INFLUENCE OF SPIDER MITE-INDUCED FEEDING STRESS ON THE SUSCEPTIBILITY OF PEPPERMINT TO VERTICILLIUM WILT

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Abstract

A 3-year field study to evaluate whether spider mite leaf injury contributes to the susceptibility of peppermint to Verticillium wilt was concluded in 1999. Field treatments were combinations of *Verticillium dahliae* inoculum and high or low mite populations. Mite populations, mite predator populations, wilt incidence, *V. dahliae* soil populations, hay yield, and oil yield and character were evaluated. Plots were re-inoculated with *Verticillium* after the 1997 field season which did not yield sufficient wilt symptoms. Mint and wilt response variables were not influenced by mite populations in any year. Wilt incidence was significantly higher in high inoculum plots in both 1998 and 1999. The high *Verticillium* inoculum treatment alone reduced hay and oil yields. The percent of menthofuran, menthol, and pulegone was higher in low mite treatments in 1998. But all other treatments did not influence oil characteristics in either 1998 or 1999. This field trial yielded no consistent interaction between spider mite population and wilt incidence.

Introduction

There is good evidence that peppermint susceptibility to Verticillium wilt (*Verticillium dahliae*) is increased when the plants are stressed. For example, Santo (1990, 1991) and others have reported that nematode-induced stress increases the severity of Verticillium wilt, and more recently (Smith, personal communication) suggested that herbicide-induced stress may increase severity of Verticillium wilt. It also has been reported that interactions with spider mite feeding injury and water stress influences peppermint susceptibility to Verticillium wilt (Hollingsworth 1981, Hollingsworth and Berry, 1982, 1983). Since there is good laboratory evidence that peppermint under stress is more susceptible to Verticillium wilt, we undertook a 3-year field study in central Oregon to evaluate the influence of spider mite-induced stress on Verticillium wilt. The intent of this project was to evaluate how spider mite feeding injury on leaves (DeAngelis et al., 1982, 1983a,b) contributes to the susceptibility of peppermint to Verticillium wilt under field conditions (Hollingsworth and Berry, 1982).

Materials & Methods

Experimental plots (20 x 20 ft) were established April 1996 at the Central Oregon Agricultural Research Center in Madras in cooperation with Fred Crowe. Verticillium wilt inoculum was applied October 31, 1996 at a rate of 3.0 to 3.5 microsclerotia (MS)/gm soil. All plots were tilled to incorporate the inoculum into the soil. The following treatments were established in the plots: (1) Verticillium wilt + high mite density (>5 mites/leaf), (2) Verticillium wilt + low mites (<5 mites/leaf), (3) no Verticillium wilt + high mite density, and (4) no Verticillium wilt + low mite density. However, the incidence of Verticillium wilt was very low during 1997. It was suspected that the lab-produced inoculum may have died in the
field prior to substantial plant infection. In the fall of 1997, an additional 3.5 MS/gm of soil were distributed over the plots, which were then tilled to incorporate the inoculum. The incidence of Verticillium wilt in the plots was adequate in 1998 based on the number of strikes observed in 1998. Plots were tilled again in the fall of 1999 to increase further the level of wilt. However, wilt levels in 1999 were only modestly higher than in 1998 (Table 1). This observation is discussed further below.

Verticillium wilt strikes were estimated in all plots in early, mid- and late July and early August by walking around the plot perimeter and with 2 to 3 passes through the plots. Wilt symptoms were noted (leaf curling and/or yellowing, stunted growth, dead stems and foliage, etc.) and were considered distinct infections if stems or clusters of stems were separated by at least 1 foot. Colored flags were placed at new wilt loci that were observed on subsequent sample dates. Some stems were sampled to confirm infection by Verticillium wilt. The cumulative number of wilt loci per plot at harvest was recorded as the final wilt rating.

To detect and estimate Verticillium wilt in the soil (Harris et al., 1993), two soil-core subsamples per plot were collected to 15 cm deep, mixed well, and allowed to air dry for 1 month to rid the soil of Verticillium conidia and hyphae. Subsamples were passed through a grinder to pulverize, and rocks larger than one cm were removed. From each subsample, 25 gm of soil were shaken and dispersed in water containing a small amount of detergent for one hour, then passed by wet sieving through 60- and 200-mesh screens to reduce soil volume and any competitive organisms. The residue remaining on the screen was re-suspended with continuous agitation in 100 ml of water, and 2 ml of this suspension was spread evenly onto a modified pectate agar semi-selective growth medium in a Petri plate, 10 plates per subsample. Plates were held at room temperature and observed after 2 weeks. Colonies distinctive of Verticillium were counted and data were expressed as the number of CFU (Colony Forming Units) per gm of soil per plot, after averaging the two subsamples. Because of the 1-month wait to rid the soil of conidia and hyphae, all CFU presumably represents microsclerotia or aggregates of microsclerotia.

All plots were harvested August 12, 1998 and August 19, 1999 with a 42-inch-wide swather by cutting a 20-ft swath length from each plot. Fresh weight of the hay was taken and approximately 12 lb from along the swath was placed into a gunny sack and air-dried. Hay was distilled in modified mini-stills (Thacker-Mckellop inverted cone separators, Newhouse Manufacturing, Redmond, OR) in early September according to the methods outlined by Hughes (1952). Yield was expressed as the amount of dry hay and oil per acre (Table 1). Oil samples were analyzed by gas chromatography by A. M. Todd, Kalamazoo, MI in 1998, and by RCB International, Ltd., Albany, OR in 1999 (Table 2).

Spider mites and predators were counted with a 16x hand lens in all plots. In 1999, samples were taken on 6/17, 6/30, 7/15, 7/29, and 8/12, just prior to harvest. Three plants were selected randomly in each plot and 3 leaves were examined on each plant (top, middle, and bottom leaves, n = 9 leaves). An action threshold of 5 mites/leaf was used to determine whether or not treatment was necessary. In 1998, Comite and Asana were applied to reduce the mite and predator populations. No acaricide treatments were applied in 1999. However, two spotted spider mites were added to treatments 1 and 3 on June 30 and July 29, 1999 in an attempt to increase the spider mite density.
Results and Discussion

Peppermint wilt symptoms result from (a) new root infections each season and (b) winter survival of rhizomes infected from previous seasons, perhaps increased by fragmentation of infected rhizomes during tillage. Symptoms may be increased by seasonal stresses. Soil assay recovery of CFU was much lower than expected in either 1998 or 1999 (data not shown) based on the number of microsclerotia applied in 1997, which may be explained in several ways: first, our lab-produced inoculum still may have only weakly survived once placed in the soil. If so, this inoculum still may have been sufficient to provide root and rhizome infection, but the inoculum may not have survived to contribute much to wilt levels in subsequent years. In this case, carryover of *V. dahliae* in rhizomes may have been sufficient to provide adequate seasonal wilt levels. Alternatively, the lab-grown inoculum may have remained viable in plots but we may have had difficulty with soil assay recovery efficiency, which is known to be highly variable among soil types. We have been able to assay soils elsewhere on the COARC-Madras farm, but we do not yet have experience in all parts of the farm. Irrespective of how to interpret low soil recovery of *V. dahliae*, relatively little wilt occurred in low wilt plots and relatively abundant wilt occurred in high wilt plots. Thus, our infestation was adequate to provide sufficient wilt symptoms in 1998 and 1999 as a basis for measuring interactions between spider mites and wilt incidence in each year.

At the beginning of the mite-sampling period in 1999 (June), all treatments had a uniform infestation of twospotted spider mites and predator mites (Figure 1). However, by the end of June (June 30), spider mites averaged <1.0/leaf, so additional spider mites were distributed in treatments 1 and 3 in an attempt to increase the population of spider mites. Low densities of spider mites in all plots were evident during July, so additional spider mites were distributed in treatments 1 and 3 on July 29. At harvest time, the density of spider mites and predator mites averaged <1.0/leaf. In 1998, the spider mite densities were successfully manipulated in all treatments, and spider mite-induced stress was evident in treatments 1 and 3 during late June and July (Figure 2). In 1999, spider mite densities exceeded the action threshold in mid-June only and remained below the action threshold during the growing season. As a result of the low spider mite densities in 1999, it is unlikely that any spider mite-induced stress occurred.

In 1998 and 1999, the incidence of peppermint plants expressing Verticillium wilt symptoms was higher in the Verticillium wilt-infested plots, and in 1999 there was a significant interaction (p<0.0324) between Verticillium wilt (measured as wilt strikes in 400 ft²) and spider mites (Table 1). In 1998, Verticillium wilt significantly reduced the fresh hay weight (p<0.05) compared with the treatments without Verticillium wilt (Table 1). However, the high densities of spider mites did not affect fresh hay weights. There were no significant effects of either spider mites or Verticillium wilt on oil yield in 1998 (Table 1). In 1999, fresh hay weight was slightly lower where Verticillium wilt was high, but was not significantly different from the treatments without Verticillium wilt (p< 0.05). In contrast to 1998, oil yields in 1999 were significantly depressed in treatments with high Verticillium infestation (p<0.05) (Table 1).

In 1998, high spider mite densities in treatments 1 and 2 significantly affected the oil characteristics, particularly menthofuran, esters, menthol, and pulegone (Table 2). Results showed that high spider mite densities decreased the levels of pulegone. This result supports
earlier research (DeAngelis et al., 1983c), that indicated that spider mite-induced feeding injury decreases the amount of pulegone in injured leaves, suggesting that spider mite feeding injury causes a more rapid oil maturation. However, in 1999, there were no significant effects of spider mite density on oil characteristics (Table 1). There were no significant differences in the oil characters with respect to the inoculum levels of Verticillium in any of the treatments during the course of this experiment.

We have collected data for three complete growing seasons, and our results indicate that there was a significant interaction between spider mites and incidence of verticillium wilt in 1999 at the end of the experiment (Figure 3). We also demonstrated that Verticillium wilt alone reduced fresh hay weight and oil yields by the end of 3 years. In 1998, spider mite induced feeding injury altered the amount of pulegone, but we did not find any significant effect of verticillium wilt on oil characters.

Even though we were unable to document consistently a strong interaction between spider mite feeding injury and incidence of Verticillium wilt, it is still possible that spider mites influence wilt incidence. Perhaps the effect of spider mite feeding is less than we were able to measure within the power of our experimental design, or spider mite feeding did not cause plant stress to occur at high enough levels at an appropriate time to induce a differential wilt response.

![Graphs showing interaction of verticillium wilt strikes and relative spider mite populations in 1998 and 1999 in peppermint plots treated with different densities of V. dahliae and spider mites. The P value represents the probability of obtaining F ≤ 0.05 for the interaction of mites by verticillium wilt.](image)

Figure 3. Interaction of verticillium wilt strikes and relative spider mite populations in 1998 and 1999 in peppermint plots treated with different densities of V. dahliae and spider mites. The P value represents the probability of obtaining F ≤ 0.05 for the interaction of mites by verticillium wilt.
Literature Cited


Figure 1. Densities of spider mites and mite predators in peppermint plots treated with different densities of verticillium wilt, 1999.

Figure 2. Densities of spider mites and mite predators in peppermint plots treated with different densities of Verticillium wilt, 1998.