 Harvesting time

Amount and composition of essential oil is strongly dependent on developmental stage of the plant (ontogeny), and therefore harvesting time is one of the most important factors influencing mint oil quality.

Harvesting a crop early or late resulted in a low yield of leaves as well as the essential oil content because at an earlier or later stage of harvesting, the crop was immature or over mature resulting in a poor yield of herb and oil content (Shah & Gupta 1989). According to Chalchat and co-authors (1997), harvesting should be carried out after flowering to obtain oil that contains a high amount of menthol. Ulseth (1994) found that the poorest oil quality was obtained from mints that were in a stage of early blooming or were not yet blooming at all. Oil content in stems was very poor and present only in younger parts (Croteau & Gershenon 1994).

According to Clark and Menary (1984), besides the timing of harvest, the numbers of harvests per year greatly influence yield, and composition of oil. The essential oil from the first harvest was richer in menthol than that of the second harvest.

In the second harvest all the leaves were young with a higher menthone and lower menthol content (Saxena & Singh 1995). Similarly, Court and co-authors (1993) found that the amount of menthol increased as the plant matured.

According to Ram and Kumar (1999), there was a difference between the yield and quality of essential oil in seven mint cultivars in the first and the second harvest. For corn mint, the maximum oil content was reached during the flowering period; however, high temperature was found to decrease the oil yield (Duriyaprapan *et al.* 1986). According to Ram and Kumar (1999), the oil content of the first or second harvest of *M. arvensis* depends on the variety.

The concentration of carvone in Scotch spearmint was higher in oil of the first harvest compared with the second harvest (Kothari & Singh 1995). There is a remarkable difference in oil composition between leaves at different age, especially juvenile and mature leaves (Duriyaprapan & Britten 1982). A greater proportion of oil is obtained from the leaves, especially from leaves at the upper part of the plant canopy, but not at the top because leaves at the age of 0–2 weeks contributed 16% of leaf dry matter compared with 27% of oil yield. This may explain why the maximum essential oil production of mint plants is attained during the flowering period when almost no top growth occurs.

The intermediate and lower leaves reached their highest essential oil content at approximately the time when plants were starting to bloom. The upper leaf pairs completed their development after floral initiation (Burbott & Loomis 1967).

According to Voirin and co-authors (1990), the young (apical) leaves of peppermint mainly contained menthofuran, pulegone and menthone. Menthol increased rapidly while menthone decreased in the older leaves. Menthone was higher in the young leaves and menthol appeared as the main component of the oldest leaf pairs.

2 The aim of study

The general aim of the study was to examine the optimal conditions for the cultivation of mint in Northern Finland. The specific aims of the study were (first) to examine the differences in the oil content of several mint species and origins and (second) to compare the effect of various factors such as plant density, liming, propagation methods, harvest date and number of harvests per year on cultivation success and quality and quantity of the produced plants

3 Materials and methods

The results expressed in this dissertation are based on five separate experiments. Only a brief outline is given here and the details are found in the original papers (I–V).

3.1 Study years and area

The experiment was established in 1994–2000 in Ruukki (Northern Finland) at the North Ostrobothnia Research Station of MTT Agrifood Research Finland, 64°40'N and 25°05'E. The soil texture was fine sand containing 3–12% of organic matter.

3.2 Plant material and treatments

Since different mint types, origins and harvest dates (bud stage on July 20th in comparison with flowering stage on August 14th) have an important role on the essential oil content, studies were made into the influences of harvest dates and different mint types on oil composition. The stolons of seven mint species and origins (Table 2) were stored in a cool place (+4° C) through the winter months (November–April). The stolons were transferred to a greenhouse in the last week of April and were grown there for six weeks before they were transplanted in the experimental field in 1995, and the variations in the extract composition were compared (I).

Table 2. Plant material and treatments

Experiment	Plant material (mint species/origin)	Treatments
(I) 1995–1996	*M.* x *piperita* L. (Chinese, Bulgarian, Hungarian, USA, Polish and Finnish origin) *M. canadensis* L. (corn mint, Chinese origin) *M. arvensis* L. var. *sachalinensis* (Sachalin mint Hungarian origin)	Different mint species and origin and harvesting time
(II) 1994–1996	*M. x piperita* L. (Hungarian origin)	Plant density (10, 20 and 30 cm x 50 cm)
(III) 1997–1998	*M.* x *piperita* L. (USA and Bulgarian origin)	Propagation method (conventional and micropropagation)
(IV) 1998–2000	*M.* x *piperita* L. ('Black Mitcham' Cultivar, Egypt origin) and *M. arvensis* L. var. *sachalinensis* (Hungarian origin)	Liming levels (0, 4, 8, 12, 16 tons ha^{-1})
(V) 1998	*M.* x *piperita* L. ('Black Mitcham', Egypt origin), *M. canadensis* L. corn mint, (Chinese origin) *M. arvensis* L. var. *sachalinensis* (Sachalin mint Hungarian origin), *M. spicata* L. (Egypt origin)	Harvesting time (once at the end of August, compared with two cuttings. First, cut at the beginning of Aug. and second cut mid Sep.)

The effect of plant spacing at 10, 20 and 30 cm x 50 cm (with rows 50 cm apart with inter-row spacing of 10, 20 and 30 cm apart) on the plant growth, herbage yield, essential oil production and composition of *M*. x *piperita* was studied. The stolons were planted as in experiments I. The experiments were conducted for two years during 1994–1995 and 1995–1996 (II).

The quality and quantity of oil obtained from *M*. x *piperita* from three different origins and a spearmint, *M. spicata*, were compared in micropropagated and conventionally propagated plants in 1997–1998. In conventional propagation, the crop was developed from the stolons as in experiments I and II but in micropropagation, nodal cuttings were cultured on Murashige and Skoog's (1962) (MS) medium for shoot regeneration and the woody plant medium (WPM) of Lloyd & McCown (1980) for rooting. After preliminary growth in a greenhouse, both micropropagated and conventionally propagated plants were transplanted in the experimental field on June 15th (III).

The effect of five liming (10% Mg and 19% Ca) levels, 0, 4, 8, 12, and 16 tons ha^{-1}, on the herb and essential oil yield and menthol and menthone content of two micropropagated mints (the micropropagation method described in publication III) were studied during 1998–2000 (IV). The mint species are presented in Table 4 (IV).

In the field experiment, plants cut only once at the end of August were compared with those of two cuttings. The first cut was at the beginning of August and the second in mid September. The mint species are given in Table 2 (V).

3.3 Soil analyses

Soil analyses were carried out in September. The samples were taken from each replication, mixed together, and sent to the Soil Analysis Service of Finland,

"Viljavuuspalvelu Oy". The field was fertilized in spring and during growing time in mid July (I, II, III and V).

Samples for soil analyses were taken in May 1998 before liming and fertilization and in September 1998 and May 2000 after liming and fertilization. Five subsamples were taken from each subplot replication (liming level) and mixed in order to obtain one sample of approximately 0.5 l from each liming level. Both the pH and EC were measured (IV).

3.4 Fresh yield, oil yield and oil content determination

After the harvest, the fresh yield was weighed and then dried (at +40^{o} C). Random samples (2 x 200 g) were used to assess the dry matter content. To isolate and measure essential oil concentration, the mint leaves (50 g) were dried at +30^{o} C and then mixed with 700 ml of water, which was subsequently hydrodistilled for 2 h at 120^{o} C at atmospheric pressure. The essential oil content was then measured (I–V).

The oil composition was analyzed from leaves because the analysis of many samples from oil requires time, it is slow process and it must be done almost on the same day as the harvest. (Oil distillation is slow and labour-intensive and has to be distilled the same day as harvested). Analyses from dried leaf oil may weaken the oil quality because of high temperature (100 ^{o} C) and oil should be stored so that its quality does not suffer. Frozen oil (oil from frozen leaves) could be analyzed when there is time for doing so. Due to these reasons, all the oil analyses in these experiments were done from leaves, as they could be stored frozen and taken from the freezer when needed.

Leaf samples were randomly collected from different parts of plants (5–10 plants, 1 leaf per each plant) and from plants that had been drawn by lots and were selected to represent leaves of all ages. The fresh samples were then stored in a freezer for later analysis of oil content. For analysis, 5–10 leaves (10–20 g) were thawed at room temperature for 10 minutes and then crushed in liquid nitrogen. A crushed sample of 180–200 mg was weighed and 1.0 ml of methylene chloride containing styrene (304 ng μl^{-1}) as an internal standard was added. Methylene chloride was used to extract oil from the leaves because it is easy to dry it with anhydrous Na_2SO_4. However, quantitative analyses were done only for some of the components because reference compounds were not available for all components. The samples were extracted in an ultrasonic bath for 5 minutes and filtered. Turbid extracts were dried with anhydrous Na_2SO_4 before their introduction to a gas chromatograph. The constituents of the essential oil were analyzed and identified by GC-MS (I–V).

3.5 Root and stolon observation

When finishing the experiment, root and stolon samples were taken from each plot in the year 2000 in order to observe their development. Root density, the number of stolons as well as the weight of roots and stolons were measured (IV).

3.6 Statistical analyses

Analyses of variance (ANOVA) and Kruskal-Wallis's test were performed using SPSS-Computer Program (I). ANOVA and student-Newman-Keuls test (SNK-test) were used in the data analyses (II) and analyses were performed using the SAS software. All statistical analyses based on used experimental design, which was the randomized complete block design (III) or the traditional split-splot desing (IV–V) (Gomez and Gomez 1984).
In the repeated measurements ANOVA (III–V) the correlation of observations from the same plot was taken into account in used statistical models. This was done modelling the covariance structure for the repeated measurements. The best structure was selected using Akaike's information criteria (Gumperetz and Brow 1993). The parameters of the models were estimated by the restricted maximum likelihood method (REML) using the SAS software (8.2, Mixed procedure) (III–V).

Assumptions about normality were checked after ANOVA using graphical methods: box-plots of residuals (I–V). Scatter plots of residuals and predicted values were used to test the constancy of error variance, when ANOVA were used (Neter *et al.* 1996) (I–V).

4 Results

A short overview of the results is presented here. For details, please refer to the original articles.

4.1 Main components of the oil

The 16 compounds identified in oils of *M. x piperita* (peppermint) of different origins were α-pinene, sabinene, β-pinene, myrcene, limonene, 1.8-cineole, cis-p-menth-2-en-1-ol, menthofuran, menthone, menthol, pulegone, piperitone, menthyl acetate, β-bourbonene, γ-cadinene and β-caryophyllene (Table 1 in I). In general, the menthol content in M. x piperita origins was low (9.8–26.2%). Egyptian origin contained the highest menthol and optimum oil percentage in comparison with other peppermint origins. The menthol content of different peppermint origin varied between 10% to 63% and menthone content 12% to 76% (I–V). The content of some peppermint origins were even higher than European Pharmacopoeia standard (30–55%) (European Pharmacopeias 1997). Menthol is reported 31–65% and menthone 25–30% in peppermint (Duriyaprapan & Britten 1986, Ram & Kumar 1997).

The compounds identified in oils of *M. canadensis* (corn mint) were α-pinene, sabinene, β-pinene, myrcene, limonene, 1.8-cineole, menthofuran, menthone, menthol, pulegone, piperitone, menthylacetate, β-bourbonene, γ-cadinene and β-caryophyllene (Table 1 in I). Menthol content in peppermint was 60.8–69.8% of oil content. In Sachalin mint, the compounds identified in oils were the same as in corn mint. The menthol content varied in corn mint between 66% and 88%. Menthol is reported 72–86% in corn mint (Duriyaprapan & Britten 1986, Ram & Kumar 1997) and menthone content was 10% to 20%. The mint menthol content in Sachalin was from 83 to 84 and menthone from 7 to 14% (I, V). Menthol is reported at 76% in Sachalin mint (Rohloff *et al.* 2000).

The variation of the percentage composition for main components at the two stages of growth, namely at budding (July 20th 1995) and during harvest (August 14th 1995) (I, Table 1).

Micropropagated peppermints had a higher menthol content during the first year ($p<0.01$). In the second year, however, the results obtained by the two methods were practically identical (III).

The menthol content of USA and Egypt ('Black Mitcham') origins was higher than that of Bulgarian origin (Table IV in III).

In experimentation with *M.* x *piperita* of Hungarian origin and different plant densities, the components identified were α-pinene, β-pinene, 3-octanol, limonene, eucalyptol, menthone, meomenthol, isomenthone, menthol, isomenthol, pulegone, piperitone, menthylacetate, β-bourbonene and β-caryophyllene (II). There was no effect of density on oil composition.

The menthol content of Sachalin mint (82%) was higher than that of peppermint 'Black Mitcham' (50%) but Sachalin mint was lower (9%) in menthone than peppermint (22%). Liming had no significant effect on menthol and menthone content (IV).
Neomenthol and menthyl-acetate were identified in both peppermint and corn mint, the amount of neomenthol was higher at the onset of flowering (1.08) than at full bloom (0.25) or during flowering bud (0.00%) formation (V). The content of menthyl-acetate was low in experiments with different mint origin (I).

The menthol content in peppermint was significantly higher ($p=0.01$) at full bloom in comparison with the onset of flowering. Carvone content in spearmint was significantly ($p<0.1$) higher at the onset of flowering than at the stage of flower bud (beginning of August), but it was significantly lower than in the second harvest before flower buds. The ratio of menthol/menthone increased in all species when the mints were cut at the end of August in comparison with the beginning of August ($p=0.01$) (V).

In this study, levels of menthofuran and pulegone were low (I–V). Menthofuran is reported at 5–6 and pulegone at 0.85–2% in peppermint (Maffei & Mucciarelli 2003, Özel & Özgüven 2002). Pulegone was not detected in isolated oils in experiments with conventionally produced and micropropagated peppermint and spearmint.

4.2 Oil content and oil yield

The variations in oil content for peppermints of different origin were not statistically significant ($p=0.05$). The maximum amount of oil was obtained from *M. x piperita* of Chinese origin (2.26%) and the minimum oil content was measured for *M. x piperita* of USA origin (1.3%). *M. canadensis* produced 2.5% of oil (I). The oil content of Sachalin mint was 1.8% and corn mint 2.48%. The oil content of Sachalin is reported to be 1.08–1.75% (Rohloff *et al.* 2000) and corn mint at 0.94–2.08% (Singh et al 1986, Gasic et al 1992).

No significant differences were found in the comparison between plant densities. However, the highest oil concentrations were in 30 cm densities in the first growing season. (1.82%, 1.92% and 1.99% for 10 cm, 20 cm and 30 cm, respectively) (II). The oil yields in different densities differed slightly, but only in the second year. The highest yields were in 10 cm densities (II, Table 3).

Conventionally propagated plants had a significantly higher percentage (mean 2%) of oil ($p=0.04$) than micropropagated (mean 1.6%) ones in the first year. However, in the

second year, these differences were considerably smaller (p=0.02) (III, Table III). In the first year, menthofuran was detected in conventionally propagated peppermints but it was not detected at all in micropropagated peppermints.

There were no differences in the oil content of different peppermint origins. Liming did not affect the oil content of Sachalin mint (p=0.38 and in 1999, p=0.16 in 1998) and peppermint (p=0.16 and in 1999, p=0.71 in 1989) (IV). The oil content in peppermint was 0.97–1.53% and in Sachalin mint 1.6–2.0%, depending on the year and liming level. Significant (p=0.02) higher oil yield was achieved with liming Sachalin mint in 1998. In 1999, although the oil yield was higher with liming than without it in both peppermint and Sachalin mint, the differences were not significant because of wide random variation (IV).

When the plants were cut at the beginning of August or in the second cut in mid September, the oil contents of peppermint and Sachalin mint were higher than when they were cut only once at the end of August (p=0.03). A sharp decrease in oil content in *M. spicata*, and a slight decrease in corn mint were observed in the second cut in mid September (V, Table 5). The oil yield was higher when the plants were cut at the end of August due to the higher herb yield and higher plant height (V).

4.3 Fresh and dry yields

The fresh and dry yields were highest in 10 cm density and lowest in 30-cm density. The fresh and dry yields in different densities in the first growing season had a statistically significant difference (fresh yield p=0.001 and dry yield p=0.004 in 1994) or indicative difference (fresh yield p=0.050 and dry yield p=0.054 in 1995). In the second growing season in 1995, statistically significant differences were not found in yields, but in 1996 there was a significant difference (p=0.010) between different densities in fresh yields as well as in dry matter yields (p=0.031). The highest fresh yields were almost 35 metric tons, and dry yields over 6 metric tons in the second growing season in 1996 in 10 cm density (II).

In the experiment with the propagation method, although the total dry yield in 1997 was significantly higher with conventional propagation than it was with micropropagation, the dry yield of leaves failed to exhibit a significant difference (Tables 1 and 2 in III).

Liming clearly increased the Ca and Mg content of the soil. There are no recommended soil nutrient levels for herbs in Finland. According to Soil Analysis Service Ltd (1997), a target level of Ca content of 1400–2000 mg l^{-1} and Mg content of 120–200 mg l^{-1} is satisfactory in fine sands soil for outdoor vegetables. The Ca and Mg levels in the experimental field were initially lower than the ranges recommended by Soil Analysis Service but the satisfactory values were achieved after liming (IV).

Liming had the most pronounced effect on fresh yield. In 1999 but not in 1998, the fresh yield of Sachalin mint was significantly and peppermint almost significantly higher with liming in comparison with no liming (IV, Table 4).The yield in both species was higher with liming at the level of 4 t ha^{-1} in comparison with no liming, in the second and third years (1998–2000). The highest yield in Sachalin mint was achieved with a liming

of 4 or 8 t ha^{-1} in the second year after application (soil pH 6–6.5). The average yield for three years shows that liming with 4 t ha^{-1} gave almost the same yield as with 8 and 12 t ha^{-1} (IV).

The dry matter content for Sachalin mint in 1998, 1999 and 2000 was 17–18%, 18–19%, and 17–19% and for peppermint, it was 13–14%, 19–20%, and 19–21% respectively. Liming had no effect on the percentage of dry matter. Differences in dry matter were seen between different species ($p<0.0001$) as well as between different years ($p<0.0001$) (IV).

The herb and leaf yields were significantly higher in all four species, when harvested once at the stage of full bloom in comparison with the first harvest or the total yield of the first and second harvest ($p <0.001$) (V).

The dry yield in North Ostrobothnia was on average 5.8, 3.1, 3.0 and 3.9 t ha^{-1} for 'Black Mitcham', Sachalin mint, corn mint and spearmint respectively (Aflatuni *et al.* 1999 b). According to Tapalov et al (1991) and Ram & Kumar (1998), the dry yield of peppermint was 4.0 t ha^{-1} in Bulgaria and India. According to Singh et al (1986) and Rao (2000), the dry yield of corn mint was 4.5–6.0 t ha^{-1}.

4.4 Leaf/stem ratio

In the experiment with different densities, the highest leaf/stem ratio was found in the widest inter-row spacing in each growing season and year, even though the differences were not significant in the second growing seasons. The leaf/stem ratio was also negatively correlated with plant density (II, Table 2).

In the experiment with propagation methods, the leaf/stem ratio in dry matter was significantly higher in the first year, but was reduced to an insignificant level in the second year ($p<0.001$). In 1997, micropropagated mints of different origin contained more leaves than conventionally propagated mints but in 1998, the differences were no longer significant (III, Table 2).

4.5 Development of roots

The effect of liming on the weight and density of roots was not significant, but Sachalin mint roots were longer ($p <0.001$) in limed plots when the pH was 6–6.5 than in unlimed plots (pH 5.5). In peppermint, the difference in root length was not statistically significant (IV).

5 Discussion

Mint plants are one of the most interesting research plants; they are between medicinal and aromatic plants. There is a numerous research on mint, however, only few reports are available on Finnish environment conditions (Aflatuni *et al.* 1999 a). Several mint species, origins and cultivars are cultivated around the world, but in this study only some of the most important mint species and a few available mint origins.

5.1 Variation in main components of the oil

Mint essential oil contains about 15 basic components, and several additional trace substances (Dolia *et al.* 1999). Although 16 components were identified in the different mint species in this study, only a few of the main components are discussed here. There is no universal standard for the concept of essential oil quality or for an easily definable characteristic. The European standard is different from the American standard (Alexandrov & Zinchenko 2003) and the selection of *Mentha* plants of different origins was made on the basis of higher menthol and lower menthone content. The components of peppermint oil vary slightly from year to year. This may be mostly due to changes in weather conditions and the effect of weather on chemotypes of mints (Clark & Menary 1980 a, Bruneton 1995). Peppermint of different origins contains almost the same components, and differences are found in the percentage of components. The menthol content in peppermint of Chinese origin was lower and limonene was higher than that of USA origin (I, III) and 'Black Mitcham' (III). The Chinese peppermint contains higher limonene than the 5% European Pharmacopoeia standard (European Pharmacopeias, 1994). In the present work, peppermint of USA and Egyptian origins contained the higher level of menthol and oil percentage than the other peppermint origins. These (menthol levels of both mints) were slightly higher than reported by Duriyaprapan and Britten (1986) and Ram and Kumar (1997).

The differences between the menthol content of corn mint and Sachalin mint were not significant. Although there are some reports about Sachalin mint oil content (Rohloff *et al.* 2000 and Rohloff 2002) and many reports concerning corn mint oil (Duriyaprapan & Britten 1986, Ram & Kumar 1997), it seems there is no report concerning the comparison

between corn mint and Sachalin mint menthol content (Rohloff *et al.* 2000 and Rohloff 2002). In this work, carvone was detected in Sachalin mint but not in corn mint (I). This may weaken the quality of Sachalin mint as a source of menthol in comparison with corn mint.

The pulegone, menthofuran and menthyl acetate amounts were lower in the oils of all mints (I–V) in comparison with earlier studies (Maffei & Mucciarelli 2003, Özel & Özgüven 2002). The main reason for that could be that in this study, the oil content of the various mints was determined only from the leaves. Pulegone and menthofuran are usually found in high amounts in flowers (Alexandrov & Zinchenko 2003). Menthofuran is an undesirable monoterpenoid component of peppermint (*Mentha* x *piperita*) essential oil that is derived from the alpha, beta-unsaturated ketone (Bertea *et al.* 2002). The other reasons for the low content of menthofuran are the long day, low temperature and rather high moisture level of air during the growing period in North Finland; the content of menthofuran raises under stressful environmental conditions (high temperatures, drought, and low light intensity) (Clark & Menary 1980 a).

The menthofuran content of different peppermint origin and the carvone content of spearmint in conventionally and micropropagated plants in the second year were found to be about the same, although differences are higher in the first year of growing in the field. These results are consistent with the reports of Holm and co-authors (1989).

In the experiment with different mint origins (I), the menthol contents in all *M.* x *piperita* origins were low. The reason is that all the sample leaves were collected from the upper half of the plant stem, which is known to be low in menthol and high in menthone (Voirin *et al.* 1990) whereas in the other experiment (III), in which there were three peppermints of the same origin, the leaf samples were randomly collected from leaves of all ages. Thus, the menthol content was higher and the menthone content was lower.

Conventionally propagated plants showed a higher menthol percentage in first year of planting but not in second year, which is consistent with the reports of Chaput and co-authors (1996). According to Holm and co-authors (1989), the micropropagated peppermints had a higher content of menthol during the first year than the conventionally propagated plants did, and in the second year, the content of menthol was about the same as that in conventionally propagated plants. This was perhaps the impact of medium in which the plants were propagated. Differences between other components have also been reported in different propagation. For example, according to Phatak and Heble (2002), rooted micropropagated shoots accumulated pulegone as the main constituent instead of menthol followed by isopulegone, menthone and isomenthone. According to them, the peak area of pulegone in the terpenoid profile of the essential oils from Mentha arvensis cultured tissues was much higher (shoots 80%, rooted shoots 80% and field cultivated tissue culture plants 18.1% of terpenoid peak area) whereas in conventionally propagated mother plants, the pulegone peak was only 0.6%. When the plantlets were transferred to the soil and grown in a field, they showed a similar terpenoid pattern as that of the mother plant. These results indicate a possible inhibition in the conversion of pulegone to menthol at the early stages of differentiation.

There were significant changes between menthol and menthone at different harvesting times (beginning and end of August) (V, Table 2). Menthol concentration seems to increase with the growing season (Singh *et al.* 1988, Court *et al.* 1993, Özel & Özgüven 2002). When the second cut in autumn is compared with the first cut in summer,

peppermint has been shown to produce oils with higher menthol content (V) (Piccaglia *et al.* 1993), which is especially important in Finnish environment conditions because mints must be harvested later in August in order to achieve higher menthol content.

Changes in pH, Ca and Mg levels in soil due to different levels of liming, did not have any effect on the oil content and proportion of menthol and menthon (IV). In literature, there is no evidence that liming can have an effect on the essential oil of plants as with N, Ca and Mg (Letchamo 1993, Piccaglia *et al.* 1993, Alkire & Simon 1995, Suh & Park 1999). According to Maia and co-authors (2001), high levels of Ca and Mg, and a low level of P caused no significant alternations in the quality of *Mentha arvensis* L. but in solutions with low concentrations of N, the menthol content in the essential oil increases as the Mg concentration decreases. According to this experiment, liming to raise pH is mostly for reasons other than mint oil quality.

5.2 Variations in the oil content and oil yield

The oil contents of peppermint varied slightly from year to year mostly due to variations in yearly growing weather conditions. This is consistent with the reports of Muller and co-authors (1997) and Sharma and co-authors. (1992). Peppermint of Chinese origin had the highest oil content and USA origin lowest (I). The oil content of Egyptian and Bulgarian origin was almost the same as that of Chinese origin (III). Chinese origin had higher oil, however, Chinese origin is not recommended because it has lower menthol than USA and Egyptian ('Black Mitcham') origin. The oil content of corn mint was higher than that of Sachalin mint (I, V) (Singh *et al.* (1986), Gasic et al (1992) and Rohloff *et al.* (2000)). Thus, corn mint is recommended for cultivation in Northern Finland.

In our experiments, the plant distance did not affect oil concentration. According to Singh and co-authors (1986), *M. arvensis* had highest oil concentration in 45 X 80-cm, but the differences to 45 x 40, 45 x 60 were not significant. However, due to the highest biomass in the highest plant distance (50 x 10-cm), the oil yield was higher in the second growing season (II). These results are consistent with the reports of Rao and co-authors (1984) about *M. citrata* and Shalaby and Razin, (1992) about *Thymus vulgaris*.

When propagation methods were compared, the differences between the oil content of micropropagated and conventionally propagated plants in the second year of growing in the field were considerably smaller, although conventionally propagated plants had a significantly higher percentage of oil than did the micropropagated plants in the first year (III). Similar results have been reported by Holm and co-authors (1989). According to Guedes and co-authors (2003), the essential oil yield obtained in vitro shoots of *Hypericum androsaemum* L. was lower than the minimum value obtained from the in vivo cultivated plants. Either the different conditions of growth or the immaturity of the in vitro shoots compared to those of in vivo plants may be responsible for the respective low content of essential oil. On the other hand, Hirata and Murakami (1990) obtained almost equal amounts of essential oils from *M. spicata* in vitro plantlets and the mother plants. In this study, there could be two reasons for the higher oil content in the mother plants: one is that perhaps the plants were not exactly of the same maturity at the time of

harvesting and second, the influence of growth substances ma have had an effect only during the first year of growing in the field.

In this work (IV), liming had no effect on the oil content of Sachalin and peppermint. The wide random variation in oil yield in Sachalin mint and peppermint in different pH is due to some other factors in the experimental field conditions, such as soil mineral fertilization (Piccaglia & Marotti 1993) rather than genetic differences, since the plant material used in this experiment was micropropagated and thus genetically affairs uniform (IV). According to Maia and co-authors (2001), higher levels of Ca and Mg and a low level of P enhance the levels of oil content in *Mentha arvensis*.

The oil content reached the maximum at the onset of flowering in peppermint: The stage of harvesting did not alter oil content of corn mint significantly. Similar findings were reported by Sharma and Tyagi (1991) and Piccaglia and co-authors (1993). The oil content of corn mint was almost constant at different harvesting times, while there were significant changes in peppermint, spearmint and Sachalin mint. However, the oil yield of all mints was higher when the plants were cut once at the end of August (V), due to higher herb yield and higher plant height (V).

We can state that there was a high variation in the oil content between the different mint species of different origin. The harvesting time affected the oil content but planting density, liming and propagation methods had less effect or no effect at all on the oil content.

5.3 Variations in fresh and dry yields

The highest fresh and dry yield and dry matter contents were obtained with the highest inter-row spacing (10 cm) (II). A similar pattern was also found e.g., in both pelargonium species (Rao 2002, Naghdi Badi *et al.* 2004) and thyme (*Thymus vulgaris* L.) when 15, 30 and 45-cm row spacing were compared (Shalaby & Razin 1992). However, there were no significant differences in the dry matter contents of mints due to plant density in any of the growing seasons (II). Plants grown at optimal density intercept radiant energy effectively, resulting in increased peppermint growth (Burbott & Loomis 1967). The proportion of the weight of leaves above ground was highest at the lowest planting density. A plant space of 20 x 50 cm (with rows 50 cm apart and with inter-row spacing of 20 cm, 10 plants m^{-2}) can be recommended for Finland. There were no significant differences between the different densities in the second growing season in yield, and amount of weeds at 30 x 50 cm was higher than at 10 x 50 cm and 20 x 50 cm spacing. The seedling costs are higher and there could be a danger of fungi and diseases at the 10 x 50 cm than at 20 x 50 cm. However, Singh and co-authors (1986) found that *M. arvensis* had better smothering effect of crop on weeds in 40 cm row spacing, when 40, 60 and 80 cm row spacing were compared.

The yield in peppermint and Sachalin mint was higher with liming at 4 t ha^{-1} in the second and third years (1998–2000) in comparison with no liming. Many studies have reported the increase of yield with liming (Shukla *et al.* 1997, 1998, Ernani *et al.* 2002). The highest yield was achieved after the third year of liming with the liming of 4 or 8 t ha^{-1} (soil pH 6–6.5) in fine sand in Northern Ostrobothnia. The average yield for three

years shows that liming with 4 t ha^{-1} gave almost the same yield as with 8 and 12 t ha^{-1}. Matusiewicz (1972) studied peppermint of the Mitcham variety in five combinations of soil pH, ranging from 4.7 to 6.9 for 3 years. He concluded that the plants developed best and gave the highest crop of plant material at soil pH ranging from 5.6 to 6.2. In addition to low pH, the reason for low yield in this study could also be Ca and Mg deficiency, which limited the yield when liming was not applied (IV).

In an experiment with liming in 2000, the yields were much lower than in the two previous years due to weeds, weak overwintering and plant aging. Galambosi (1995) also found a decrease in the yields of mint in Finland. Moreover, according to Hornok (1992), peppermint plantation should be maintained only for two, or rarely, for three years. Peppermint should not be cultivated in the same field for four years due to increases in weeds, insects and diseases. However, all plants in our experiment were healthy and no signs of disease were observed (IV).

The herb yield and leaf yield were significantly higher in all four species, when harvested once at end of August during full bloom compared with the yield from the first harvest at the beginning of August or the total yield of the first and second harvests. This is in accordance with the results of Court and co-authors (1993) concerning the harvest date for peppermint.

Therefore, according to the results obtained in this experiment, in order to achieve the optimum dry-yield and oil yield, peppermint (‘Black Mitcham’ or USA origin) or Sachalin mint must be planted in 20 x 50 cm spacing with a liming of 4 t ha^{-1} and a recommended harvest once at the end of August.

In this study, the herbage yield of peppermint was higher than, or as high as, in India or Bulgaria due to using better cultivation techniques than before. However, the herbage yield of corn mint was slightly lower than in India. According to Galambosi and co-authors (1994), peppermint and corn mint in Sorksar produced more fresh and dry yield than it did in Finland (in Ruukki and Mikkeli).

6 Conclusions and future prospects

There is high variation in quality and quantity between peppermints of different origin, and in compositions of main components between different mint species. Among the peppermints of different origins studied, USA origin and Egypt origin ('Black Mitcham') peppermint contained the highest menthol level and gave highest oil yield. Corn mint and Sachalin mints both had high menthol content but carvone was identified in Sachalin mint, which may weaken the quality of Sachalin mint as source of menthol. It is recommended to cultivate corn mint rather than Sachalin mint because of the higher menthol and oil content.

Plant density had no significant effect on oil composition. At row spacing 30 x 50 cm growth of a markedly higher amount of weeds was observed. On the other hand, seedling costs and danger of fungi and diseases may increase at 10 x 50 cm row spacing. Consequently, 20 x 50 cm spacing is commended for Northern Ostrobothnia.

When choosing the right type of mint for cultivation in Finland, other factors influencing the cultivation successes are: soil pH, the propagation method, planting density and harvesting time. By applying 4 t ha^{-1} of lime, pH rose from 5.5 to 6, in fine sand soil with 6–12% organic matter content. Liming almost doubled the fresh herbage yield of both Sachalin mint and peppermint. Liming had no effect on the oil content and proportion of menthol and menthon. If the pH value is lower than 6, or Mg and Ca levels are low, liming at the level of 4–8 t ha^{-1} for sandy soils in Finland is recommended in order to achieve higher fresh yield and higher oil yield.

In the first year, there were no differences in the leaf dry matter yield between micropropagated and conventionally propagated plants, but the menthol of conventionally propagated plants was significantly higher than that in micropropagated plants. In the second year, the leaf dry matter yield of micropropagated plants was higher than that of their conventionally propagated counterparts. Micropropagation is a feasible method when rapid multiplication is essential; for example, when there is a shortage of plant material or elite plants. In Northern conditions, poor overwintering may occasionally produce an insufficient number of seedlings. Thus, micropropagated plants, being more uniform in size and shape, are easier to transplant than conventionally propagated ones are.

Harvesting of peppermint only once at the stage of full bloom (end of August) gave the maximum oil yield of good quality. Furthermore, there is not enough time for the leaves to mature for the second cut. The dry yield and carvone content in spearmint was also significantly higher when harvested once at the onset and stage of flowering than when harvested with the first harvest at the stage of flower buds. According to this study, the optimum harvesting time in Northern Ostrobothnia is at full bloom at the end of August.

In order to get better results from leaf oil composition, leaf samples should be taken from the lower part, which contains more menthol and represents the oil content of most parts of mint. There is still a great challenge to find the most suitable origin and variety of peppermint, corn mint and spearmint suitable for cultivation in the Finish environmental conditions. The effects of liming on pH and Mg and Ca levels and soil condition as whole are slow and a longer period of study is needed for reliable results. At least three follow-up years are needed in order to understand better the optimum harvesting time.

The low contents of menthofuran found in this study could be due to the long day, low temperature and rather high moisture level during growing season in North Finland. This study shows that it is possible to achieve as high as or even higher oil quality and dry yield of mint in Northern Ostrobothnia than in central Europe (as in Hungary) or southern Asia (as in India). However, this requires following of cultivation methods as described above (right type of mint, soil pH, planting density, harvesting time and propagation method). In addition, mints must be cultivated only for two and a maximum of three years in the same place and there must be rotation to avoid weeds and insects. This may also require using herbicides and pesticides or other methods or techniques in the case of biological cultivation.

Although the present study contains some new information and gives a significant amount of information about the chemical composition of the studied species and origins in Northern Finland, much still remains to be studied.

Chemical analyses of oil from leaves must be taken into account more thoroughly. It would be interesting to study whether there are any differences between the two methods of analysis of mint oil (extraction from leaf samples as is described in the present study and from hydrodistillation).

During this study, it was observed that weeds are the greatest problem with mint cultivation. Unfortunately currently there are no accepted herbicides in Finland fro this purpose. Future research and accumulation of knowledge, especially about weeds and pest control, will enable extensive and economic mint cultivation in Finland.

References

Aflatuni A (2003) The use of plant origin substances against *Galerucella sagitaria*. Proceeding of the Nordic Association of Agricultural Scientists 22nd Congress "Nordic Agriculture in Global Perspective", July 1-4 2003, Turku, Finland. Available at www.njf.dk/njf/reports/njfreports.htm.

Aflatuni A (1999 a) Development of propagation methods for mints under Nordic conditions. In: Salo R (ed) Mint Research in Finland. Symposium of Mint Research, Jokioinen, 8.12.1999. Publications of Agricultural Research Centre of Finland, Series A 66, 74–81.

Aflatuni A (1999 b) The comparative study of mint species grown in Northern Finland. In: Salo R (ed) Mint Research in Finland. Symposium of Mint Research, Jokioinen, 8.12.1999. Publications of Agricultural Research Centre of Finland, Series A 66, 74–81

Aflatuni A, Galambosi B, Kemppainen, R, Niskanen, M & Jauhiainen L (1999) Performance of mint species in different climates and in organic cultivation. Publications of Agricultural Research Centre of Finland, Series A 53. Jokioinen. 61

Alexandrov AN & Zinchenko AA (2003) Essential oil quality and standards, with special reference to Mentha oils. The magazine page. Online. http://www.users.globalnet.co.uk/~nodice/new/magazine/magazine.htm

Alkire BH & Simon JE (1995) Response of midwestern peppermint (*Mentha x piperita* L.) and native spearmint (M. *spicata* L.) to rate of nitrogen fertilizer 1. Acta Horticulturae 426: 537–549.

Alonso WR, Rajaonarivony JIM, Gershenzon J & Croteau R (1992) Purification of 4*S*-limonene synthase, a monoterpene cyclase from the glandular trichomes of peppermint (*Mentha* × *piperita*) and spearmint (*M. spicata*). Journal of Biological Chemistry 267: 7582–7587.

Atanasov Z, Slavov SI, Koseva D, Decheva R & Gargova N (1979) Application of single and compound mineral fertilizers to peppermint. Plant Science 1: 61–65.

Banthorpe DV (1996) *Mentha* species (mints): in vitro culture and production of lower terpenoids and pigments. In: Bajaj PS (ed) Biotechnology in Agriculture and Forestry, vol. 37 Medicinal and Aromatic plants IX. Springer-Verlag, Berlin, 202–225.

Bertea CM, Schalk M, Karp F, Maffei M & Croteau R (2002) Demonstration that menthofuran synthase of mint (*Mentha*) is a cytochrome P450 monooxygenase: Cloning, functional expression, and characterization of the responsible gene. Biochemistry and Biophysics 390: 279–286.

Brandt WH, Lacy ML & Horner CE (1984) Distribution of verticillium in stem of resistant and susceptible species of mint. Phytopathology 74: 587–591.

Bremer K, Chase MW & Stevens PF (1998) An ordinal classification for the families of flowering plants. Annals of Missouri Botanical Garden 83: 531–553.

Bruneton J (1995) Pharmacognosy, Phytochemistry, Medicinal Plants. Lavoisier Publ. Londres, New York, Paris, 405–466.

Budavari S, O'Neil MJ, Smith A & Heckelman PE (eds) (1989) The Merck Index. An Encyclopedia of Chemicals, Drug, and Biologicals. (11. edition). Merck & Co., Rahway.

Burbott AJ, & Loomis WD (1967) Effects of light and temperature on the monoterpenes of peppermint. Plant Physiology 42: 20–28.

Burke CC, Wildung MR, Croteau R (1999) Geranyl diphosphate synthase: cloning expression and characterization of this prenyltransferase as a heterodimer. National Academy of Sciences USA 96**:** 13062–13067.

Caissard JC, Faure O, Jullien F, Colson M & Perrin A (1996) Direct regeneration in vitro and transient GUS expression in *Mentha x piperita*. Plant Cell Reports 16: 67–70.

Calvo MC & Sanchez-Gras MC (1993) Accumulation of monoterpenes in shoot-proliferation cultures of *Lavendula latifolia*. Medical Plant Science 91: 207-212.

Cameron JS, Hancock JF & Flore JA (1989) The influence of micropropagation on yield, components, dry matter partitioning and gas exchange characteristics of strawberry. Scientia Horticulturae 38**:** 61–67.

Chalchat JC, Garry RP & Michet A (1997) Variation of the chemical composition of essential oil of *Mentha piperita* L. during the growing time. Journal of Essential Oil Research 9: 463–465.

Chambers HL & Hummer KE (1994) Chromosome counts in the *Mentha* collection at the USDA-ARS national germplasm repository. Taxon 43: 423–432.

Chaput MA, San H, Hys Lde, Grenier EH, David H & David A (1996) How plant regenerate from *Mentha x piperita* L. and *Mentha x citrata* Ehrh. Leaf protoplasts affect their monoterpene composition in field conditions. Journal of Plant Physiology 149: 481–488.

Chialva F, Ariozzi A, Decastri D, Manitto P, Clementi S & Bonelli D (1993) Chemometric investigation on Italian peppermint oils. Journal of Agricultural and Food Chemistry 41: 2028–2033.

Clark RJ & Menary RC (1979) The importance of harvest data and plant density on the yield and quality of Tasmanian peppermint oil. Journal of the American Society of Horticultural Science 104: 702–6.

Clark RJ & Menary RC (1980 a) 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. Australian Journal of Plant Physiology 7: 685–92.

Clark RJ & Menary RC (1980 b) The effect of irrigation and nitrogen on the yield and composition of peppermint oil (*Mentha piperita* L.). Australian Journal of Agricultural Research 31: 489–498.

Clark RJ & Menary RC (1980 c) Environmental effects on peppermint (*Mentha piperita* L.) II. Effects of temperature on photosynthesis, photorespiration and dark respiration in peppermint with reference to oil composition. Australian Journal of Plant Physiology 7: 693–697.

Clark RJ & Menary R C (1984) The effect of two harvest per year on the yield and composition of Tasmanian peppermint oil (*Mentha piperita* L.). Journal of the Science of Food and Agriculture 35: 1191–1195.

Clark GS (1998) Menthol- an aroma chemical profile. Perfumer & Flavorist 23: 33–46.

Court WA, Roy RC & Pocks R (1993) Effect of harvest date on the yield and quality of the essential oil of peppermint. Canadian Journal of Plant Science 73: 815–824.

Croteau R, Burbott AJ & Loomis WD (1972) Biosynthesis of mono and sesquiterpenes in peppermints from glucose ^{14}C and $^{14}Co_2$. Phytochemistry 11: 2459–67.

Croteau R & Loomis WD (1972) Biosynthesis of mono and sesquiterpenes in peppermint from mevalonate-2 ^{14}C. Phytochemistry 11: 1055–1066.

Croteau R (1986) Biochemistry of monoterpenes and sesquiterpenes of the essential oils. Horticulture and Pharmacology 1: 81–133.

Croteau R & Gershenzon J (1994) Genetic control of monoterpene biosynthesis in mints (*Mentha*: Lamiaceae). Recent Advances in Phytochemistry 28: 193–229.

Diemer F, Caissard JC, Moja S & Jullien F (1999) *Agrobacterium tumefaciens*-mediated transformation of *Mentha spicata* and *Mentha arvensis*. Plant, Cell, Tissue and Organ Culture 57: 75–78.

Dolia VS, Mozul VI, Karpenko VV (1999). Research of mint plant essential oils. Visnik Farmacii 2:158–159.

Dorman HJ, Kosar M, Kahlos K, Holm Y & Hiltunen R (2003) Antioxidant prosperities and composition of aqueous extracts from Mentha species, hybrids, varieties and cultivars. Journal of Agricultural and Food Chemistry 51:4563–4569

Duhan SPS (1979) Application of N and growth affecting chemicals on the productivity and quality of essential oil from *Mentha piperita* L. Ph.D. Thesis submitted to Kumaon University, National India.

Duhan SPS, Bhattacharya AK & Husain A (1977) Effect of N and its methods of application on the herb and quality of corn mint. Indian Perfume 21: 135–138.

Duriyaprapan IS & Britten EJ (1982) The effect of age and location of leaf on quantity and quality of corn mint oil production. Journal of Experimental Botany 33: 810–814.

Duriyaprapan S, Britten EJ & Basford KE (1986) The effect of temperature on growth, oil yield and oil quality of corn mint. Annals of Botany 58: 729–736.

EL-Keltawi NA & Croteau R (1986) Single-node cuttings as a new method of mint production. Scientia Horticulturae. 29: 101–105.

Ernani PE, Bayer C & Maestri L (2002) Corn yield affected by liming and tillage system on an acid Brazilian oxisol. Agronomy Journal 94: 305–309.

ESCOP (1992) Proposal for a European monograph on the medicinal use of Menthae Piperitae aetheroleum peppermint oil. In: Proposal for European Monograph on the Medicinal Use. Vol 3.

European Pharmacopeias (1994) 2nd ed. European Treaty Series No 50. Maissonnueve S.A, France, 405–457.

European Pharmacopeias (1997) 3rd ed. Strasbourg, France: Council of Europe, 1298–1300.

Fageria NK. & Baligar VC (1999) Growth and nutrient concentrations of common bean, low land rice, corn, soybean, and wheat at different soil pH on an Inceptisol. Journal of Plant Nutrition 22: 495–1507.

Fahlen A, Welander M & Wennersten R (1999) Effects of light-temperature regimes on plant growth and essential oil yield of selected aromatic plants. Journal of the Science of Food and Agricultural 73: 111–119.

Franzios G Mirotsou M, Hatziapostolou E, Kral J, Scouras ZG & Mavragani-Tsipidou P (1997) Insecticidal and genotoxic activities of mint essential oils. Journal of Agricultural Food and Chemistry 45: 2690–2694.

Földesi D & Havas T (1979) Peppermint (*Mentha piperita*) propagation by rooted stolon shoots. Herba Hungarica 18: 63–73.

Galambosi B (1989) Cultivation possibilities of essential oil plants in south Finland. Journal of Essential Oil Research 1: 161–4.

Galambosi B (1992) Mausteiden tuonti Suomeen 1981-1991. In: Aro H & Galambosi B (eds). Mauste- ja rohdoskasvien markkinointi, julkaisuja 23, 7-17. Helsingin Yliopiston Maaseudun tutkimus- ja koulutuskeskus, Mikkeli.

Galambosi B, Aflatuni A, Nemeth E. & Bernath J (1994) Yield and essential oil content of mint species grown in Finland and Hungary. In: Production of herbs, spices and medicinal plants in the Nordic countries. Mikkeli, Finland, 2-3 August 1994. Proceedings of NJF seminar no. 240, 61–62.

Galambosi B (1995) Mauste- ja rohdosyrttien luonnonmukainen viljely. Helsinki: Printing center (Painatuskeskus). 234 ISBN 951-37-1530-2.

Gasic O, Mimica-Dukic N & Adamovic D (1992) variability of content and composition of essential oil of different *Mentha arvensis* L. var. *piperascens* cultivars. Journal of Essential Oil Research 4:49–56.

George EF & Sherrington PD (1984). Plant Propagation by Tissue Culture. Handbook and Directory of Commercial Laboratories. Eastern Press, Reading, Berks, 709 pp.

Gershenzon J, McConkey ME & Croteau RB (2000) Regulation of monoterpene accumulation in leaves of peppermint. Plant Physiology 122: 205–213.

Gomez KA & Gomez AA (1984) Statistical Procedures for Agricultural Research. 2nd ed. John Wiley & Sons, New York.

Good R (1974) The Geography of the Flowering Plants, 4th ed. London, Longman, 518 pp.

Gowan MC Taylor HMM & Willingham J (1991) Influence of row spacing on growth, light and water use by sorgum. Journal of Agricultural Science 116: 329–339.

Grahle A & Hoeltzel C (1963) Photoperiode Abhängigkeit und Bedeutung für des ätherischen Öl bei *Mentha piperita* L. Naturwisessenschaften 50: 552.

Green RJ (1963) Mint farming. ARS, USDA Agricultural Information. Bulletin 212.

Green RJ (1975) Peppermint and spearmint production in the United States progress and problems. International Flavour & Food Addition 6: 246–247.

Guedes AP, Amorim LR, Vicente AMS, Ramos G & Fernandes-Ferreira M. (2003) Essential oils from plants and in vitro shoots of *Hypericum androsaemum* L. Journal of Agricultural and Food Chemistry. 51:1399–1404.

Guenther E (1961) The peppermint oil industry in Oregon and Washington states. Perfumery Essential Oil Record 52: 632–42.

Gul P (1994) Seasonal variation of oil and menthol content in *Mentha arvensis* Linn. Pakistan Journal of Forestry 44: 16–20.

Gumperetz ML & Brow C (1993) Repeated measures in randomized block and split- splot experiments. Canadian Journal of Forest Research 23: 625–639.

Gupta R (1991) Agrotechnology of Medicinal Plants. In Wijesekera ROB (ed) The Medicinal Plant Industry CRC Press, 43–57.

Hay RK & Waterman PG (ed.) (1993) Volatile Oil Crops: their Biology, Biochemistry and Production. ISBN 0-582-00557-4. Longman Scientific & Technical, New York.

Hedge C (1992) A global survey of the biogeography of the Labiatae. In Harley RM & Reynolds T (eds) Advances in Labiatae Science. Royal Botanic Gardens, Kew 7–17.

Hegnauer R (1989) Chemotaxonomie der Pflanzen. Eine Übersicht über die Verbreitung und die systematic Bedeutung der Pflanzenstoff. Basel, Boston, Berlin. lISBN 3-7643-1895-3.

Heywood V H (ed.) (1978) Flowering Plants of the World. Oxford, Oxford University Press, 119 pp.

Hirata, T & Murakami S (1990). Volatile monoterpenoid constituents of the plantlets of *Mentha spicata* produced by shoot tip culture. Phytochemistry 29: 493–496.

Holm V, Hiltunen R, Jokinen K & Törmälä T (1989) On the quality of the volatile oil in micropropagated peppermint. Journal of Flavour and Fragrances 4: 81–84.

Hornok L (1974) Effect of nutrition supply on crop as to the quality of *Mentha piperita* L. and its essential oil content. Publications Universitatis Horticulturae 38: 75–82.

Hornok L (1983) Influence of nutrition on the yield and content of active compounds in some essential oil plants. Acta Horticulturae 132: 239–247.

Hornok L (1992) Peppermint (*Mentha piperita* L.) in cultivation and processing of medicinal plants. Akademiai Kiado, Budapest, 187–196.

Ibrahim AM, Kainulainen P, Aflatuni A, Tiilikkala K & Holopainen J (2001) Insecticidal, repellent, antimicrobial activity and phytotoxicity of essential oils: With special reference to limonene and its suitability for control of insect pests. Agricultural and Food Science in Finland 10: 243–259.

Jain M, Banerji R, Nigam SK, Scheffer JJC & Chaturvedi HC (1991) In vitro production of essential oil from proliferating shoots of *Rosmarinus officinalis*. Planta Medica 57: 122–124.

Jullien F, Diemer F, Colson M & Faure O (1998) An optimizing protocol for protoplast regeneration of three peppermint cultivars (*Mentha x piperita*). Plant Cell, Tissue and Organ Culture 54: 153–159.

Järvi A, Pessala R, Hupila I, Simojoki P, Huhta H, Virri K, Kemppainen R, Aflatuni A & Galambosi B (1994) Yield potential of cold tolerant culinary herbs (*Arthemisia dracunculus*, *Levisticum officinale*, *Mentha* x *piperita*, *Agastache foeniculum*) grown in different latitudes in Finland. In Ahonen S (ed) Proceedings of NJF seminar no. 240, Production of Herbs, Spices and Medicinal Plants in the Nordic Countries. Mikkeli, Finland, 53.

Kalyan S, Ram P and Singh JP (1989) Effect of nitrogen and inter and intra row spacing on herb and oil yield of transplanted corn mint (*Mentha arvensis* L.). Annales of Agricultural Research 10: 258–261.

Kjonaas R, 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. Archives of Biochemistry and Biophysics 220: 79–89.

Kokkini S, Karousou R & Lanaras T (1995) Essential oils of spearmint (carvone-rich) plants from the Island of Crete (Greece). Biochemical Systematics and Ecology 23: 287–297.

Konrad M (1997) Agronomic measures for better utilization of soil and fertilizer phosphates. European Journal of Agronomy 7: 221–233.

Kothari SK & Singh UB (1995) The effect of row spacing and nitrogen fertilization on scotch spearmint (*Mentha gracilis* Sole). Journal of Essential Oil Research 7: 287–297.

Krasnyansky S, Ball TM & Sink KC (1998) Somatic hybridization in mint: identification and characterization of *Mentha piperita* (+) *M. spicata* hybrid plants. Theoretical and Applied Genetics 96: 683–687.

Krasnyanski S, May RA, Loskutov A, Ball TM & Sink KC (1999) Transformation of the limonene synthase gene into peppermint (*Mentha piperita* L.) and preliminary studies on the essential oil profiles of single transgenic plants. Theoretical and Applied Genetics 99: 676–682.

Kähäri J, Mäntylahti V & Rannikko M (1987) Soil fertility of Finnish cultivated soils 1981–1985. Soil Analysis Service. 105 pp.

Lacy ML & Horner CE (1966) Behavior of *Verticillium* in the rizhosphere and on roots of plants susceptible, resistant and immune to wilt. Phytopathology 56: 427–430.

Lawrence BM (1985) A review of the world production of essential oils (1984). Perfumer & Flavorist 10: 1–16.

Lawrence BM & Shu CK (1989) Peppermint oil differentiation. Perfumer and Flavorist 14: 21–30.

Lawrence BM (1992) The Spearmint and peppermint industry of North America. In Verlet N (ed) 3rd International Conference on Aromatic and Medicinal Plants, Nyons, France, December 2-4, 1991, 59–90.

Letchamo W (1993) Nitrogen application affects yield and content of the active substances in chamomile genotypes. New Crops, 636–639.

Lloyd G & McCown B (1980) Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Combined Proceedings of the International Plant Propagators Society 3: 421–427.

Maffei M, Mucciarelli M & Scannerini S (1994) Are leaf area index (LAI) and flowering related to oil productivity in peppermint. Flavour and Fragrances 9: 119–124.

Maffei M, Canova C, Bertea CM & Scannerini T (1999) UV-A effects on photomorphogenesis and essential-oil composition in *Mentha piperita.* Journal of Photochemistry and Photobiology 52: 105–110.

Mahmoud SS & Croteau RB (2001) Metabolic engineering of essential oil yield and composition in mint by altering expression of deoxyxylulose phosphate reductoisomerase and menthofuran syntheses. Proceedings of the National Academy of Science 98: 8915–8920.

Maia NB, Bovi OA, Marques MOM, Granja N do P, & Carmello QAC (2001) Essential oil production and quality of *Mentha arvensis* L. grown in nutrient solutions. Acta Hoticulturae 548: 181–187.

Marotti M, Piccaglia R, Giovanelli E, Deans SG & Eaglesham E (1994) Effects of planting time and mineral fertilization on peppermint (*Mentha* x *piperita* L.) essential oil composition and its biological activity. Journal of Flavour and Fragrance 9: 125–129.

Matabwa CJ & Rowell DL (1997) The factors limiting crop production on the Mubangwe farm, Malawi. Soil Use and Management 13: 107–110.

Matusiewicz E (1972) The reaction of *Mentha x piperita* to soil pH. Prace Komisji Nauk Roliniczch i Komisji Nauk Lesnych PTPN 33: 211–220.

McConkey M, Gershenzon J, Croteau R (2000) Developmental regulation of monoterpene biosynthesis in the glandular trichomes of peppermint (*Mentha* × *piperita* L.). Plant Physiology 122: 215–223.

Muller RF, Berger BM, Vegen O & Cakir C (1997) Seasonal variation in the chemical compositions of essential oils of selected plants growing wild in Turkey. Journal of Agricultural and Food Chemistry 45: 4821-4825.

Murashige T & Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15: 473–497.

Murray MJ, Marble P, Lincoln D & Hefendehl FW (1988) Peppermint oil quality differences and the reasons for them. Flavors and Fragrances: Proceeding of the 10th International Congress of Essential oils, Fragrances and Flavors, Washington, DC, U.S.A., 16-20 November 1986, p 189–208.

Myers RJK & Foale MA (1981) Row spacing and population density in grain sorghum a simple analysis. Field Crops Research 4: 147–154.

Naghdi Badi H, Yazdani D, Mohammad Ali S & Nazari F (2004) Effects of spacing and harvesting time on herbage yield and quality/quantity of oil in thyme, *Thymus vulgaris* L. Industrial Crops and Products 19.232–236.

Nelson CE, Mortensen MA & Erly RE (1971) Evaporative cooling of peppermint by sprinkling. Washington Agricultural Experiment Station. Bulletin 539.

Nemeth E & Pham TV (1995) Vegetative propagation of four species of *Mentha*. Gartenbauwissenschaft 60: 34–37.

Neter J., Kutner M., Nachtsheim C. & Wasserman W. (1996). *Applied Linear Statistical Models,* Fourth Edition. Irwin, Chicago, 1310 pp.

Nijjar GS (1990) Optimizing *Mentha* oil yield from 'Shivalik-88' variety of *Mentha arvensis*. Indian Perfume 34: 186–189.

Niu X, Lin K, Hasegawa PM, Bressan RA & Weller SC (1998) Transgenic peppermint (*Mentha piperita* L.) plants obtain by co cultivation with *Agrobacterium tumefaciens*. Plant Cell Reports 17: 165–171.

Oudhia P (2003) Traditional and medicinal knowledge about pudina (*Mentha* sp. family: *Labiatae*) in Chhattisgarh, India. Botanical. Online, http://botanical.com.

Pank F (1974) The influence of different planting material on the yield potential of peppermint (*Mentha piperita*) in the first growing year. Pharmazie 29: 344–346.

Patra NK, Khanuja SPS, Shasany AK, Singh HP, Sing VR, Hasan T, Alok K, Singh HB, Mengi N, Tyagi NK, Naqavi AA, Sushi K, Tanveer H, Kalra A & Kumar S (2000) Proceeding of the national seminar on the research and development in aromatic plants: current trends in biology, uses, production and marketing of essential oils. Journal of Medicinal and Aromatic Plant Sciences 22: 263–277.

Phatak SV & Heble MR (2002) Organogenesis and terpenoid synthesis in *Mentha arvensis*. Fitoterapia 73: 32–39.

Piccaglia R, Dellacecca V, Marotti M & Giovanelli E (1993) Agronomic factors affecting the yields and the essential oil composition of peppermint (*Mentha* x *piperita* L.). International symposium on medicinal and aromatic plants, Tiberias on the Sea of Galilee, Israel, 22-25 Mar. 1993. Acta Horticulture 344: 29–40.

Piccaglia R & Marotti M (1993) Characterization of several aromatic plants grown in northern Italy. Journal of Flavour Fragrance 8:115–122.

Prasad S & Saxena MC (1980) Effect of date of planting and row spacing on the growth and development of peppermint (*Mentha piperita* L.) in Tarai. Indian Journal of Plant Physiology 23: 119–126.

Ram M & Kumar S (1997) Yield improvement in the regenerated and transplanted mint *Mentha arvensis* by recycling the organic wastes and manures. Bioresource Technology 59:141–149.

Ram M. & Kumar S (1999) Optimization of interplant space and harvesting time for high essential oil yield in different varieties of mint *Mentha arvensis*. Journal of Medicinal and Aromatic Plant Sciences 21: 38–45.

Randhawa GS & Satinder K (1996) Optimization of harvesting time and row spacing for the quality oil in corn mint (*Mentha arvensis* L.) varieties. Acta Horticulturae 426: 615–622.

Randhawa GS, Mahey RK, Sidhu BS & Saini SS (1988) Effect of growth regulators on emergence, herb and oil yields of *Mentha* species. Vth ISHS symposium on medical and aromatic spices plants. Acta Horticulturae 188: 169–173.

Rao BRR (2002) Biomass yield, essential oil yield and essential oil composition of rose-scented .geranium (Pelargonium species) as influenced by row spacings and intercropping with corn mint (*Mentha arvensis* L .f. *piperascens* Malinv. ex Holmes). Industrial Crops and Products 16: 133–144.

Rao BRR, Singh SP, Rao EVSP, Rajeswara-Rao BR & Prakasa-Rao EVS (1984) Effect of row spacing and nitrogen application on biomass yield, essential oil concentration and essential oil yield of bergamot mint (*Mentha citrata* Ehrh.). Indian Perfume 28: 150–152.

Ravishankar GA & Venkatarman LV (1988) Rapid multiplication of plants from cultured axillary buds of *Mentha piperita*. Philippine Journal of Science 117: 121–129.

Rech EL & Pires MJP (1986) Tissue culture propagation of *Mentha* sp. by the use of axillary buds. Plant Cell Reports 5: 17–18.

Rohloff J, Skagen EB, Steen AH, Beisvåg T and Iversen TH. Essential oil composition on Norwegian peppermint (*Mentha x piperita L*) and Sachalin mint (*Mentha sachalinensis* (Briq.) Kudo). Acta Agriculturae Scandinavica. Sect. B 50:161–168.

Rohloff J (2002) Essential oil composition of Sachalin mint from Norway detected by solid-phase microextraction and gas chromatography-mass spectrometry analysis. Journal of Agricultural and Food Chemistry. 50:1543–1547.

Salo R (ed) Mint research in Finland (1999) Symposium of Mint Research, Jokioinen, 8.12.1999. Publications of Agricultural Research Centre of Finland, Series A 66, 173.

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

Saxena A & Singh JN (1995) Effect of irrigation, mulch and nitrogen on yield and composition of corn mint (*Mentha arvensis* L. subsp. Haplocalyx var. piperascens) oil. Blackwell Wissenschafts-Verlag - Berlin ISSN 0931-2250, 183–188.

Seigler DS (1998) Plant Secondary Metabolism. Kluwer Academic Publishers. Boston/ Dordrecht/London, pp 759.

Shah SC & Gupta LK (1989) Response of mentha species to different harvesting intervals. Progressive Horticulture 21: 148–150.

Shalaby AS & Razin AM (1992) Dense cultivation and fertilization for higher yield of thyme (*Thymus vulgaris* L.). Journal of Agronomy and Crop Science 168: 243–248.

Sharma S & Tyagi BR (1991) Character correlation, path coefficient and heritability analyses of essential oil and quality components in corn mint. Journal of Genetics 45: 257–262.

Sharma S, Tyagi BR, Nagivi AA & Thrkur RS (1992) Stability of essential oil yield and quality characters in Japanese mint (*Mentha arvensis* L.) under varied environmental conditions. Journal of Essential Oil Research 4: 411–416.

Shasany AK, Khanuja SPS, Dhawan S & Kumar S (2000) Positive correlation between menthol content and in and in vitro menthol tolerance in *Mentha arvensis* L. cultivars. The Journal of Biosciences 25: 263–2000

Shukla PK, Haseeb A & Srivastava NK (1997) The relation between soil pH and the reproduction/damage potential of *Pratylenchus thornei* on growth and oil yield of *Mentha spicata*. Nematologia Mediterranea 25: 25–28.

Shukla PK, Haseeb A & Srivastava NK (1998) Influence of pH on reproduction and damage potential of *Pratylenchus thornei* on *Mentha x piperita*. Fundamental and Applied Nematology 21: 103–105.

Singh K, Singh V & Kohtari SK (1986) Effect of planting materials and spacing on herb, oil and sucker production in *Mentha arvensis* L. Annales of Agricultural Research 7: 313–316.

Singh VP & Singh DV (1986) Accumulation pattern of major chemical constituents in *Mentha* species with advancement of crop age and nitrogen levels. Acta Horticulturae 188: 86–94.

Singh AK, Naqvi AA, Singh K & Thakur RS (1988) Transformations of menthol, menthone and menthyl acetate in corn mint with relation to age of plant. Current Science 9: 480–481.

Singh VP, Chaterjee BN & Singh DV (1989) Response of mint species to nitrogen fertilization. Journal of Agricultural Science 113: 267–271.

Sivropoulou A, Kokkiki S, Lanaras T & Arsenakis M (1995) Antimicrobial activity of mint essential oils. Journal of Agricultural and Food Chemistry 43: 2384–2388.

Slavov SI (1985) The effect of fertilizer application on peppermint productivity. Plant Science 1: 61–65.

Small E (1997) *Mentha*-mint family (Lamiacae). In: Culinary Herbs. Ottawa, Ontario, Canada. NRC Research Press, 351–372.

Srivastava NK & Luthra R (1994) Relationship between photosynthetic carbon metabolism and essential oil in peppermint under Mn-stress. Journal of Experimental Botany 47:1127–1132.

Stevens RJ & Laughlin RJ (1996) Effects of lime and nitrogen fertilizer on two sward types over a 10-year period. Journal of Agricultural Science 127: 451–461.

Suh EJ & Park KW (1999) Effect of magnesium on the content and composition of essential oil of basil cultivars grown in hydroponics. Korean Society for Horticultural Science 40: 336–340.

Taiz L & Zeiger E (1998) Plant Physiology. 2nd ed. University of California. Sinauer Associates, Inc., Publishers, 792 pp.

Tan KH (1994) Environmental Soil Sience. New York, 304 pp.

Tapalov V, Zheljazkov V & Kolarov 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 Hungarica 1-2: 60–67.

Thompson DP (1989) Fungitoxic activity of essential oils components on food storage fungi. Mycologia 81: 151–153.

Tucker AO (1992) The truth about mints. The Herb Companion 4: 51–52.

Tullihallitus (1984-1996) Ulkomaankauppa. Suomen viralliset tilastot 1984-1996. Helsinki: Tullihallitus. Finnish National Board of Customs.

Ulseth AI (1994) Cultivation and essential oil analysis from different cultivars of peppermint (*Mentha* x *piperita*). In Ahonen S (ed) Proceedings of NJF seminar no. 240. Production of herbs, spices and medicinal plants in the Nordic countries, 73. Mikkeli, Finland.

Valdeyron GB Dommee & Vernet P (1977) Self-fertilisation in male-fertile plants of a gynodioecious species: *Thymus vulgaris* L. Heredity 39: 243–9.

Van der Watt HVH, Barmad RO, Cronje IJ, Dekker J, Croft GJB & van der Walt MM (1991) Amelioration of subsoil acidity by application of a coal-derived calcium fulvate to the soil surface. Soil Chemistry and Physics 350: 146–148.

Voirin B, Brun N & Bayet C (1990) Effects of day length on the monoterpene composition of leaves of *Mentha piperita*. Phytochemistry 29: 749–755.

Wiersema HJ & Leon B (1999) World Economic Plants: A Standard Reference. CRC Press.

Wink M (2003) Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry 64: 3–19.

Yao AYM & Shaw Rh (1964) Effect of plant population and planting pattern on distribution of net radiation. Agronomy Journal 56: 165–168.

Zambori N & Tetenyi P (1988) Studies on the stimulation of the stolon development of peppermint (*Mentha piperita* L.). Herba Polonica, 34: 129–135.

Zheljazkov V & Topalov V (1996) Effect of planting time and density on yields from rooted mint cuttings. Journal of Herbs, Spices & Medicinal Plants 4: 15–24.

Özel A & Özgüven M (2002) Effect of different planting times on essential oil components of different mint (*Mentha* spp.) varieties. Turkish Journal of Agriculture and Forestry 26: 289–294.