Propagation of Mint (*Mentha*)

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An essential oil industry based on production of high value peppermint oil for export is emerging in the upper reaches of the river valleys of northeast Victoria. Research into essential oil production at the Ovens Research Station (ORS) by the Department of Agriculture, Victoria, commenced in 1976. This paper gives an overview of the production process, including methods of propagation.

TAXONOMY

The genus *Mentha*, in the family Labiatae, contains a large number of species and has a geographic distribution over most continents. Most species are herbs and aromatic. The mints have been known over the centuries for their use in herbal treatments and oil extraction. The first references to the use of mint leaves date from the time of the Pharaohs.

The *Mentha* genus is divided into two subgenera *Pulegium* and *Menthastrum*. *Pulegium* is characterised by poorly developed stolons, reclining habit, and axillary flower spikes. The subgenus *Menthastrum* typically has well developed stolons and is mostly perennial. *Mentha* species most commonly used in steam distilled essential oil extraction in today's essential oil industry are derived from the subgenus *Menthastrum*.

Native Spearmint. *Mentha spicata* (2n=48) which is possibly a hybrid of *M. longifolia* (2n=24) × *M. suaveolens* [syn: *M. rotundifolia*] (2n=24).

Scotch Spearmint. *Mentha cardiaca* (2n=72) a name recently changed to *M.* × *gracilis*. The major spearmint components are carvone (55% to 65%) and limonene (13% to 20%). Spearmints are most often found in chewing gums and other confectionery lines.

Peppermint. *Mentha* × *piperita* (2n=72) is believed by some to be derived from a triple cross between *M. aquatica* (2n=96) and *M. spicata* (2n=48) (itself a possible hybrid). It is important to note that *M.* × *piperita* is sterile. Peppermint oil typically contains menthol (43% to 50%), menthone (15% to 25%), and menthylacetate (2% to 7%) and is the true peppermint of commerce. Main uses for peppermint include confectionery, food flavouring, and pharmaceutical products.

Corn Mint. *Mentha arvensis* (2n=96) produces an oil containing many compounds in common with peppermint oil but with menthol levels much higher at 85%. The oil produced is inferior to peppermint oil but is used in mixtures with peppermint oil to produce a cheaper oil.

COMMERCIAL PRODUCTION

This discussion will focus on peppermint because its value and production is by far the largest of the four species listed above. Peppermint oil production has historically been situated in the USA with the main producing states Wisconsin,

Michigan, Washington, Oregon, and Idaho. These states have a latitude above 40°N. This fact provides an insight into one of the conditions necessary to produce high quality peppermint oil. The peppermint plant uses daylength to determine its flowering time and hence foliage maturity. Broadly speaking, oil quality can be tied to the flowering stage of the plant.

Another determinant which controls oil quality is diurnal temperature fluctuation in the month preceding harvest (January-February in Australia). In Australia the production of peppermint is restricted to two regions: Tasmania, which offers daylengths similar to production areas in the U.S.A.; and the upper reaches of the river valleys in north eastern Victoria, which have clear hot days and clear cool nights, due to their proximity to mountain ranges.

If either of the two above conditions are not met, it is virtually impossible to produce an oil which has the correct balance of components. The normal chemical pathway involves the reduction of pulegone to menthyl acetate, through the intermediates menthone and menthol. Production of menthofuran, an undesirable compound, from pulegone occurs in plants which are not grown under the proper climatic conditions.

PRODUCTION CYCLE

Planting. The peppermint plant being a sterile hybrid must be propagated vegetatively. This has a negative side because the entire commercial crop in a region could possess the same genotype. Such a monoculture has inherent problems with disease susceptibility.

Field propagation of peppermint is relatively easy due to the stoloniferous habit of the peppermint plant. Stolons can be broken up and spread over the ground in spring or (for best results) in autumn, lightly incorporated and then irrigated. Commercial planting equipment achieves a multiplication ratio of approximately 1 : 7. This equipment consists of modified double-row potato digger which lifts the plants or stolons into either a truck or directly into a mint root planter. The planter flails or strips the stolons into short segments containing both roots and leaves. The segments are then dropped down hoppers into furrows opened and closed by tines. A roller follows to smooth out the soil layer. Irrigation is applied as soon as possible following completion of planting.

A less sophisticated but satisfactory method involves the use of a converted spinning-disc fertiliser spreader to evenly apply the mint stolons, followed by light discing and irrigation.

Growing Stages. Following autumn sowing of mint stolons and proper weed control, the mint shoots appear in September and October. Attention to nutrition, weed, pest, and disease control as well as irrigation are the growers' main activities until harvest approaches in late January to mid February. By this time the plants are approximately 1 m tall and fill all the available space in a field.

Harvest and Distillation. The mint field is mown and windrowed to allow wilting for a 1/2 to 1 day. Wilting provides a means of cost reduction as less water in the plants means lower transport costs and lower fuel costs at distillation time.

The mint herbage is then forage harvested into large enclosed tubs which hold about eight tonnes of herbage and may hold the crop from 1/3 ha. These tubs are

transported to the boiler site and become part of the still when steam is connected to one end and a condenser to the other. Peppermint oil is collected in a receiving can. The expected yield of peppermint oil is approximately 70 kg/ha.

New Cultivar Breeding. Gamma-ray irradiation to induced artificial mutations is the main method used to produce new cultivars. Several new strains have been developed using this technique.

The main cultivar released in Victoria is Todd's Mitcham, a cultivar with some tolerance to a specific type of verticillium wilt (*Verticillium dahliae* var. *menthae*)—not identified to date in Australia. This root disease has altered the way mint is grown in the U.S.A. It is spread by soil and water movement and stolon transfer. Soil disturbance has been reduced to a minimum for fear of spreading the disease across fields. Planting material is now produced in certified nurseries geographically remote for the production centres.

MICROPROPAGATION OF *MENTHA* SPECIES

Micropropagation of peppermint is a simple technique using conventional tissue culture methods. Firstly, shoot and shoot tip culture was undertaken using excised plant material imported in tissue culture from the National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon, U.S.A.

Shoot tips are placed on Murashige and Skoog (MS) medium which is commonly used in the micropropagation of herbaceous plants. MS medium contains macro- and micronutrients, vitamins, sucrose, and agar. Other additives can be used but for peppermint propagation the basic MS medium was found to be satisfactory except for several difficult lines requiring added hormones.

The flasks contain 30 ml of medium which provides sufficient nutrient to last the plantlet for up to 6 weeks. In the case of peppermint, turn around time is 3 to 5 weeks between subculturing. Subculturing at 4 weeks typically produces a multiplication rate of four (four new plants from one plant every 4 weeks). NCGR in Oregon uses a very similar protocol with similar results.

Import Procedure. The Department of Agriculture had initial importation problems because of Australia's quarantine regulations relating to the type of container used to maintain sterile conditions. In an attempt to reduce container damage and limit the risk of contamination these regulations state that plants imported via tissue culture must be sent in rigid, clear plastic containers preferably with screw top lids/seals. Unfortunately, the NCGR only sends tissue cultured plants in soft, clear, heat-sealed, plastic containers called STAR-PAK. STAR-PAK is a more suitable method of transportation as it possesses some flexibility and occupies a smaller volume.

Storage. Peppermint plantlets store and travel very well for periods of up to a year under refrigeration at 2 to 4C. Although they can still be successfully propagated after extended storage, some cultivars do show a loss of vigour after such an extended period.

Media. In an attempt to better use the plant material received, and to reduce plant loss in the initial propagation phases, basic research was undertaken into the use of hormones in propagation media. The cytokinin, benzylaminopurine (BAP), was used to increase shoot multiplication from the limited plant material

available. A suitable rate for *Mentha* species was found to be 0.1 µM. The auxin, indole-3-butyric acid (IBA), was used at different rates to induce root initiation of plants difficult to propagate. Treatments of 0.1, 1 and 10 µM were tried with 1.0µM found to be most suitable. With the addition of these hormones the general multiplication rate was 4×.

Once in culture the peppermint plants were grown under similar regimes of temperature and daylength as most other herbaceous plants. Temperatures of 20 to 24C and a daylength of 16 h were found to be best for peppermint multiplication.

CONCLUSION

The preceding comments have outlined the growing and propagating requirements for the emerging peppermint oil industry in north east Victoria. The industry is now in its fourth year of exporting to the flavour and fragrance houses around the world. It is anticipated that the industry will continue to evolve and produce a wider range of essential oils and to take its place on the world scene as a producer of essential oil of the highest quality.