FIELD PERFORMANCE OF MINT PROPAGATED BY VARIOUS MEANS'

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Abstract

For this field trial, peppermint and spearmint rooted cuttings were propagated in various ways, some of which might result in measurable shifts in field growth, development, yield, or oil character in comparison to other propagation methods. If differences were to occur, the most likely cause(s) would be clonal genetic variation (when only one or a few selected plants were used as the basis for propagation of many plants) or alteration in disease status (when plants were meristem tip cultured or otherwise treated in a manner that might eliminate viruses or other systemic infection). The several propagation treatments were not necessarily exclusive with respect to their potential genetic or disease effects — i.e. in some treatments, both clonal selection and disease freedom might occur, either of which, or both of which, might effect growth, development, yield, and oil character. In general, whether any measurable differences for various propagation methods occurred in the field were of interest. The nature of any such differences would be investigated later.

In this first year of planting, plants from all propagative techniques established well in the field. Plants were managed in ways that might prevent transmission of virus and bacteria among plots. Strong differences in vigor (peppermint and spearmint), timing and extent of flowering (peppermint and spearmint), and stolon development (peppermint) were observed ($p<0.05$). Harvest weights seemed to differ, especially for peppermint, but means were not statistically separable ($p<0.05$). Specific treatment differences are not discussed, as the identity of treatments remains coded at this time. Some caution is urged in interpreting this first year data, as there is the potential for field differences to have arisen from cultural effects during greenhouse production and shipping, especially for peppermint where two different propagation companies were involved, and where plants for one treatment arrived in two stages.

Introduction

Commercial propagation of rooted mint cuttings has changed in recent years. In addition, heretofore unknown micro-orpnisms have been identified within mint plants that might have effects on mint growth and performance. It could become routinely important to distinguish genetic variation within the plants themselves from pathogenic infection by viruses or other micro-organisms that may or may not reside in mint plants. Another issue is whether mint rootstock should routinely be passed through a meristem tip culture or tip culture phase to eliminate any risk that the common soil-borne and rhizome-borne pathogen fungus, *Verticillium dahliae,* might be carried along in propagation stock, although the risk of this is low already. There is some advantage in maintaining mint axenically (free of all micro-organisms) in a culture test tube *(in vitro* culture) as a source of stock plants, rather than keeping stock plants in greenhouse beds or collecting new stock plants from the field — although such culture in itself will not exclude viruses.

With respect to axenic or *in vitro* culture of mint in test tubes, problems typically arise when bacteria living within the plant (endogenous bacteria) eventually proliferate and contaminate test tube plant cultures (Buckley et al. 1995, Reed et al. 1995). The natural distribution of such bacteria in commercial mint production, and the possible influence by such organisms on growth and development of mint in the field has been raised. Certain endophtyic bacteria are occasionally influential in some plant systems, so at least theoretically, their exclusion from (if detrimental) or retention in (if beneficial) rootstock destined for the field could be important. It is argued here, however, that the endogenous bacteria associated with mint are far less likely to be the cause of growth differences in the field than are either virus(es) as discussed below or genetic variation in the mint plant (also addressed below). Bacteria almost certainly routinely enter mint plants through cut ends of rhizomes, through wounds of various sorts, or into leaf air spaces. The two citations above are for mint (Buckley et al. 1995, Reed et al. 1995), and resulted from work under the direction of Dr. Barbara Reed. She observed many kinds of bacteria associated with mint, none of which were common plant pathogens, but all of which were common soil inhabitants. Dr. Reed did not perceive the presence of absence of bacteria to impact mint growth in her studies, except in tissue culture. No individual mint plant harbored more than one or two bacterial

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species. No bacterial type was predominant — many types were each found in low frequency. No bacteria were found in some plants, and no bacteria were found in high population within any plants. Dr. Reed's only concern was to eradicate any bacteria that proliferated after mint plants were placed into tissue culture, under conditions that favored the growth of the bacteria such that the mint cultures were overwhelmed and unsustainable. She considers the endogenous bacteria associated with mint to be strictly a tissue culture problem in the laboratory. This may translate into a hurdle for maintaining *in vitro* propagative stock for commercial mint propagators, but bacterial status of rootstock in the field is likely unimportant (Barbara Reed, USDA-ARS, Corvallis OR, personal communication). While there are endogenous bacteria that do affect plant growth (usually adversely), these are typically quite different than the ones Dr. Reed found Screening mint rootstock for bacteria and subsequently identifying the various types is tedious, expensive work, which we feel is an unjustified effort unless some stronger indication emerges that bacteria play a larger role in mint performance in the field.

A previously undetected Capilloviruses virus was recently detected in peppermint. This plant virus group is typically transmitted by vegetative propagation and typically only causes growth reduction rather than necroses, distortions, color changes or other debilitating symptoms often associated with many plant viruses. We suspect that this virus is routinely propagated with normal grower handling of field grown rhizomes, and likely is routinely transmitted with greenhouse grown rooted cuttings unless extreme measures (e.g. meristem tip culture) are taken to eradicate it (Crowe 1993, 1994, and Crowe & Lommel 1995). Observations by us and others (L. Welty, Montana State Univ., personal communication) indicate that this virus may in fact prove beneficial in mint. Even though bay yields were lower, oil yield was higher in non-meristemmed mint. However, some growers have reported better growth and yields with meristemmed mint.

Our virus findings have not yet been completed to the point where the virus has been reinserted into truly virus-free mint in order to verify that it actually causes the observed differences in plant growth. This is an objective of research in progress, which has been delayed by the need to establish truly virus free test plants for the transmission studies.

As an alternative hypothesis to explaining the above growth differences, it is possible that by using a single selected mother plant (meristem tip cultured or otherwise) as the base for all later propagation, genetic clonal variation inadvertently occurs. This could be true for axenic culture or *in vitro* systems, too, if most rooted cuttings were derived from only one or a few mother plants.

For the field trial reported here, and for a similar field trial in Montana, peppermint and spearmint rooted cuttings were propagated in various ways, some of which might result in measurable shifts in field growth, development, yield, or oil character in comparison to other propagation methods. If differences were to occur, the most likely cause(s) would be clonal genetic or alteration in disease status. Treatments included in central Oregon are described in detail below. The several propagation treatments were not necessarily exclusive with respect to their potential genetic or disease effects — i.e. in some treatments, both clonal selection and disease freedom might occur, either of which, or both of which, might effect growth, development, yield, and oil character. In general, whether any measurable differences for various propagation methods occurred in the field were of interest. The nature of any such differences would be investigated later.

Ideally, all material used for field testing comparisons would have come from a single propagator and materials to be compared would have been handled comparably during propagation and shipment. Indeed, in our past field study, our inability to acquire both meristemmed and non-meristemmed mint from a single propagator was cause to discount all information on size and vigor of plant materials shipped from different suppliers, and to discount all growth comparisons in the first year in which materials were planted When the plantings grew differently in the second and later years, we were confident that such differences were not due to carryover of different greenhouse management.

In 1996, all spearmint treatments and four of five peppermint treatments were from Summit Labs, with one additional treatment from Lake Seed Co.

Objectives

1. Determine whether methods of propagation affect field performance of peppermint and spearmint.
2. Gather clues as to whether observed differences in plant growth might be due to virus infection status or to genetic alteration (stable or semi-stable) during propagation.

Methods

Treatments included in the 1996 planting were as follows, with sources of the material in parentheses. Treatments in central Oregon may vary somewhat from those included in related Montana field trials.

Black Micham Peppermint Treatments: The mother plant mentioned below was tested by S. Lommel to be infected with the Capillovirus mentioned above.

a. (Summit Labs) **Heat treated and meristem tip cultured from single mother plant.** Using a single, heat treated mother plant from the MIRC greenhouse beds, fully differentiated daughter plants were regenerated in test tube from meristem tip. Both heat treatment and meristem tip culture assist in the elimination of viruses from regenerated plants (Wright 1988). Regenerated daughter plants were relocated to the greenhouse. Several of these putative virus-free daughter plants were used as sources of rooted cuttings planted into shipping trays received in central Oregon. The probable virus status of the several daughter plants regenerated has not yet been established.

b. (Summit Labs) **Rooted cuttings from same single mother plant as in section a.** The same mother plant as above was the source for a step-wise increase of rooted cuttings into shipping trays. These plants are almost certainly virus infected, but this has not yet been confirmed.

c. (Summit Labs) ***In vitro* nodal propagation from same single mother plant as in section a.** The mother plant above was used to create several aseptically-maintained daughters *in vitro* in test tubes. From these daughters, nodes were rooted briefly in aseptic medium *in vitro* and, when rooted, were transferred to shipping trays. These plants likely are infected with the virus, but this has not yet been confirmed_

d. (Summit Labs) **Recent *in vitro* nodal propagation from many plants.** [Hundreds of plants originating from MIRC beds were established *in vitro* in the winter of 1995-96. Nodes were cut and rooted aseptically for a few weeks in early spring of 1996, then transferred to greenhouse flats. These were then re-established *in vitro,* from which nodes were rooted *in vitro* and then transferred to shipping trays. These plants likely are infected with the virus, but this has not yet been confirmed.

e. (Lake Seed) ***In vitro* propagation from many plants (4 yr. *in vitro).*** This propagator established Black Mitcham *in vitro* in 1992 from one or a very few plants, but did not meristem tip culture. The plants have remained in culture since then. Nodes were rooted *in vitro* prior to transferring to shipping trays. The plants have not yet been tested for virus infection status.

Scotch Spearmint Treatments: The mother plant used by Summit Labs was tested by S. Lommel to be infected with the Capillovints mentioned above. Treatments were identical as for a, b, c, and d above, but no plants were available from Lake Seed or other propagators.

In 1996, plants were rooted in potting medium in individual plastic flat cells at Summit Labs and Lake Seed Co., shipped on June 17 from Colorado and Montana, and received June 18 in Madras. Prior greenhouse management is not listed here, but slight variations would be expected among propagators, and slighter variation for each propagator with respect to length of time within rooting cells, light incidence, fertility, potting mix, etc. All plants were in excellent condition upon arrival in Madras.

The experimental design was a randomized, complete block with four replications. Within plots, plants were placed 15 inches apart into 20 ft open furrows and covered with soil 2.5-3 inches deep on June 18. Irrespective of length of root ball and stems, plants were laid along the furrow, with only the top-most foliage protruding above the soil surface. This allowed for greater rooting area along stems. There were five furrows per plot spaced 20 inches apart. As mint grew, plots were considered to be 9 ft wide x 20 ft long. There were 5 ft separations between plots within replications, and 10 ft separations between replications. Due to an under-supply of Sturunit meristem tip cultured peppermint, plants were received in two shipments. Sicty-four percent of the meristem tip cultured peppermint plants were planted on June 18 leaving alternate gaps; gaps were planted on August 7 with additional meristemmed plants.

Within 3 weeks of planting, a half-rate of Sinbar (terbacil, DuPont) herbicide (3/4 lb ai/ac) was applied to peppermint, but not spearmint. All plots were hand-weeded as necessary during the season. After harvest, another 3/4 lb ai/ac of Sinbar was applied to both spearmint and peppermint. Plots were aerially sprayed once with Comite (propargite) at mid-season.

For all plant sampling, and for harvest, care was taken to avoid transmission of virus and other micro-organisms between plots. For flower stem collection, stems were snipped with scissors that were sterilized between plots with disinfectant. At harvest, swather cutter bars and related pieces involved with cutting and bringing hay into the swath were sprayed with 0.05 percent NaOCL between each plot. No viral or bacterial analysis was attempted from field grown material in 1996, although in retrospect this probably should have been done.

The total plot width and length was harvested on September 9, for both peppermint and spearmint.

To date, treatments remain coded, such that the field investigator in central Oregon (F. Crowe) is not aware of the type of propagation (except for Summit's meristem tip cultured plants which were in

short supply, and plants from Lake Seed Co., which arrived by separate shipment).

Results

As indicated above, the virus infection status of plants in various treatments has not yet been determined.

Peppermint: Treatments are coded P1 through P5. Ignoring the fewer plants placed into the one treatment, a perfect stand was achieved in all treatments — 100 percent of those planted on June 18 were present and growing on July 29. Evidence for differing field performance among treatments is seen in Table 1. No buds were observed on July 22, but by July 29, buds were seen in treatment P1 but in no other treatments ($p<0.05$). Vigor rating for July 22 (not shown) was similar to July 29 for which there were strong differences ($p<0.05$). Above ground stolon development showed some differences on July 29 ($p<0.05$). Careful excavation suggested that underground rhizome development somewhat matched ground surface development of stolons for each treatment, although data were not specifically collected. In general, plants within plots seemed quite uniform in growth and development.

Harvest results are shown in Table 1 as the mean dry weight (lb/ac) per treatment. The peppermint was not distilled. In the meristem tip cultured treatment, the plants received late were well-established by harvest, but did not have sufficient top growth to contribute to harvest yield. Further, there was no top growth on plants in any treatment that intersected the top growth of adjacent plants, which might have resulted in competition for sunlight and reduced growth per plant. Thus, each plant in each treatment was considered to have contributed equally toward harvest yield in 1996, irrespective of stand. For presentation in this table, harvest yield for the meristem tip cultured treatment was corrected by the stand proportion for the first planting date, so the harvest data reflect yield for a 100 percent stand as for other treatments. When the data were analyzed either with or without such correction, no statistically significant differences were found in either case ($p<0.05$).

Spearmint: Treatments are coded S lthrough S4. Survival averaged 95 percent, with no statistical differences among treatments (jy<0.05). Evidence for differing field performance among treatments is shown in Table 2. Well-developed lower buds were present on plants in two of the four treatments on July 22 ($p<0.05$). Also on July 22, vigor in one treatment was substantially less than in other treatments ($p<0.05$). It was felt that excessive early flower development might be to the detriment of root and rhizome growth. To redirect growth from flowers to vegetative growth [illegible] and weighed (Table 2). By July 29, uniform but very early development of bud initials were present on plants in all treatments. Extensive foliar growth had occurred during the previous week, and no flowers were removed at this point. In contrast to peppermint above, no rhizome/stolon development was seen at ground surface level on July 22 or July 29, but this was irregularly present on August 7, 1996. Careful excavation suggested that underground rhizome development was comparable to surface development of surface stolons. In general, plants within treatments seem quite uniform in growth and development.

Harvest results are shown in Table 2 as the mean dry weight (lb/ac) per treatment. No statistically significant differences were found among harvest weights ($p<0.05$). The spearmint was not distilled.

Discussion

All rooted cuttings from all treatments established well in 1996. Spearmint and peppermint derived by different propagation means show some marked early and mid-season variability in vigor, growth, development and yield following planting in field plots in central Oregon. The various treatments remain coded at this time and are not identified_ Caution should be used in inferring that all field variation is due to propagative technique and/or pathogenic status, as some or all variation could be due to greenhouse management/shipping variables, especially between the two propagation companies in the peppermint trial. Further, in the case of the meristem tip cultured peppermint, we may not have been correct to adjust the data as we did for stand differences, although we believe this was the appropriate way to consider the data. As any theoretical greenhouse or establishment effects should be gone by 1997, future differences may be more meaningful than those seen this establishment year.

Final comment on previous meristem tip culture trial, 1992-1996

The previous field trial, in which Black Mitcham that had been meristem tip cultured was compared with Black Mitcham that had not been meristem tip cultured, was retained in 1996 with minimal attention. No differences were seen with respect to several growth or harvest parameters, although originally meristem tip cultured plants were substantially greener in early to mid June than originally non-meristemmed plants. It may be recalled that growth in originally meristemmed plants was greater, and oil yields were lower than in non-meristemmed plants. However, these differences subsided over several years while at the same time virus began to appear in originally meristemmed plants that had not been present in the earliest years of the trial.

We initially thought that the meristem tip

a trace of virus particles might have been left in the meristemmed plants, resulting in eventual reappearance of the slow-growing virus over several years. We now suspect that our harvest processes, especially swathing, may have spread this virus between and among mint plants and across plots. We made no attempt to disinfect the swather cutter bars or other surfaces exposed to plant sap during harvest or other field operations during 1992-1995. Our new trial reported above is being handled substantially differently in this respect.

Growers of commercially available meristemmed mint may note a similar slow reversion of meristemmed mint to the non-meristemmed type of growth if they move swathers, tubs/choppers or other equipment that might spread expressed plant sap between fields of meristemmed and non-meristemmed mint.

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Table 1. Means for ratings of peppermint development and yield in the propagation trial in Central Oregon, following planting of rooted cuttings on June 18, 1996, using the Fisher LSD Procedure

Treatment	No. Plants with Buds Present per Plot[1] 7/29/96	Stolon Development[2] 7/29/96	Vigor of Top Growth[3] 7/29/96	Dry Hay Yield at Harvest[4] (lb/ac) 9/9/96
P1	26.5 b[5]	6.0 a	6.6 a	1,768 a
P2	0 a	8.4 **b**	7.7 ab	1,961 a
P3	0 a	8.7 b	9.3 be	2,098 a
P4	0 a	8.3 b	8.4 abc	1,642 a
P5	0 a	9.0 b	10.0 c	2,088 a

1. The number of plants (of 80 per plot) on which at least a single bud was seen to be developing.
2. Rhizome/stolon development at ground surface on a scale of 1 (none) to 10 (abundant and aggressive).
3. Vigor (visual rating, considering number and length of branches, of stems and foliage) on a scale of 1 (very little) to 10 (abundant and aggressive).
4. Entire plot was harvested with a forage swather, weighed, with sub-samples weighed and dried to determine the proportion of dry to fresh weight.
5. Means followed by the same letter are not significantly different ($p<0.05$).

Table 2. Means of ratings for spearmint development and yield in the propagation trial in Central Oregon, following planting of rooted cuttings on June 18, 1996, using the Fisher LSD Procedure

Treatment	Buds Present per Plot[1] 7/22/96	Dry Wt of Flower Stems + Buds[2] 7/22/96	Vigor of Top Growth[3] 7/22/96	Vigor of Top Growth[3] 7/29/96	Dry Hay Yield at Harvest[4] 9/9/96
Si	2.8 a[5]	4.8 a	8.5 b	9.8 b	2,091 a
S2	52.3 b	105.0 b	8.5 b	9.3 ab	2,323 a
S3	4.8 a	7.6 ab	9.3 b	9.8 b	2,344 a
S4	41.0 ab	75.3 ab	4.8 a	7.8 a	2,163 a

1. The total number of stems with buds seen within a plot. Some plants had more than one stem in bud.
2. Oven dry wt (grams) of buds and stems removed.
3. Vigor, including number and length of branches, of stems and foliage on a scale of 1 (very little) to 10 (abundant and aggressive).
4. Entire plot was harvested with a forage swather, weighed, with sub-samples weighed and dried to determine the proportion of dry to fresh weight.
5. Means followed by the same letter are not significantly different ($p<0.05$).