PEPPERMINT VARIETY TRIAL, CENTRAL OREGON, 2001

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Summary

Mint varieties and selections were planted in 1999 from rooted cuttings into side-by-side *verticillium* wilt-infested and noninfested trials at the Central Oregon Agricultural Research Center (COARC) in Madras. In 2001 the selection 'B90-9' outyielded other varieties in both infested (59.7 lb/acre) and noninfested (72.3 lb/acre) trials. 'Black Mitcham' and the selections 'M83-14', and 'M90-11' yielded similarly in both infested and noninfested trials. 'Todds' and the selections '84M0107-7', '87M0109-1', and 'McKellip' yielded more in the noninfested than in the infested trial. No wilt was observed in selection '84M0107-7'. Wilt was present but at a relatively low incidence in infested plots for 'Todds' and the selections 'M83-14', '87M0109-1', and 'M90-11'. Wilt incidence in infested 'Black Mitcham' plots was moderate. Wilt incidence in selection 'B90-9' and in the selection 'McKellip' (derived from 'Black Mitcham') was intermediate between that of 'Todds and 'Black Mitcham'. 'Black Mitcham' and 'Todds' were considered to have "Madras-quality" composition. Percentage composition of a number of key oil components for selections 'B90-9', 'M83-14', '92(B-37XM0110)-1', '87M0109-1', and 'McKellip' were not out of range for "Madras quality", although 'Todds' and some selections had reduced menthofuran compared to 'Black Mitcham'.

Introduction

Peppermint variety trials initiated in central Oregon and elsewhere in 1994 and 1995 were the first public, replicated, and randomized such trials under uniform management and in which statistical comparisons could be made. The Oregon State University (OSU)-COARC field at Madras was not cropped with irrigated crops until 1990, and even now has a substantial area that is not infested with measurable levels of any strain of *Verticillium dahliae*. This allowed us in 1994 to artificially infest part of the trial area with a uniform level of only the mint strain of *V. dahliae*. This was an advantage over most fields in which *V. dahliae* populations are non-uniform, and in which mixtures of strains can confuse interpretation of soil assay measurements. This and other trials have proven useful for wilt and yield comparisons, and should become more useful as a new generation of mini stills are developed that may provide near-commercial character for the oil distilled from small plots. A second trial was initiated in 1999, again in a field believed to be noninfested with *V. dahliae* on the OSU-COARC farm at Madras. Half of the trial was infested with mint strains of *V. dahliae* in fall 1999. The growth, yield, oil quality, and *Verticillium* wilt susceptibility of seven entrees and two standard varieties will be evaluated from 2000 to 2003.

Methods

Plots were established from rooted cuttings in the summer of 1999. The trial area is split into an artificially infested area and a noninfested area as per 1994-1998. Plot sizes are 10 ft x 20 ft. All plots have been managed identically. Trials were maintained as per commercial practices with respect to fertility, pest and weed control, and irrigation.

Nine entrees were included. The Mint Industry Research Council (MIRC) submitted six entrees: '84M0107-7', 'M90-11', '87M0109-1', 'M83-14', '92(B37xM0110)-1', and 'B90-9'. A privately developed variety from L. McKellip (labeled 'McKellip 98') was also included. Standard varieties included 'Black Mitcham' and 'Todds'. The field was divided into two randomized complete block design trials: one half of the field was noninfested and the other half was infested with *V. dahliae*. Each variety is replicated four times within each trial.

In 1999, *V. dahliae* inoculum was produced in the laboratory by growing *V. dahliae* on a modified, minimal agar (Puhalla 1979) overlain with sterile, uncoated cellophane. After a 3-week incubation, microsclerotia were harvested by blending the cellophane in sterile water and washed on a 38-μm sieve. The dried concentrated inoculum was ground with sterile sand with a mortar and pestle. The concentration of microsclerotia/g inoculum was estimated and mixed with enough sterile sand to yield about four microsclerotia/g soil, a slightly higher initial rate than in 1994. As in the previous trial, the sand and *V. dahliae* microsclerotia mixture was spread over half of the field on plot surfaces after fall dormancy. At that time, all plots were tilled to both distribute rhizomes and place inoculum within the rooting zone of the mint.

In mid-summer 2000, *V. dahliae* soil populations were estimated. Soil was collected from three random locations within each plot in mid-July and air-dried for 1 month to eliminate ephemeral conidial spores and hyphae of *V. dahliae* (Butterfield and DeVay 1977) prior to analysis (Harris et al. 1993). After drying, subsamples were ground with a mortar and pestle to pulverize the soil and remove rocks larger than 1 cm in diameter. From each subsample, 25 g of soil were shaken and dispersed in water for 1 hour. The soil was wet sieved through 60- and 400-mesh soil screens to reduce soil volumes and many competitive organisms. Residue remaining on the 400-mesh screen was suspended in 100 ml water and 2 ml of this suspension was spread onto a semi-selective modified pectate agar medium in a petri plate. Ten plates were prepared per subsample. A total of 5 g of soil was plated per subsample. After a 2-week incubation in the dark at room temperature, colonies distinctive of *V. dahliae* were counted. The population data were expressed as the number of colony-forming units (CFU) per gram of soil.

Mint performance and oil character were assessed as in 1994-1998. Wilt severity was represented as per the recommended MIRC wilt rating with the proportion of plants in plots allocated to various wilt classes ranging from no wilt through severe wilt. The plots were harvested at about 10 percent bloom in 2000 and 2001. Subsamples from each plot were collected and air-dried in gunnysacks. Oil from these subsamples was distilled using the McKellip-Newhouse mini still located at OSU-Madras. RCB International, Ltd. in Albany, Oregon performed the oil component analysis in 2000, and I.P. Callison did this in 2001. Other evaluations were also made.

All response variables underwent an analysis of variance (ANOVA) using the general linear model, PROC GLM, of SAS version 7.0 (SAS Institute 1988). The noninfested and infested halves of the field were analyzed as separate experiments. Due to an unexpected level of wilt in the noninfested trial, discussed further in the results and discussion sections, four analyses were performed for each response variable. ANOVA was performed on the dataset (1) containing all plots, (2) without plots in the western blocks, and (3) containing all plots but relabeling the western block in the noninfested half of the field as infested. An analysis of covariance, using PROC GLM, was performed on the complete dataset with *V. dahliae* soil population as the covariate. Treatment means were separated by Fisher's protected least significant difference (LSD) test.

Results

As the 2000 growing season progressed, a disproportionate amount of wilt symptoms were evident in the western edge of the trial in both the infested and noninfested halves of the field. The plots most affected were those in the two blocks, one noninfested and one infested, that compose the western edge of the field. Following consultation with Cliff Pereira, statistician at Oregon State University, we performed a series of analyses to determine which approach yielded the most useful and meaningful data, taking into consideration this unbalanced disease incidence.

The *V. dahliae* soil population data were not significant as a covariate for any of the response variables. Therefore, soil populations in the plots could not account for the high proportion of wilt in those plots. We then compared the results from an analysis of the complete dataset with the analysis (1) of a dataset with the western edge blocks removed, and (2) of a dataset with the western edge block in the noninfested half of the field relabeled as infested because all plots displayed high disease severity. Neither the removal of the blocks nor the relabeling of the noninfested block changed the trends in the data with respect to differences in means among the varieties. Therefore, we chose to present results from the more conservative analysis of the dataset with the western blocks removed from both the noninfested and infested trial. This consequently reduced the total number of replications per variety to three in each trial.

The estimated means of net wilt at harvest, spring ground cover, mid-season vigor, mildew, fresh hay yield, and oil yield are shown in Table 1 (infested) and Table 2 (noninfested). Powdery mildew severity, hay yield, and oil yield are displayed in Table 1. The full MIRC wilt ratings are shown in Table 3 (infested) and Table 4 (noninfested). Oil character analyses is not shown in this report, but we hope to include these analyses in the companion MIRC report.

In 2000, the selection 92(B37xM0110)-1 proved to be inferior with respect to nearly all measured parameters, and this trend continued in 2001. The yield of 92(B37xM0110)-1 was only 14.7 lb/acre in the infested trial, and 25.1 lb/acre in the noninfested trial, attributable to poor winter survival and high wilt incidence. Hay weights between 2000 and 2001 varied substantially, with different varieties/selections ranking differently in the

2 years. In 2000, there no few significant yield differences among varieties/selections, but in 2001 the selection ‘B90-9’ outyielded other varieties in both infested (59.7 lb/acre) and noninfested (72.3 lb/acre) trials. ‘Black Mitcham’ and the selections ‘M83-14’, and ‘M90-11’ yielded similarly in both infested and noninfested trials. ‘Todds’ and the selections ‘84M0107-7’, ‘87M0109-1’, and ‘McKellip’ yielded more in the noninfested than in the infested trial.

No wilt was observed in selection ‘84M0107-7’. Based on the lack of any observed wilt incidence for this selection in the more highly infested western tier of plots, we might expect the apparent tolerance/resistance of selection ‘84M0107-7’ to hold up at higher infestation levels. This is supported by wilt data from the Willamette Valley trials, reported elsewhere. (Note, however, the apparent depressive effect of *verticillium* on yield of this and certain other selections or varieties, even in the absence of wilt symptoms.) Wilt was present but at a relatively low incidence in infested plots for ‘Todds’ and the selections M83-14’, ‘87M0109-1’, and ‘M90-11’. Wilt incidence in infested ‘Black Mitcham’ plots was moderate. Wilt incidence in selection ‘B90-9’ and in the selection ‘McKellip’ (derived from ‘Black Mitcham’) was intermediate between that of ‘Todds’ than ‘Black Mitcham’.

Stand, as measured by percentage groundcover in the spring, was lower in infested than in noninfested trials, for all varieties and selections. In the presence of *V. dahliae*. Stand was particularly depressed in the selection ‘92(B-37XM0110)-1’, and less so for ‘Black Mitcham’. In some plots, the selection ‘92(B-37XM0110)-1’ was particularly poor, and may be classified as nearly wilted out even in the beginning of 2001; none of the individual ‘Black Mitcham’ plots are that severely damaged yet.

Only the variety ‘Black Mitcham’ and the related selection ‘McKellip’ showed any mildew, which has not been severe in central Oregon in either 2000 or 2001.

Partial oil composition is shown in Tables 5 and 6. Oil composition in 2001 was representative of Madras-quality oil for the ‘Black Mitcham’ and ‘Todds’ varieties [8.1-9.1percent total heads, 41.0-41.2 percent menthol, 13.5-21.1 percent total menthone, 3.6-7 percent menthofuran, 1 percent pulegone, and 7.7-8.9 percent total esters], although ‘Black Mitcham’ was slightly high for menthofuran (5.7-7.0 percent) vs ‘Todds’ (3.6-4.5 percent). Percentage composition for both of these standard varieties and for the selections did not vary substantially between infested and noninfested trials. Percentage composition of these components for selections ‘B90-9’, ‘M83-14’, ‘92(B-37XM0110)-1’, ‘87M0109-1’, and ‘McKellip’ did not vary much from that of ‘Black Mitcham’ and ‘Todds’, although (as with ‘Todds’) some had reduced menthofuran compared to ‘Black Mitcham’. Total menthone was high for selections ‘84MO107-7’ (45.3 percent) and ‘87M0109-1’ (27.8 percent). Menthofuran was low for selections ‘84MO107-7’ (1.2-1.5 percent), ‘92(B-37Xmo110)-1’ (2.1-2.8 percent), and ‘87M0109-1’ (2.0-3.2 percent). Pulegone was high for selection ‘M90-11’ (5.1 percent). Total esters were low for selection ‘84M0107-7’ (3-3.2 percent).

Discussion

There are probably several different infestation levels within the entire variety trial. The first level is the artificial inoculum in the infested half of the field. A second level is contamination from a previous trial immediately to the west that was infested with a mint isolate of *V. dahliae*. Infected plant debris and infested soil was likely dragged by machinery into the western portion of what is now the variety trial. This would account for the higher wilt severity in the westernmost block of plots in both the infested and noninfested trials. Once these western plots were removed from the data analysis, there were only low levels of wilt, which did not differ among the varieties.

In 2000, the first full production year of this trial, there were differences in stand among the varieties in both the noninfested and infested trials. In both trials, the lowest stand was observed in '92(B37xM0110)-1'. In both trials in 2000, this variety also exhibited the lowest height, dry hay, and oil yield. This trend continued for 2001, and it is likely that selection '92(B37xM0110)-1' will "wilt out" or winter kill (these phenomena can be related) within another year or two.

Selection 'B90-9' appears promising with respect to yield; it performed substantially better than all other selections and the two standard varieties, 'Black Mitcham' and 'Todds'. Selection 'B90-9' seems to show a level of wilt tolerance somewhere between 'Black Mitcham' and 'Todds', although a few more years may make its relative tolerance more clear. (In the Willamette Valley trial, this selection has been intermediate between 'Murray' and 'Black Mitcham', which supports this speculation.)

Selection '84M010707' appears highly promising with respect to wilt tolerance, showing no wilt in central Oregon (and only slight wilt in the Willamette Valley trial). Yield of this selection seems comparable to that of 'Todds' and 'Black Mitcham' in central Oregon. Without oil characterization, we can't say what the oil is like at this time. Other selections have not shown to be either superior or inferior at this time to standard varieties, but several more years' performance may be required to evaluate them.

The private selection 'McKellip', field selected from 'Black Mitcham', yielded well in the noninfested trial (better than 'Black Mitcham', and second ranked overall) but fell down in ranking in the infested trial; its wilt tolerance seemed intermediate between 'Black Mitcham' and 'Todds'. It, too, needs more time to be fully evaluated.

In 2001, percentage composition of a number of key oil components for selections 'B90-9', 'M83-14', '92(B-37XM0110)-1', '87M0109-1', and 'McKellip' did not vary much from that of 'Black Mitcham' and 'Todds', although (as with 'Todds') some had reduced menthofuran compared to 'Black Mitcham'. Of course, the partial oil composition shown in Tables 5 and 6 does not suggest totally equivalent flavor with standard varieties. 'Black Mitcham' and 'Todds' were considered to have Madras-quality composition in 2001, although menthofuran in 'Black Mitcham' was slightly elevated over that of 'Todds' and some of the selections. As in other years, the timing of harvest

may have substantially affected oil composition, but this was not investigated–all varieties/selections were cut on the same date.

It is anticipated that this trial would last for four full-season production years, 2000-2003. Depending on initial wilt levels and crop management practices, 'Black Mitcham' and other varieties might "wilt out" within this period of time. This is about how long some varieties take to show whether periodic tillage is beneficial (in the absence of *V. dahliae*).

Acknowledgements

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Table 1. Second year peppermint production and verticillium wilt disease incidence on infested soil. Central Oregon peppermint variety trial, 2001.

Variety	Verticillium Wilt Infection 8/1/01 (%)	Ground Cover 5/22/01 (1-5)	Vigor Rating 7/31/01 (1-5)	Mildew Rating 7/31/01 (%)	Hay Yield Fresh Wt 8/5/01 (lbs/ac)	Oil Yield 8/5/01 (lbs/ac)
Black Mitcham	18.0	79.9	1.5	1.3	22,775	36.0
Todds	2.3	94.0	1.2	0	25,430	39.1
84MO107-7	0	92.0	1.0	0	39,722	34.1
M83-14	2.7	90.0	1.0	0	28,085	42.3
B90-9	7.7	86.7	1.3	0	23,626	59.7
92(B-37XM0110)-1	24.7	43.3	3.2	0	16,946	14.7
87M0109-1	1.7	96.3	1.0	0	33,935	45.0
M90-11	3.0	94.7	1.0	0	27,210	47.5
McKellip	4.3	87.0	1.2	1.3	23,709	33.9
LSD (.05)	15.6	12.5	0.5	0.5	13,000	26.0

Table 2. Second year peppermint production and verticillium wilt disease incidence on non-infested soil. Willamette Valley peppermint variety trial, 2001.

Variety	Verticillium Wilt Infection 8/1/01 (%)	Ground Cover 5/22/01 (1-5)	Vigor Rating 7/31/01 (1-5)	Mildew Rating 7/31/01 (%)	Hay Yield Fresh Wt 8/5/01 (lbs/ac)	Oil Yield 8/5/01 (lbs/ac)
Black Mitcham	0.3	90.7	1.5	1.3	31,259	38.8
Todds	1.0	96.7	1.2	0	35,574	45.7
84MO107-7	0.7	95.3	1.2	0	45,576	49.4
M83-14	0	91.3	1.0	0	32,836	42.5
B90-9	0.7	88.3	1.2	0	29,164	72.3
92(B-37XM0110)-1	9.7	55.0	2.7	0	28,625	25.1
87M0109-1	0	95.0	1.2	0	40,635	51.0
M90-11	0	93.7	1.0	0	34,018	47.7
McKellip	2.7	86.7	1.0	1.3	31,404	59.6
LSD (.05)	3.4	6.6	0.5	0.5	7,225	21.4

NS = Not statistically significant
Vigor rating: 1 = Excellent / 5 = Poor. Acceptable less than or equal to 2.0
Ground cover: 1=100% / 5 = 20%.

Table 3. Second year peppermint verticillium wilt disease incidence by verticillium class on infested soil. Central Oregon peppermint variety trial. August 1, 2001.

Variety	Verticillium wilt severity scale[1]				
	1 Healthy	2	3	4	5 Severe
	(%)				
Black Mitcham	81.9	0	2.6	7.0	8.5
Todds	97.7	0	1.0	0.7	0.7
84MO107-7	100.0	0	0.0	0.0	0.0
M83-14	97.3	0	1.3	1.0	0.3
B90-9	92.3	0	1.0	3.0	3.7
92(B-37XM0110)-1	75.3	0	4.3	10.7	9.7
87M0109-1	98.3	0	0.7	0.3	0.7
M90-11	97.0	0	1.3	1.0	0.7
McKellip	95.7	0	1.3	1.3	1.7
LSD (.05)	14.7	NS	2.1	5.1	6.1

Table 4. Second year peppermint verticillium wilt disease incidence by verticillium class on non-infested soil. Central Oregon peppermint variety trial. August 1, 2001.

Variety	Verticillium wilt severity scale[1]				
	1 Healthy	2	3	4	5 Severe
	(%)				
Black Mitcham	99.6	0	0	0.0	0.3
Todds+A3	99.0	0	0	0.3	0.7
84MO107-7	99.3	0	0	0.0	0.0
M83-14	99.7	0	0	0.0	0.3
B90-9	99.3	0	0	0.3	0.3
92(B-37XM0110)-1	90.3	0	1.0	2.0	6.7
87M0109-1	100.0	0	0	0.0	0.0
M90-11	100.0	0	0	0.0	0.0
McKellip	97.3	0	0	0.3	2.3
LSD (.05)	3.4	NS	0.1	0.8	4.5

[1]Verticillium wilt severity scale

1. Healthy
2. Mild chlorosis upper leaves
3. Distinct crescent leaves, mild stunting, chlorosis
4. Severe chlorosis, severe stunting
5. Very severe stunting, 60% or more of foliage necrotic

Table 5. Second year peppermint oil partial compositional analysis from infested soil. Central Oregon peppermint variety trial, 2001.

Variety	Total Heads (%)	Menthol (%)	Total Menthone (%)	Mentho-furan (%)	Pulegone (%)
Black Mitcham	9.2	42.8	13.5	5.7	1.1
Todds	8.9	42.2	18.7	3.6	1.0
84MO107-7	6.0	27.5	45.3	1.2	1.0
M83-14	9.1	39.0	20.7	4.7	2.0
B90-9	10.6	44.6	13.2	5.9	0.9
92(B-37XM0110)-1	7.0	47.7	16.9	2.1	1.0
87M0109-1	7.5	32.6	27.9	2.0	1.6
M90-11	8.7	37.4	21.4	4.2	2.1
McKellip	8.7	43.8	14.3	6.7	1.4
LSD (.05)	3.1	6.4	3.9	1.7	1.2

Table 6. Second year peppermint oil partial compositional analysis from non-infested soil. Central Oregon peppermint variety trial, 2001.

Variety	Total Heads (%)	Menthol (%)	Total Menthone (%)	Mentho-furan (%)	Pulegone (%)
Black Mitcham	9.1	41.0	16.1	7.0	1.0
Todds	8.1	42.2	21.1	4.5	1.0
84MO107-7	6.6	28.9	43.2	1.5	1.0
M83-14	7.5	42.9	20.5	5.3	1.3
B90-9	9.3	44.0	14.9	7.8	1.1
92(B-37XM0110)-1	6.5	46.9	21.4	2.8	0.6
87M0109-1	7.3	33.1	27.8	3.2	1.6
M90-11	10.3	32.1	20.7	6.8	5.1
McKellip	8.7	40.3	16.6	8.8	1.4
LSD (.05)	1.8	3.9	2.1	1.4	0.7