FIELD PERFORMANCE OF MINT PROPAGATED BY VARIOUS MEANS, 1997-1998

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Abstract

Field performance of Black Mitcham peppermint in 1997 and 1998 was similar for test plots planted with virus-infected propagation stock derived from (i) a single plant held *in vitro* [*in vitro* refers to rooting and storage in a test tube in the absence of microorganisms] (ii) a single plant not held *in vitro* from the same mother plant as the previous treatment and (iii) multiple plants held *in vitro.* Materials were from Mint Industry Research Council (MIRC) propagation sources. Among these three treatments, however, there was a consistent trend toward higher hay and oil yield for plots planted with plants derived from the single, virus-infected plant held *in vitro.* There was no obvious adverse or beneficial field performance associated with either *in vitro* or single plant sources. However, so few single plants were used and no conscious selection for different types was conducted in the choice of plants, consequently these data do not discount the possibility that clonal selection might result in treatment differences.

For peppermint, plants in two additional treatments were initially tested to be virus free: In one, plants propagated from a meristem tip cultured plant taken from the same MIRC mother plant as the single sourced treatments above (this is necessarily an *in vitro* process). In the second, Black Mitcham plants had been privately propagated for several years *in vitro,* but the process for developing virus freedom was uncertain. In 1996 to 1998, plants in both putative virus-free treatments were more vigorous in spring through summer, grew longer stems, and lodged later than virus-infected mint. In 1996, but not 1997 or 1998, there was a statistically greater mean hay yield ($P < 0.05$) for plots with putative virus free mints than for plots planted with virus-infected mints. Mean oil yields among all treatments could not be separated ($P < 0.05$) for any year, nor were there consistent trends.

In a companion spearmint field trial, planted with the same treatments excluding the private virus-free source, no treatment means could be separated ($P < 0.05$) for any character in 1997 or 1998, except for reduced stem length in 1998 for the putative virus free material. Some of these findings may be substantially different from those reported elsewhere.

The infection status of the putative virus free MIRC-source of meristem tip cultured peppermint and spearmint, initially tested as virus free in 1996, was reconsidered in 1998, when lab tests suggested virus was present in the field grown plants. However, the peppermint originating from the private source of initially virus free material continued to test free of virus in the field in 1998. That the two sources of putative virus free peppermint tested differently in 1998 suggests that the MIRC-sourced material (either peppermint or spearmint) was never truly virus free, and that virus did not spread in the field either mechanically or via some vector. This further raises the possibility that a failed meristem tip culture process accounted for reappearance of virus after several years in the field in our earlier 1992 to 1995 trial. In both the 1992 to 1995 trial and the

current trial, spring vigor and longer stems continued to occur in plots planted with initially virus free material, even as virus reappeared.

A reliable assay for Trichovirus now is available from North Carolina State University. However, this virus seems to replicate very slowly. Based on both laboratory and field data, it can take a year or more following failed a meristem tip culture process for the titer to rise high enough for positive testing. While the meristem tip culturing process used for generating virus free material for this trial was very standard, it appears that the appropriate variations in laboratory techniques for reliably freeing plant material of Trichovirus (and the time course for subsequent verification of virus freedom) have yet to be specified.

Background

Clonal propagation typically involve mass transfer of plant parts, retaining genetic type of the mother stock, but also usually transferring any systemic infections by viruses or other pathogens. Special techniques such as meristem tip culture can free plants of systemic pathogens, again while retaining the genetic type (Murashige and Skoog, 1962; Wright, 1988). Verification is required to guarantee that meristem tip culture successfully eliminated systemic pathogens (Wright, 1988).

Clonal variants genetically distinct from the original type plant can occur if stable genetic changes have occurred in the plant parts used for propagation. Some plants are more prone to clonal offshoots than others. For example, sweet potatoes so frequently develop off types that the industry must annually reselect for the original varietal type when choosing propagation material (F. Crowe, 1974 to 1977, personal observation; D. Hall, Univ. Calif. Davis, 1974, personal communication). For true potatoes and garlic, noticeable clonal offshoots occur more rarely. Selection of new garlic and potato clones requires close inspection of hundreds of thousands of plants in commercial or seed fields, plus several years of field trials to determine whether the initially perceived differences persist (J. Scudder, Montana seed potato grower, 1989, personal communication; E. Kurtz, Am. Dehydrated Onion and Garlic Assoc., 1978, personal communication). For peppermint, the frequency of clonal formation is not clear, although some private clonal selection efforts exist in the industry. There is some concern, however, that inadvertent selection could occur during routine greenhouse selection of a few plants to redevelop mother bed stock, or when only one or a few plants are held as nuclear stock in test tubes, or when a few plants are selected from which to derive meristem tip cultured plants. Further, there is some concern that simply holding plants for extended periods in test tubes might induce genetic changes from the original type.

From 1992 to 1995, commercially propagated, meristem tip cultured Black Mitcham peppermint was initially found to be free of a Trichovirus that was present in non-meristem tip cultured Black Mitcham (Crowe and Lommel, 1995). The virus free Black Mitcham matured early, was highly vigorous, and produced more hay but less oil when harvested late in the summer compared to virus infected Black Mitcham (Crowe, 1994, 1995). Growth of virus free mint became stunted when the Trichovirus was used to re infect virus free mint (S. Lommel, No. Carolina St. Univ., 1998, personal communication). Preliminary data from Montana suggests that meristem tip cultured

spearmint grew more vigorously but also yielded more oil than Trichovirus infected peppermint (L. Welty, Montana St. Univ., and S. Lommel, No. Carolina St. Univ., 1998).

Research Goals & Objectives

Field trials in central Oregon and Montana were established in 1996 with peppermint and spearmint propagated in various ways, primarily by a common propagator, to determine whether field performance might vary.

Methods

Peppermint treatments included all five listed below; Scotch spearmint treatments included only the first four described below. The first four treatments included plants from the MIRC stock sources at Summit Labs, Ft. Collins, Colorado. Plants for the fifth treatment were provided by Late Seed, Ronin, Montana. Peppermint and spearmint were located in separate trials, with randomized block experimental designs. Until October 1997, treatments were handled as a "blind" study, with treatments unknown to field staff.

Virus Free, Single: Meristem tip cultured from a single, virus-infected MIRC Black Mitcham mother plant. The regenerated plant used for propagation was initially tested virus free. Not held *in vitro* after regeneration. Treatment codes: Summit Spearmint = 20112, COARC = S-1. Summit Peppermint = 20111, COARC = P-3.

Not *in vitro*, Single, Virus Infected: Rooted Cuttings from the same single, virus infected mother plant as in the previous treatment. Not held *in vitro.* Treatment Codes: Summit Spearmint = 20103, COARC = S-4. Summit Peppermint = 20102, COARC = P-1.

In Vitro, Single, Virus infected. In Vitro nodal propagation taken from the same single mother plant as in the treatments above. Nodes from an aseptically maintained mother plant a few daughters from this plant were rooted briefly in aseptic medium and transferred to greenhouse flats during propagation. Treatment Codes: Summit Spearmint = 20109, COARC = S-2. Summit Peppermint = 20108, COARC = P-2.

In Vitro, Multiple, Virus Infected. Recent *in vitro* nodal propagation from many MIRC Black Mitcham plants. Less likely to result in inadvertent selection of a clonal variant. Treatment Codes: Summit Spearmint = 20106, COARC = S-3. Summit Peppermint = 20105, COARC = P-4.

Virus Free, *In Vitro*. *In vitro,* private propagator commercial stock for 4 years, commercially meristem tip cultured Black Mitcham. Prior to that, initially determined to be virus free. Treatment Codes: Lake Seed Peppermint = Lake, COARC = P-5.

In 1996, plants were propagated commercially as above in potting medium in individual plastic flat cells, shipped mid-June from Colorado and Montana, and received in excellent condition the next day in Madras, Oregon. Plants were placed 15 in. apart into 20 ft open furrows and covered with 3 in. soil on June 18. Treatments were randomized within 4 replications. Plants were laid along the furrow for greater rooting area. In 1997 and 1998, plots were grown as per commercial management. Spearmint was sprayed each season with Tilt to prevent mildew development. Plots were harvested

on August 7, 1997, and August 12, 1998. Hay was air dried in gunny sacks, and distilled in mini stills.

To reduce risk of virus spread with plant sap, tools, rubber gloves, and cutting surfaces of equipment were disinfected with 5% household bleach when working among plots. Plant tissue samples were collected from all plots in late June of each year, and sent to North Carolina for virus RNA analysis as per methods described in the North Carolina report.

Results

Peppermint. In 1996, P-3 and P-5 (both initially virus free) were higher in vigor, yielded greater hay weights ($P < 0.05$). Oil data were not taken in 1996. In 1997, virus free mint (P-3 and P-5) had longer and weightier stems than other treatments, lodged later, but no other statistical differences were observed ($P < 0.05$). Treatment data in 1998 was comparable to 1997, except for some variation among treatments ($P < 0.05$) for compositional proportions of Menthofuran and Menthol (Tables 1 and 2).

In 1998, treatment P-3 was tested positive for Trichovirus and P-5 tested negative. Testing in 1997 gave mixed results (both positive and negative results, or indeterminate results) for treatment P-3, and negative for P-5. All initially virus infected materials continued to test positive. The tester plants considered virus free in North Carolina, used to compare with our field grown material, were from a different meristemmed plant than the P-3 material, but originating from Summit's MIRC material. These tester plants remained virus free according to various testing methodologies not described here.

Spearmint. Some flower initiation differences were seen in 1996 ($P < 0.05$) [data not shown], but these did not persist into 1997 and 1998. These were probably attributable to undetermined temporary cultural handling differences. In general, no statistically significant differences ($P < 0.05$) were seen in 1997 or 1998, except for some minor features (Tables 1 and 2).

In 1998, treatment S-1 tested positive for Trichovirus, as did the material from all other treatments. In 1997, S-1 plants gave mixed results in virus analysis. The tester plant considered free of virus in No. Carolina, used to compare with our field grown material, was from the original S-1 material from Summit. According to the virologists in North Carolina, it was possible that their tester material could be virus infected. But if so, the titer remains so low even after several years that it cannot be detected, so it remains useful for comparative purposes. This raises concerns about how one determines true virus freedom for these Trichoviruses, and how long it may take for titer to become enough elevated in the field before true virus freedom is certain.

Discussion

We do not find these data conclusive concerning the choice of single Vs multiple plants as greenhouse propagative material. Similarly, we do not find these data conclusive concerning the choice of *in vitro* Vs non *in vitro* plants. Based on our very limited study, there appeared to be no clear adverse or beneficial field performance

associated with these choices. This does not mean that selection of novel growth types cannot result in clonal separation.

Peppermint initially thought to be virus free but which later tested positive (MIRC/Summit) and peppermint which remained virus free (Lake Seed) both grew with the greater vigor and delayed lodging expected based on earlier investigations (Crowe, 1994, 1995). They did not yield greater hay or less oil as found earlier, but this could have been due to statistical "noise" in the trial area resulting from erratic occurrence of verticillium wilt, or perhaps we harvested too early for yield differences to manifest (Crowe, 1994, 1995).

That the Summit meristemmed peppermint and spearmint both tested positive for Trichovirus after being in the field, whereas the Lake material re-tested negative, suggested that no field spread of virus occurred, either mechanically or vectored. Instead, it appears that the initial meristem tip culture process failed to eliminate the virus. The Trichovirus group is notoriously slow growing, and it simply may take an extended period of time for the titer to become elevated enough to detect with available methods (S. Lommel, personal communication, 1998). This complicates verification of virus freedom or infection for meristem tip culture or other virus eradication procedures. Unfortunately, it also complicates determination of virus infection or freedom from material regenerated from tissue culture in the MIRC Biotech Program. It may further complicate Spearmint certification for virus freedom if freedom from Trichovirus proves beneficial in this species. Because the Trichovirus infection appears beneficial to peppermint (Crowe, 1994, 1995), lack of a quick and reliable detection method is less problematic for non tissue cultured peppermint propagative stock.

Literature Cited

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Table 1. Growth and harvest data in 1997 and 1998 for spearmint and peppermint originally propagated in various ways and planted in 1996 at the OSU-COARC.

	VIGOR		STEM HEIGHT		FRESH HAY		OIL YIELD	
Peppermint	(visual rating)		(cm)		(lb/A)		(lb/A)	
Propagation Source	5/6/97	5/27/98	6/10/97	6/16/98	8/5/97	8/12/98	8/5/97	8/12/98
P-1. Not *In-vitro,* single	3.0 a	2.6 b	33.8 a	35.5 d	26900	18995	85.7	48.3
P-2. In-vitro, single	3.0 a	2.9 b	32.9 a	38.4 b	28800	26354	90.1	63.9
P-3. Virus-Free, single, not held *in-vitro* after regeneration	4.2 b	3.9 a	36.6 b	44.6 a	31100	23165	87.9	51.8
P-4. In-vitro, multiple	3.2 a	2.7 b	32.5 a	35.9 c	25600	21360	86.9	57.6
P-5. Virus-Free (uncertain method); held *In-vitro*	4.8 a	3.7 a	36.7 b	47.1 a	27800	21516	82.1	49.8
F-value	4.58	4.65	3.66	83.62	0.54	0.30	0.19	1.16
F-test for 5%	3.26	3.26	3.26	3.26	3.26	3.26	3.26	3.26

	VIGOR		STEM HEIGHT		FRESH HAY		OIL YIELD	
Spearmint	(visual rating)[b]		(cm)		(lb/A)		(lb/A)	
Propagation Source	5/6/97	5/27/98	6/10/97	6/16/98	8/5/97	8/12/98	8/5/97	8/12/98
S-1. Virus-Free, single, not *held* in-vitro	4.0	3.1	36.6	39.2 ce	30700	25669	84.8	61.0
S-2. In-vitro, single	4.0	3.1	36.6	40.4 be	31800	25965	77.3	63.6
S-3. In-vitro, multiple	3.8	3.1	36.6	41.5 ab	34800	27458	82.0	64.7
S-4. Not *In-vitro,* single	3.5	3.0	37.8	42.6 a	35700	27474	86.1	58.5
F-values	0.86	0.20	0.11	8.06	0.60	0.71	0.19	1.60
F-test for 5%	3.49	3.49	3.49	3.49	3.49	3.49	3.49	3.49

aAnalysis of Variance F-value. F-test is significant if the F-value obtained is greater than the F-test at significance level 0.05 from an F-distribution table.
[b]Visual integration of plant vigor, including height, breadth and abundance of foliage. 0 = lowest; 5 = highest vigor.
'Means followed by the same letter are not significantly different at P 0.05 according to Fisher's protected least significant difference test.

Table 2. Oil component analysis for spearmint and peppermint in 1998 originally propagated in various ways and planted in 1996 at the OSU-COARC.

	OIL COMPONENT (% Area)						
Peppermint Propagation Source	**Heads**	**Menthone**	**MF**	**Ester**	**Isopulegol**	**Pulegone**	**Menthol**
P-1. Not *Invitro,* single	10.1	14.4	10.7 Aa	5.98	0.40	2.60	36.9 B
P-2. Invitro, single	9.35	13.8	9.28 B	5.89	0.41	2.36	39.3 A
P-3. Virus-Free, single, not in-vitro after regeneration	9.60	13.3	9.17 B	6.70	0.45	2.18	39.1 A
P-4. Invitro, multiple	9.79	13.1	8.85 B	6.59	0.44	2.14	39.2 A
P-5. Virus-Free (uncertain method); held *Invitro*	9.87	13.7	9.14 B	6.14	0.42	2.57	38.6 AB
P-value[b]	0.0515	0.5683	0.0059	0.6005	0.1968	0.4543	0.0895
Propagation Source	0.0768	0.6044	0.0117	0.5457	0.1988	0.4040	0.0551
Block	0.0704	0.4072	0.0131	0.5167	0.2339	0.4397	0.3023

	OIL COMPONENT (% Area)									
Spearmint Propagation Source	**Heads**	**Menthone**	**Menthol**	**Iso-menthol**	**Dihydro-carvone**	**Carvone**	**B-Pinene**	**Limonene**	**Cineole**	**3-Octanol**
S-1. Virus-Free, single, not in-vitro after regeneration	32.3	1.20	0.27	0.05	1.28	57.1	1.46	27.4	1.19	1.28
S-2. In-Vitro, single	33.4	1.18	0.27	0.06	1.23	56.5	1.47	28.4	1.22	1.19
S-3. In-Vitro, multiple	31.7	1.12	0.34	0.05	1.26	58.0	1.41	26.9	1.18	1.24
S-4. Not In-Vitro, single	30.7	1.13	0.34	0.05	1,20	59.1	1.37	26.1	1.17	1.29
P-value[b]	0.6219	0.7332	0.3563	0.8698	0.3559	0.6148	0.6114	0.5703	0.3690	0.1299
Propagation Source	0.3512	0.7414	0.4348	0.8314	0.9182	0.4185	0.4485	0.3627	0.6177	0.4502
Block	0.8460	0.5473	0.2686	0.7017	0.1363	0.7001	0.6469	0.7091	0.2063	0.0601

'Means followed by the same letter are not significantly different at P 5 0.05 according to Fisher's protected least significant difference test.
[b]Probability of obtaining F S 0.05.