PEPPERMINT PERFORMANCE AND WILT INCIDENCE, AS INFLUENCED BY SELECTED CULTURAL RESEARCH PARACTICES AND INOCULUM DENSITY OF *VERTICILLIUM DAHLIAE*

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Abstract

In 1995, a new soil assay, the "Harris assay", was used to determine the 1994 summer inoculum density of microsclerotia (MS) of *Verticilhum dahliae* from the field trial at the COARC, Powell Butte. The field for the trial had been (a) artificially infested with a range of infestation levels of *V. dahliae* in 1989, (b) planted with 'Todd's' peppermint in 1990, (c) managed with either tillage without flaming or with flaming without tillage for three production seasons through 1993 until peppermint in the highest initial infestation "wilted out", and (d) removed from peppermint in the spring of 1994 (while irrigation was continued). From initial infestation in 1989 at 0. 0.01, 0.1, 1.0 and 5.0 MS/g soil, plots that been flamed but not tilled in 0-1993 were assayed in the summer of 1994 to be 0.37, 0.02, 0.00, 0.12, and 0.74 MS/g soil, respectively. Plots that had been tilled but not flamed in1990-1993 were assayed to be 0.79, 0.07, 0.26, 5.70, and 18.60 MS/g soil, respectively. The reliability of the Harris assay is discussed relative to assays used previously, and to wilt disease and mint performance during 1990-1993.

The higher recovery of MS from initially non-infested plots in which wilt never occurred seems anomalous and likely represents MS from a strain of *V. dahliae* nonpathogenic to peppermint, one which does not appear to have been recovered from plots in which the peppermint strain of *V. dahliae* was added.

Based on the Harris assay data, it appears as if flaming without tillage resulted in a drop in inoculum density for most initial inoculum levels, whereas tillage without flaming resulted in a three-to-sevenfold increase over initial infestation levels. In general, the "Horner" hypothesis, at times questioned during 1990-93 because results of previous assays was confusing, seems to be confirmed by these latest results. Not only did flaming without tillage result in less wilt in all but the highest infestation levels compared to tillage without flaming, it also resulted in less carryover of soil inoculum of *V. dahliae,* and perhaps an actual lowering of soil inoculum. These results also seem to suggest that the high proportion of "wilt out" seen at the highest infestation level in flamed but not tilled plots resulted largely from a combination of reduced stand together with carryover of infection within rhizomes, rather than from re-infection from soil. "Wilt out" in tilled but not flamed plots probably resulted from these sources in addition to increasing soil inoculum.

Peppermint was killed from plots in 1994, but the integrity of plots was maintained and plots were kept irrigated to allow MS to be released into soil from decaying peppermint. Plots are ready for replanting of a second peppermint crop to determine the impact of residual inoculum from the first crop. Plots may be split to test some additional parameter.

Introduction

On this long-term project, readers are referred to reports of progress on this project from 1990 through 1994 (Crowe, 1992, 1993, 1994, 1995). The initial intent was several-fold: (1) To determine the general ranges of infestation of microsclerotia (MS) of peppermint strains of *V. dahliae* that induce various levels of verticillium wilt, and thus the type of soil assay most likely to detect these ranges, (2) to determine whether the "Horner" model of tillage vs. flaming that had been worked out for the Willamette Valley [i.e. tillage worsens wilt by incorporation of MS and increasing soil inoculum, flaming kills inoculum in the stem and keeps wilt from increasing (Horner and Dooley 1965, and McIntyre and Horner 1973)] could be applied to a moderately tolerant variety such as 'Todd's' in central Oregon (3) to determine whether winter damage of consistently untilled peppermint might be more adverse to production in central Oregon than damage due to wilt aggravated by tillage, (4) to determine actual changes in soil inoculum levels of MS over time, which had only been assumed previously but not measured, using soil assays proven useful in other regions, and (5) to serve as a basis for evaluation of inoculum density and "wilt potential" in commercial field soils using the inoculum density vs. disease loss relationships determined from this field trial.

Plots at the COARC, Powell Butte field were fumigated with methyl bromide in 1987, and the widely used wet sieving version of the "Butterfield" soil assay (Butterfield and DeVay, 1997, Joaquim *et al.,* 1988, Nicot and Rouse, 1987) in 1989 suggested that few MS of *V. dahliae* were present. Field history suggested that any MS present likely were associated with potatoes and not with peppermint. Points 1, 2, and 3 were largely assessed during 1990-1993, following uniform infestation of large field plots and planting of 'Todd's' peppermint in the spring of 1990. In general, wilt incidence increased on tilled but not flamed peppermint, and remained static on flamed but not tilled peppermint for all initial levels of infestation (0, 0.01, 0.1, 1.0 and 5.0 MS/g soil), except at the highest level of initial infestation (5.0 MS/g soil). At the highest initial level, peppermint "wilted out" for both tilled and flamed plots during the third year, as defined by great reduction in stand and yield compared to other treatments. The pattern of "wilt out" was different in the highly infested flamed plots (lowered spring stand, moderately high seasonal wilt symptoms) vs. "wilt out" in tilled plots (stand uniformly maintained near 100 percent by rootstock redistribution each spring, but very high incidence of seasonal wilt symptoms).

A major stand decline occurred in highly infested flamed but not tilled plots in the winter cold periods of 1990-91. In this treatment combination, the peppermint never really recovered full stand, perhaps due to in-season activity by *V. dahliae*. The winter injury likely was associated with lack of winter hardiness on wilt-infected rhizomes in the fall of 1990. Lack of winter hardiness was never observed at initially lower levels of infestation. Undoubtedly, the winter kill also occurred in the highly infested plots that were spring tilled, but any such winter kill was obscured by redistribution of surviving rhizomes.

The above results suggested that for the 'Todd's' variety of peppermint, the economically-acceptable inoculum density level of MS, assuming uniformity of distribution, was between

1.0 and 5.0 MS/g soil, as long as peppermint was flamed and not tilled. Further, most winter damage on untilled peppermint would result on fields that were already highly infested with *V. dahliae*. The data suggested, as had C.E. Horner (Horner and Dooly, 1965 and 1966. McIntyre and Horner, 1973), that inoculum density was not increasing greatly in flamed and untilled plots, and that it could even be dropping if much of the year-to-year wilt incidence was resulting from carryover of *V. dahliae* through active rhizome infections rather than new infections through roots, as suggested by Nelson (1950). The wilt data also suggested that inoculum density for tilled peppermint might be a "moving target", assuming inoculum was building up due to tillage along with the increase in incidence of wilt symptoms (although carryover in rhizomes also could complicate this argument). Because of the problems encountered with several standard soil assays for MS during 1990-1993, these questions and points 4 and 5 above could not be addressed. Nor could these standard soil assays be assessed in growers' fields without some measure of inoculum density vs yield losses, because the results from growers' fields could not be interpreted against any standard (ignoring the fact that other strains of *V. dahliae* also were likely present in growers' fields, which further complicates the interpretation). In 1994, it was proposed that yet another assay be attempted that had proven useful in England on cooler soil where standard assays had failed (Harris, *et al.,* 1993). This Harris assay seemed to provide reasonable data on a limited series of tests in 1994. Data from 1995 is included below.

As an alternative to dependency on soil assays to verify that soil inoculum had increased, and to avoid confusing infected rhizome carryover with root infections from MS, it was proposed in 1993 that the peppermint in the Powell Butte trial be killed, that peppermint roots and rhizomes be allowed to decay for a period of time, and that the plots be replanted to peppermint for evaluation of wilt on the subsequent crop. For cotton, it takes one to two years (Huisman and Ashworth 1976) and for potatoes, it takes about two years (Davis and Huisman, personal communication), before MS become released into soil from decaying stems, so this period of time was allotted prior to peppermint replanting. As described below, these plots are now ready for replanting as proposed.

Methods

Readers are referred to previous reports for details of trial and plot establishment, and for peppermint performance, from 1989-1994 (Crowe 1992, 1993, 1994, 1995). Briefly, the trial area was fumigated with methyl bromide during 1987. Using the Butterfield wet sieving assay, a commonly used and successful standard soil assay used to assess inoculum levels in the soil in many regions (Butterfield and DeVay, 1997, Joaquim, *et al.,* 1988, Nicot and Rouse 1987), no MS were detected from this soil prior to artificial infestation. Previous field history included many years of potatoes, but no peppermint. MS from peppermint isolates collected from diseased peppermint and proven pathogenic to peppermint, were produced in the laboratory on a cellophane-pectate agar. MS were harvested and mixed with sand, and the MS-sand mixture was used to infest large plots 8 inch deep at the COARC, Powell Butte field in the fall of 1989. Infestation rates were 0 (no added sclerotia), 0.01, 0.1, 1.0 and 5.0 MS/g soil. From the beginning, it was argued that disease incidence and peppermint performance should logically be based on recovered amounts

rather than introduced amounts. In the early spring of 1990, the Butterfield soil assay again was used to determine an inoculum density. In the spring of 1990, the recovered amount using the Butterfield assay, which correlated well with the amount known to be infested. No problems were perceived with the assay at that time. However, beginning later in 1990, and persisting through 1994, recoveries using the Butterfield assay, and several variations of it, always measured a much lower than expected number of colony forming units (CFU). Between 1992-1994 the CFU measured by this assay were consistently 0 for every level of initial infestation and peppermint treatment, which suggested that inoculum had disappeared from the soil or that the assays had become unreliable. It was confusing for several years to decide how to interpret these results. In the Butterfield assay and its variations, soil is either directly plated onto growth media that are semi-selective, or the soil is first sieved to concentrate the size fraction that includes MS. Semi-selective media allow the growth of some but not all organisms. Usually the organism of interest is enough favored to allow quantification of its population. It appeared that, beginning in mid-1990, a fusarium fungus was over-growing the assay plates of the media used from either straight or sieved soil, and that this fusarium precluded growth of *V. dahliae*. Powell Butte soil was sent to a number of other labs that use variations on the Butterfield assay, or even other assays, during 1990-1993 to determine if MS could be quantified in those labs, but none were successful.
The Harris assay (Harris, *et al.,* 1993) was first tested in 1994 on a limited range of plot treatments. The data generated seemed to fit within expected relative ranges, so soil was resampled from all plots in August of 1994 and assayed with the Harris assay. Some samples again were assayed with the Butterfield technique for comparison. Sampling was comparable to earlier sampling. Two sub-samples were collected from each plot. Each sub-sample was composed of 30 one-inch soil cores taken randomly from the plot area and to 10 inches soil depth. Cores within sub-samples were mixed well, air-dried, and ground in a meat grinder if necessary to break clods and re-mix samples were stored to air-dry for at least 30 days to eliminate condial spores of *V. dahliae*. [These spores are the ones that move within the plant, and they do not persist in soil.] The main differences between the Harris assay and that of Butterfield are minor in concept, but it seems as if *V. dahliae* is highly responsive to seemingly subtle variations in assays. These subtleties are not described in detail here. Results from the 1994 sampling are shown in Table 1.

Results and Discussion

Again, readers are referred to results of previous reports (Crowe, 1992, 1993, 1994, 1995) on peppermint performance and disease incidence resulting from initial infestations of MS of *V. dahliae*.

Table 1 shows the original infestations in MS/g soil, and amounts of *V. dahliae* in colony forming units (CFU) recovered early in 1990 by the Butterfield assay and in 1994 by the Harris assay. Colonies on assay growth medium plates are initiated from MS, as long as conidia and any hyphae (moldy growth) is not present. Recovery efficiency for the Butterfield and Harris assays has not been determined for either lab or naturally-produced inoculum, so the three figures are not comparable with certainty. Not included in the table

were zero recoveries for all sample dates beginning in fall, 1990 through 1994 while the Butterfield technique was continued in use, or when the Anderson Air Sampler was used in conjunction with the Butterfield growth medium.

The recovery of MS from plots in 1994 that had received no added pathogenic MS in 1989 was higher than from the plots initially infested at 0.01 and 0.1 MS/g soil, even though not wilt occurred in "non-infested" plots. This result seems anomalous and these CFU may represent MS from a strain of *V. dahliae* nonpathogenic to mint, one which does not appear to have been recovered from plots in which the mint strain of *V. dahliae* was added. This suggests some unanticipated relationship between populations of strains. The failure of standard soil assays to detect any MS from mid-1990 through 1994 begs the question whether low pre-infestation estimates were reliable during 1989. In fact, they may not have been. Further, the high correlation between the spring 1990 assay of MS with the calculated infestation rate might be questioned since the standard methods were used which later proved unreliable. It is possible in the first few winter months, that the recently-infested, laboratory-produced sclerotia had not become highly associated with the soil organisms that later overgrew the standard assay media and precluded normal growth of *V. dahliae* during later 1990 through 1994 Or that production of peppermint itself caused a shift in soil micro flora that overcame the assay. If so, the standard assays might have in fact worked quite will for at least a few months after infestation or peppermint planting, only to eventually fail as experienced.

Even though there are questions about efficiency of assays and how the infestation levels in plots changed during the three to four peppermint production years of the trial, the Harris assay certainly demonstrates a strong relative difference in inoculum levels based on tillage vs. flaming at the endpoint of 1994. Essentially all anticipated changes were seen to have occurred as per the Horner model. Inoculum stayed low (perhaps even dropped) with a regime of flaming without tillage, and inoculum increased with a regime of tillage without flaming. And these relative levels seem well associated with the disease incidence and peppermint performance generally (Crowe, 1992, 1993, 1994, 1995).

It remains to be determined how universal the Harris assay proves to be. It may prove most useful in demonstrating relative differences in *V. dahliae* MS populations among treatments in research trials. Recent worldwide comparisons among several *V. dahliae* assays, using identical soil samples that were divided among 13 different laboratories, indicated that within-lab results were consistent, but between-lab results were rather variable (Termordhuizen, 1995). This would suggest that different labs might communicate different levels of infestation, which would complicate interpretation with respect to disease incidence. Further, in potatoes, it seems as if even the same inoculum densities can result in

a wide variation of wilt when they occur in different soil types (J. Davis, University of Idaho, personal communication), which would further complicate interpretation. These findings make it more doubtful that a commercially useful test can be devised for determination of the wilt potential in growers' fields, even though assays might be very useful research tools. And the issue of strain identification of the CFU recovered remains, in addition.

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TABLE 1: Inoculum infested and recovered from COARC, Powell Butte, 1989-1994[1].

1989, Initial Infestation[4]	Colony Forming Units of *Verticillium Dahliae* per Gram of Soil[2]								
	1990, Butterfield Assay[5]			1994, Harris Assay[3]					
				Flaming Without Tillage[6]			Tillage Without Flaming?		
MS/g	Sub-A	Sub-B	**(A+B)/2**	Sub-A	Sub-B	**(A+B)/2**	Sub-A	Sub-B	**(A+B)/2**
0 (no added MS)	8			0.4	0.34	0.37	0.84	0.84	**0.79**
0.01				0.04	0	**0.02**	0.10	0.04	**0.07**
0.1				0	0	**0**	0.28	0.24	**0.26**
1.0	1.4	1.0	**1.2**	0.20	0.04	**0.12**	5.16	5.24	**5.70**
5.0	6.0	6.6	**6.3**	0.68	0.80	**0.74**	17.96	19.24	**18.60**

1. Plots were planted to 'Todd's' peppermint in February, 1990, and peppermint was killed in March, 1994. Soil assays using the Butterfield technique yielded no colony forming units between mid-1990 and 1994, or so few that the assay was discounted.
2. Recovery is expressed as the number of colonies of *V. dahliae* which were discernable amongst soil residue on petri plates, per gram of soil represented. Assay does not distinguish among pathogenic strains of *V. dahliae.*
3. Means are from five replications, after plots were split for flaming and tillage. Soil samples were taken in August, 1994. Soil assay was as per Harris,et *al.* 1993. Ten grams soil were assayed from 30 cores per sub-sample per plot for 0, 0.01and 0.1 MS/g initial infestation, and five grams soil were assayed from 30 cores per sub-sample per plot for 1.0 and 5.0 MS/g initial inoculum.
4. Microsclerotia (MS) were uniformly applied and tilled into test plot soil in December 1989, as calculated from the MS per unit volume recovered from cellephane agar in the laboratory and the number of grams of soil contained in 8 inches depth of 10'x80' test plots.
5. Means are from 10 replications, prior to plots being split. Plots were sampled on Februay 14, 1990. Soil was assayed as per Butterfield, *et al.,* 1977. Thirty soil cores taken to 8" were combined per sub-sample. Sub-sample data represents the average number of CFU found on 20 plates on each of which seived residue from 1 gm soil was placed.
6. Plots were propane flamed following harvest in 1990, 1991, 1992 and 1993, but not tilled 1990-1993.
7. Plots were tilled in the February of 1991, 1992 and 1993, but not flamed 1990-1993.
8. Soil not assayed at lowest initial infestations.