PEPPERMINT VARIETY TRIAL, CENTRAL OREGON, 2000

Fred Crowe and Robin Parks

Abstract

A new variety trial was established at Oregon State University, Central Oregon Agricultural Research Center, Madras, in June 1999. Rooted cuttings were planted into *Verticillium-free* soil, and irrigated through the summer and fall. Establishment was very high for all varieties/lines included. In fall 1999, half of the field was infested with *Verticillium dahliae* at a rate of 4-microsclerotia/g soil. The first full production year with harvest and disease assessment was 2000. Among varieties, there was little difference in early season stand or plant height. A lower stand was observed for the entree 92(B37xM0110)-1 in both the infested and noninfested trials. In the noninfested trial, 84M0107-7 and M90-11 yielded more dry hay compared to the other varieties although oil yield did not differ among the varieties. Neither hay nor oil yields differed among the varieties in the infested trial Rhizome development was good in both trials; development was lowest in 92(B37xM0110)-1 but similar among all other varieties. In both trials, little or no powdery mildew was observed in 84M0107-7, M90-11, and 87M0109-1. Wilt severity significantly differed among the varieties in the infested trial only. The most severe wilt symptoms were observed in 92(B37xM0110)-1 and Black Mitcham. No differences in *V. dahliae* soil populations were observed in either trial.

Introduction

Variety trials initiated in central Oregon and elsewhere in 1994 and 1995 were the first public, replicated, and randomized trials under uniform management and in which statistical comparisons could be made. The 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 *V. 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.* In most fields, *V. dahliae* populations are non-uniform, and 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 non-infested 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.

Materials & Methods

Plots were established from rooted cuttings in the summer of 1999. The trial area is split into an artificially infested area and a non infested area as per 1994-1998. Plot sizes are 10 ft. x 2.0 ft. All plots were managed identically in 2000. Plots were fertilized with 280 lb nitrogen acre in early season. To manage mites, Comite (Uniroyal Chemical) was aerially applied in late June at 1.5 lb/acre.

Nine entrees were included. The Mint Industry Research Council 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-wk incubation, microsclerotia were harvested by blending the cellophane in sterile water and washed on a 38-i.tm sieve. The dried concentrated inoculum was ground with sterile sand with a mortar and pestle. The concentration of microsclerotiat inoculum was estimated and mixed with enough sterile sand to yield about 4 microsclerotialg 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 one 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 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 2 wk incubation in the dark at room temperature, colonies distinctive of *V. dahliae* were counted. The population data was expressed as the number of colony-forming units (CFU) per gram of soil.

Wilt incidence, mint performance, and oil character was assessed as in 1994-1998. Individual wilt loci, also referred to as strikes, were flagged as symptoms appeared during the season and where they could be distinguished. Wilt severity was represented as the number of strikes per plot at the end of the season. The recommended MIRC wilt rating was estimated for wilt severity at harvest as follows. The number of strikes per plot was converted to the standard MIRC Verticillium wilt rating based on an estimate of the number of stems in each plot. The number of stems in 1 ft^2 was counted in a plot area exhibiting good fall regrowth. The number of stems in the entire plot was estimated based on the early season stand evaluation. Five stems were assumed to be included in each wilt strike. A strike was assigned a severity rating of 4.5 and the rest of the stems were assigned a healthy rating of 1.

The plots were harvested on August 3, 2000. Subsamples from each plot were collected and air-dried in gunnysacks. Oil from these subsamples was distilled on September 6 using the McKellip-Newhouse mini-still located at OSU-Madras. RCB International, Ltd. in Albany, Oregon, performed the oil component analysis. In early fall 2000, rhizome development and powdery mildew were evaluated.

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 results and discussion sections later, 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 was 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 in 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 or 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 early season stand, height, rhizome development, powdery mildew severity, hay yield, and oil yield are displayed in Table 1. Wilt severity and *V. dahliae* soil population estimates are shown in Table 2. Oil character analysis is shown in Table 3.

Noninfested Trial: The stand of all varieties ranged from 99.0 to 100 percent except for M90-11 and 92(B37xM0110)-1, which averaged 5.3% and 41.4% (P = 0.0001) lower, respectively, than that of the other varieties. The height of these two varieties also tended to be lower compared to the other varieties (P = 0.0853). Yet, M90-11 produced 16.5 to 48.percent. (P = 0.0001) more dry hay compared to the other varieties. Only 84M0107-7 yielded more hay: 22.3 to 51.7% more than that of other varieties. There was no difference in oil yield among any of the varieties (P = 0.0776). The lowest rhizome development was observed in 92(B37xM0110)-

1, although this difference was not significant (P = 0.1587). Rhizome development was similar among all other varieties. Powdery mildew was most severe in B90-9, 92(B37xM0110)-1, Black Mitcham, Todds, M83-14, and McKellip 98, respectively (P=0.0024). Little or no mildew was observed on 84M0107-7, M90-11, and 87M0109-1.

Wilt was observed in noninfested plots. The amount of wilt strikes did not differ among the varieties (P = 0.7864). However, as described previously, most wilt was observed in the western-most block of the field. That block, in both the noninfested and infested trials, was removed from the analysis. As expected, *V. dahliae* was recovered from noninfested plots as well. Soil populations did not differ among varieties (P = 0.5994).

Infested Trial: The stand of all varieties, except 92(B37xM0110)-1, ranged from 91.7 to 99.7 percent. The stand of 92(B37xM0110)-1 averaged 29.0 percent lower (P = 0.0007) than that of the other varieties. The mean height of 92(B37xM0110)-1 was also lower than other varieties as well, although not significantly (P = 0.3940). Neither dry hay nor oil yield differed significantly (P = 0.2037 and P = 0.1692, respectively) among the varieties. Similar to the non-infested trial, the least rhizome development was observed in 92(B37xM0110)-1 (P= 0.0001) while development was similar among all other varieties. The ranking and level of severity of powdery mildew among the varieties were almost identical to the non-infested trial. Mildew was most severe in B90-9, 92(B37xM0110)-1, Black Mitcham, Todds, McKellip 98, and M83-14, respectively (P = 0.0001). Little or no mildew was observed on 84M0107-7, M90-11, and 87M0109-1.

V. dahliae soil populations did not differ among any of the varieties (P = 0.2521). Wilt severity differed among the varieties in the infested trial. Wilt severity, measured by strikes, in 92(B37xM0110)-1 was 46.2 to 100% higher (P = 0.0035) compared to all other varieties. There were 50.6 to 100 percent more wilt strikes in Black Mitcham plots compared to the other varieties. No wilt symptoms were observed in M90-11 and 87M0109-1. A similar ranking was obtained using our approximation of the MIRC standardized wilt ratings although means were not as separated as means for wilt strikes.

Table 1. Peppermint performance in noninfested and *Verticillium dahliae* infested variety trials, OSU-COARC, Madras, Oregon 2000.

Noninfested Variety	15 Jun Stand (% cover)	5 Jul Height (in)	16 Oct Rhizome (1_5)a	16 Oct Powdery Mildew (1_5)b	3 Aug Dry Hay (lb/acre)	Oil (lb/acre)
Black Mitcham	98.7 A[c]	17.0	1.00	2.67 AB	6187 BC	47.1
Todds	100 A	18.7	1.00	2.67 AB	6982 BC	27.2
84M0107-7	100 A	17.7	1.00	0.67 CD	9712 A	42.4
M90-11	94.3 B	16.0	1.00	0.00 D	9029 A	38.9
87M0109-1	100 A	15.7	1.00	0.00 D	7542 B	42.5
M83-14	99.0 A	18.3	1.00	2.00 BC	7362 BC	31.7
92(B37xM0 1 10)-1	58.3 C	14.7	2.00	3.33 AB	4694 D	23.8
B90-9	99.7 A	16.3	1.33	4.00 A	6016 CD	58.0
McKellip 98	99.7 A	16.3	1.67	2.00 BC	7170 BC	50.0
P-value[d]	0.0001	0.0470	0.0937	0.0046	0.0001	0.0478
Variety	0.0001	0.0853	0.1587	0.0024	0.0001	0.0776
Block	0.7887	0.0423	0.0634	0.4719	0.0039	0.0531

V. dah/iae-Infested Variety	Stand (% cover)	Height (in)	Rhizome (1-5)	Powdery mildew (1-5)	Dry hay (lb/acre)	Oil (lb/acre)
Black Mitcham	96.6 A	18.4	1.02 B	3.52 AB	6573	53.5
Todds	99.7 A	18.0	1.00 B	1.33 AB	7118	52.3
84M0107-7	98.7 A	17.0	1.00 B	1.67 CD	7646	32.0
M90-11	91.7 A	16.0	1.00 B	0.00 D	8126	54.0
87M0109-1	99.7 A	16.3	1.00 B	0.00 D	8097	47.4
M83-14	94.7 A	15.3	1.00 B	2.00 BC	6315	33.5
92(B37xM0110)-1	69.0 B	13.7	2.67 A	3.67 AB	4836	28.0
B90-9	97.3 A	15.3	1.00 B	4.00 A	6123	54.8
McKellip 98	99.3 A	15.3	1.00 B	3.00 BC	6426	56.4
P-value	0.0015	0.3582	0.0001	0.0001	0.2861	0.2526
Variety	0.0007	0.3943	0.0001	0.0001	0.2037	0.1692
Block	0.8184	0.2780	0.4014	0.0114	0.6822	0.8448

'Rhizome development rating based on runners and growth from rhizomes. Scale: 1 = excellent, 3 = moderate, 5 = worst.

[b]Powdery mildew rating scale 1-2 = mild, older leaves, 3 = moderate, old leaves to severe, young leaves, 4-5 = severe, all foliage.

Weans followed by the same letter are not significantly different at P 0.05 according to Fisher's protected least significant difference (LSD) test.

[b]Probability of obtaining F..0 05.

Table 2. Verticillium wilt severity and *Verticillium dahliae* soil populations in noninfested and infested peppermint variety trials at OSU-COARC, Madras, Oregon, 2000.

Noninfested	2 Aug	2 Aug	17 Jul
Variety	COARC Wilt rating (mean no. strikes/200ft)	MIRC Wilt rating[a] (mean infected/total stems)	*V. dahliae* Soil population (mean cfu/g soil)[b]
Black Mitcham	0.67	1.001	2.80
Todds	0.33	1.000	2.73
84M0107-7	0.33	1.000	1.67
M90-11	0.00	1.000	1.27
87M0109-1	0.00	1.000	0.87
M83-14	0.33	1.000	2.73
92(B37xM0110)-1	1.00	1.002	2.27
B90-9	0.00	1.000	2.80
McKellip 98	0.00	1.000	0.73
P value'	0.8640	0.7214	0.1161
Variety	0.7864	0.6669	0.5994
Block	0.7982	0.5957	0.0091

V. dahlie infested	COARC	MIRC	*V. dahliae*
Variety	Wilt rating (mean no. strikes/200ft2)	Wilt rating (mean infected/total stems)	Soil population (mean cfu/g soil)
Black Mitcham	6.08 B[d]	1.008 B	0.63
Todds	0.33 C	1.000 BC	1.60
84M0107-7	0.33 C	1.000 BC	0.80
M90-11	0.00 C	1.000 C	0.60
87M0109-1	0.00 C	1.000 C	0.27
M83-14	1.00 C	1.001 BC	0.40
92(B37xM0110)-1	11.30 A	1.021 A	3.53
B90-9	1.67 BC	1.002 BC	0.40
McKellip 98	3.00 BC	1.003 BC	8.93
P value	0.0075	0.0011	0.2884
Variety	0.0035	0.0005	0.2521
Block	0.8698	0.7004	0.4143

'Approximated using estimated number of stems per plot and number of wilt strikes at harvest.
[b]Colony-forming units per gram soil.
`Probability of obtaining F 0.05.
[d]Means followed by the same letter are not significantly different at P0.05 according to Fisher's protected least significant difference (LSD) test.

Table 3. Partial oil character analysis from noninfested and infested variety trials at OSU-COARC, Madras, Oregon 2000.

Noninfested

	% AREA					
Variety	Menthone	Menthol	Heads	Ketones	Pulegone	Menthofuran
Black Mitcham	20.4 EF	69.6 BC	8.22	22.3 E	1.29 CD	3.63 AB
Todds	22.4 DE	71.1 ABC	7.60	24.0 DE	1.07 CD	2.98 BC
84M0107-7	47.1 A	63.9 D	7.83	49.0 A	1.30 CD	1.05 D
M90-11	27.5 C	53.5 E	8.20	33.9 C	5.90 A	5.10 A
87M0109-1	34.1 B	61.1 D	8.58	37.7 B	3.10 B	1.77 CD
M83-14	24.6 CD	72.4 AB	7.28	26.9 D	1.73 C	3.02 BC
92(B37xM0110)-1	25.3 CD	73.4 A	7.27	26.8 D	0.73 D	2.61 BCD
B90-9	20.8 EF	68.2 C	7.66	22.8 E	1.42 CD	3.84 AB
McKellip 98	18.5 F	68.6 C	7.88	20.7 E	1.54 C	5.06 A
P-value	0.0001	0.0001	0.4220	0.0001	0.0001	0.0017
Variety	0.0001	0.0001	0.4704	0.0001	0.0001	0.0012
Block	0.4686	0.8081	0.2605	0.5753	0.6120	0.1287

V. dahliae Infested

Variety	Menthone	Menthol	Heads	Ketones	Pulegone	Menthofuran
Black Mitcham	21.0 CD	67.6 AB	9.28	23.2 CD	1.67 BC	4.19 A
Todds	25.8 CD	71.9 A	7.78	27.8 CD	1.39 BC	3.19 C
84M0107-7	45.1 A	64.1 AB	8.54	46.9 A	1.20 C	1.26 D
M90-11	24.2 CD	48.0 C	8.66	28.9 BC	4.36 A	4.45 A
87M0109-1	31.2 B	58.0 BC	8.47	33.9 B	2.21 B	1.60 D
M83-14	23.2 CD	72.8 A	7.16	25.2 CD	1.49 BC	3.34 BC
92(B37xM0110)-1	26.5 BC	72.4 A	7.84	28.1 BCD	0.92 C	1.91 D
B90-9	20.4 D	68.5 A	8.60	22.4 D	1.41 BC	4.47 A
McKellip 98	19.3 D	68.1 A	7.73	21.1 D	1.24 BC	3.86 AB
P-value	0.0001	0.0042	0.4690	0.0003	0.0001	0.0001
Variety	0.0001	0.0024	0.8909	0.0001	0.0001	0.0001
Block	0.4600	0.3186	0.0429	0.3712	0.2172	0.0666

'Means followed by the same letter are not significantly different at P 0.05 according to Fisher's protected least significant difference (LSD) test.

[b]Probability of obtaining $F_{0.05}$.

Discussion

There are most likely three different infestation levels within the entire variety trial. The first level is the artificial inoculum in the infested half of the field. The second 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. The third level of infestation may be a background population of *V. dahliae* that is perhaps throughout the entire field. A soil assay of the field was performed in February 1999 to check for any *V. dahliae* populations but, no measurable population was detected. Relatively high populations, up to 6 cfu/g soils, were recovered in noninfested plots but few or no symptoms were observed in those same plots. Therefore, this background *V. dahliae* population may be a non-mint isolate that is not highly pathogenic on mint. Potatoes have been grown to the east of this trial and therefore, it is possible a potato isolate population of *V. dahliae* could have been spread from that field into what is now the variety trial.

Although wilt was visibly more severe in the western edge of the field, pathogen soil populations could not explain this higher disease severity. A highly virulent mint strain, which requires only a low population to induce wilt symptoms, may have been moved from the previous experiment on the west side of the variety trial into the western plots, causing severe wilt from a low pathogen population. Once these western plots were removed from the data analysis, there were only low levels of wilt, which did not differ among the varieties.

Similarly, low soil populations in the western plots of the infested trial could not account for the higher disease severity in those plots. As can be expected from an artificial infestation, there was no difference among *V. dahliae* soil populations among the varieties in the infested trial. Yet, wilt severity differed among the varieties in the infested trial. The highest amount of wilt occurred in 92(B37xM0110)-1 and Black Mitcham.

In this 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, this variety also exhibited the lowest height, dry hay, and oil yield although only hay yield in the noninfested trial was significantly lower than the other varieties. The similarity in stand and height among all varieties was reflected in the dry hay and oil yields. Varieties differed significantly in dry hay yields only in the noninfested trial, yet trends were similar between both trials. M90-11, 84M0107-7, and 87M0109-1 produced the highest hay yields between the two trials, but there were no consistent trends in oil yield among varieties.

While not evaluated quantitatively in 2000, we believe M90-11 and 87M0107-7 suffered less mite damage and less moisture stress when irrigation was inadvertently reduced in July.

It is difficult to assess differences in oil composition in this trial because all varieties were harvested on the same date. Oil composition is directly affected by maturity and harvest date, and each variety may have its own optimal harvest date. Nevertheless, Menthol and Ketone composition of oil from all varieties was much higher than typical for oil from the Madras area. This suggests either that harvest was late, or that perhaps the plants were unduly stressed prior to harvest. Both may be true. The summer of 2000 was hotter than normal, and the variety trial was unintentionally underwatered at about 10 days prior to harvest. Considering long-term averages, the date of harvest, August 3, was not late for the Madras area, and no variety had excessive bloom on that date. Variety 84M0107-7 had exceptionally high Menthone and Ketone levels, and there was quite a variation among varieties with respect to Pulegone and Menthofuran. Heads composition was somewhat low for all varieties, which may be primarily influenced by mini-distillery design or management.

The plots on the western edge of the variety trial field will likely be maintained for the duration of the trial. Yet data from these plots will be omitted from the analysis of the rest the trial because of the disproportionate wilt severity.

Literature Cited

Butterfield, E. J., and J. E. DeVay. 1977. Reassessment of soil assays for *Verticillium dahliae.* Phytopathology 67:1073-1078.

Harris, D. C., J. R. Yang, and M. S. Ridout. 1993. The detection and estimation of *Verticillium dahliae* in naturally infested soil. Plant Pathology 42:238-250.

Puhalla, J. E. 1979. Classification of isolates of *Verticillium dahliae* based on heterokaryon incompatibility. Phytopathology 69:1186-1189.

SAS Institute. 1988. SAS guide for personal computers: Statistics. Version 6.0. SAS Institute, Cary, NC.

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