

Evaluate Site-Specific Flaming for the Management of Verticillium Wilt in Peppermint

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Introduction

Verticillium wilt, caused by *Verticillium dahliae*, is a limiting disease to peppermint production in Oregon and other western and mid-western states. Initial inoculum for the disease comes from microsclerotia present in soil and/or infected rhizomes. Infection by the pathogen causes chlorosis, stunting, and asymmetric growth of apical leaves, shortening of internodes, and death of shoots or the whole plant. At the end, the pathogen produces a great number of microsclerotia in infected tissues, which survive in soil and serve as inoculum for further infections. Although long term rotation and planting certified Verticillium free rhizomes reduce the initial disease level in the first peppermint crop, to date no cost effective method is available for controlling the disease in established crops. Previously, in 2009 and 2010, screening fungicides and biological control agents for this disease did not find any product that could provide satisfactory disease control in peppermint.

Verticillium wilt is a typical monomolecular disease, secondary infections are ignorable in a single cropping season and disease incidence at the end of the season is mainly determined by inoculum density in the soil and initial infection level. However; the disease incidence usually increases exponentially over multiple seasons. Usually, increases range from lower than 1% in the first year to high enough (20% or higher) to make growing the crop unprofitable in 4-5 years. This increase may be caused by the expansion of existing disease foci and increasing number of disease foci. Results from Johnson et al (2006) suggested the expansion of existing Verticillium wilt foci is slow and disease foci are generally small, incidence increase is mainly due to more disease foci. Theoretically, without new inoculum, the number of infections/disease foci should increase linearly as the inoculum density remains constant or even slow down due to viability of soilborne microsclerotia decline over years. The exponential increase of disease foci suggested that the spread of new inoculum produced in diseased plant tissues plays an important role in epidemics of this disease. McIntyre and Horner (1973) reported an average of 250,000 to 1,250,000 CFU of *V. dahliae* in a gram peppermint stem tissue. Brandt et al. (1984) recovered up to 15,000 propagules per millimeter of infected stem from susceptible mint (*Mentha piperita*) plants. Peppermint plants are very susceptible to *V. dahliae*, one microsclerotia in a gram of soil is enough to cause significant disease. A diseased plant with an average size consisting of five 3-foot long stems, could add up to 70 million *V. dahliae* propagules. This, if returned to the soil, could infest 2,400 cubic feet soil at a density of one microsclerotia per gram soil. Given the importance of *V. dahliae* propagules newly produced in the infected peppermint tissues, Horner (1965) proposed post-harvest propane flaming of peppermint residues as a method of controlling Verticillium wilt in peppermint. He reported that flaming could kill the majority of *V. dahliae* propagules in infected stems, and recommended a propane flamer, rear-mounted on a tractor moving at 2.5 to 3 mph. To reduce the fuel cost, Butler et al. (1995) investigated if satisfactory disease control could be achieved at a higher speed and found that at 2 to 2.5 mph, the number of infected stems with recoverable *V. dahliae* could be reduced 56 to 59% by flaming, but at speeds of 4 mph or higher, the effects of flaming was insignificant compared with untreated checks.

Because growers usually rotate a peppermint field out of mint production for more than ten years before growing peppermint again and use only certified *Verticillium*-free rhizomes, the incidence of *Verticillium* wilt is usually very low the first year of peppermint crop. For example, the percentage of quadrats (2 ft × 2 ft) with *Verticillium* wilt was 1.32% or lower in 3 fields with peppermint or Scotch spearmint (Johnson et al. 2006), and four or fewer infected plants (1.3 plants on average) were recorded within 2000 square feet in last year's field trial (Uppala et al. 2011). Given the very low initial wilt incidence, we proposed a new disease management strategy to slow the spread of *Verticillium* wilt in peppermint fields via site-specific flaming of individual diseased plants. By flaming only a small number of plants in a field, we can spend more time flaming each diseased plant to ensure better kill rates of *V. dahliae*. This additionally dramatically reduces fuel cost and impact to the environment. This year, we proposed to test the following three hypotheses that are required by the effectiveness of this new strategy: 1) increasing flaming time at each diseased plant, to a level that is still economic and practical, while killing most newly produced *V. dahliae* propagules in the infected peppermint tissues; 2) stolons initiated from infected mother plants play only a limited role in the spread of *Verticillium* wilt in peppermint fields; and 3) microsclerotia in infected roots and soil play only a limited role in the spread of this disease in peppermint fields.

The specific objectives in 2012 include (1) comparing the effects of different flaming treatments on viability of *V. dahliae* in stems, stolons and top soil; (2) quantifying the propagules of *V. dahliae* in different parts of peppermint stolons and stems; and (3) quantifying the distribution of microsclerotia in the soil prior to, and after harvest around disease foci.

Materials and Methods

Effects of propane flaming on viability of *V. dahliae*. To compare the effects of propane flaming treatments on the viability of *V. dahliae* in peppermint stems, stolons and soil, peppermint microplots established in 2011 were subjected to the following 7 treatments: 1) no treatment; 2) flaming the above ground parts for 10 seconds at each plant; 3) flaming the above ground parts for 20 seconds at each plant; 4) flaming the above ground parts for 30 seconds at each plant; 5) flaming the above ground parts for 10 seconds and then flaming the soil surface for 10 seconds; 6) flaming the above ground parts for 20 seconds and the soil surface for 20 seconds; 7) flaming the above ground parts for 30 seconds and the soil surface for 30 seconds. The treatments were arranged in a randomized complete block design with 5 replicates. Each plot was 5 ft × 5 ft and adjacent plots separated by a 5 ft buffer. Twenty four Black Mitcham peppermint plants were planted in each plot originally in 2011 and missing plants were replanted again this year in early May with seedlings reproduced in a greenhouse. The plots were irrigated as needed and no pesticide was applied during this study. When the peppermint plants were close to maturation, two separate plants with *Verticillium* wilt symptoms were identified and marked in each plot. Flaming treatments were then conducted on the marked plants using a handheld propane flamer (Fig. 1). The rate of propane consumption was approximately 2 gallons over a 45 minute period.

Plant parts and soil surrounding each marked plant were sampled immediately after flaming treatments. Five samples were collected from each plot (samples collected at the two marked plants were pooled and mixed together): A) the base part of peppermint stems starting from the

soil surface to 3 inch height; B) the top part of stems starting from 3 inch above the soil surface; C) the stolons above ground or near the soil surface; D) plant roots; and E) top soil within a 1 inch depth and 6 inch radius around each marked plant. The samples were assayed in the laboratory for viable *V. dahliae* propagules according to the following protocols:

Quantify *V. dahliae* in soil:

1. Wet sieve 100 cc soil using a sieve #40 on top of a #400 sieve;
2. Transfer all residues on the #400 sieve into a 500-cc beak
3. Stir up the residues with tap water, let them settle for 5 seconds, then pour floating residues back to the #400 sieve;
4. Repeat step 3 twice;
5. Collect all the residues on the #400 sieve (If needed, concentrate the collection) to make 10 ml suspension;
6. Plate 0.5 ml suspension onto each NP-10 plate, 3 plates per sample;
7. Count colonies of *V. dahliae* on each plate after incubation at room temperature for 10 days.

Quantify *V. dahliae* in plant tissue

1. Soak stems, stolons or roots in tap water for 15 minutes;
2. Rinse stems, stolons or roots with tap water to remove dirt;
3. Chop samples into 0.5-cm pieces with sterilized scissors;
4. Weigh 5g (fresh wt) chopped pieces and place them into a blend with 200 cc distilled water;
5. Blend for 2 minutes;
6. Plate 0.200 ml blended suspension and 1:100 (with sterilized water) diluted suspension on each NP-10, 3 plates per dilution;
7. Count colonies of *V. dahliae* on each plate after incubation at room temperature for 10 days.

Distribution of *V. dahliae* microsclerotia in the soil. In a commercial field in first year peppermint, five small isolated disease foci were identified and marked out one week prior to harvest. Five soil cores (1-inch diameter and 7.5 inch deep) were taken at a distance of 1 and 2 ft from the center of each focus. Each core was divided into three parts based on depths (0-2.5, 2.5-5.0, and 5.0-7.5 inch) and pooled together as one sample for each depth and distance combination. In another commercial field in 2nd year peppermint, soil samples were collected around the marked disease foci 4 weeks after harvest in the same manner as described above. All soil samples were assayed for viable propagules of *V. dahliae* in the laboratory by wet sieving and plating concentrated residues on selective medium NP-10 as described above.

***V. dahliae* propagule densities in peppermint stems and different parts of stolons.** First, in the same trial plots for flaming treatments, 5 samples of stolons, symptomless and symptomatic stems were collected from unmarked symptomatic plants (no flaming treatments were done) and assays were performed as described to quantify viable propagules of *V. dahliae*. In addition, a greenhouse experiment was conducted to determine the role of peppermint stolons in the spread of Verticillium wilt. Eight Black Mitcham peppermint plants in 1-gallon pots were inoculated

with *V. dahliae* on June 22nd, and 1 to 2 stolons were selected from each plant and were grafted each into another 1-gallon pot with sterilized soil on August 29th. The mother plants and daughter plants were monitored for symptoms of Verticillium wilt. On October 18th, stems (including some stolons) were taken from mother plants, near end of daughter plants and far end of daughter plants. For the mother plants with two daughter plants, the samples from the two daughter plants were pooled according their position. After surface sterilization with 2% bleach for 2 minutes and rinsing twice with sterilized water, ten sections from each sample were placed on NP-10 medium. After incubation at room temperature for 10 days, each section was examined under a dissecting microscope for *V. dahliae*.

Results and Discussion

Effects of propane flaming on viability of *V. dahliae*. The results revealed that flaming treatments had no significant effect on the viability of *V. dahliae* propagules in peppermint roots (Fig. 2). Regardless the flaming time, peppermint roots contained 0.33 to 0.79 million of viable propagules of *V. dahliae* in each gram fresh tissue. All flaming treatments significantly reduced viable propagules of *V. dahliae* in peppermint stems, both upper and base parts of the stems as compared with untreated controls. In both flamed and unflamed stolons, the number of viable propagules of *V. dahliae* was very low, and flaming the soil surface seemed to further reduce the viable propagules of *V. dahliae* in the stolon tissues. Without flaming treatments, the base part of peppermint stems (0.81 million CFU/gram fresh tissue) and roots (0.39 million / gram fresh tissue) contained the most viable propagules of *V. dahliae*, with no significant difference between them. The density of viable propagules of *V. dahliae* was found to be the lowest in stolons (0.08 million CFU/ gram fresh tissue), and moderate in the upper stems (0.19 million CFU/ gram fresh tissue). A 10-second flaming of plant parts and 10-second flaming of the soil surface killed almost 100% propagules of *V. dahliae* in stems and stolons, leaving fewer than 100 viable *V. dahliae* propagules per gram fresh tissue. Regardless of flaming treatments, no viable propagules of *V. dahliae* was detected in the soil except two plots where only one CFU was found in 15 g soil (data not shown).

Distribution of *V. dahliae* microsclerotia in the soil. Similar to the results obtained last year, although we used wet sieving to concentrate the *V. dahliae* microsclerotia, we did not detect viable propagules of *V. dahliae* in soil samples collected from commercial fields before or after harvest (data not shown). The results from both microplot and commercial fields, as well as the results from last year revealed consistently that the inoculum density of *V. dahliae* was extremely low in the soil. This suggested that movement of soil perhaps is not important for the spread of this disease in the field unless infected roots or other plant residues are moved with the soil. This also suggested that it may be unnecessary to heat the soil to kill most newly produced propagules of *V. dahliae* and slow the spread of the disease in peppermint fields.

***V. dahliae* propagule densities in peppermint stems and different parts of stolons.** For the extra samples collected from untreated peppermint plants in the microplots, the symptomatic stems contained most viable propagules of *V. dahliae*, around 0.79 million CFU/ gram fresh tissue (Fig. 3), which was very close to the result obtained above. The density of viable propagules of *V. dahliae* was once again very low in peppermint stolons (0.021 million CFU/gram fresh tissue), even lower than in the symptomless stems (0.12 million CFU/gram

fresh tissue). In the greenhouse experiment, 100% of the mother plants were symptomatic and positive in the test for *V. dahliae*, and the samples from the near end of stolons were also 100% *V. dahliae* positive in the test although sometimes symptomless. On the contrast, the samples from the far end of stolons were mostly (75%) symptomless and 30% negative in the test for *V. dahliae*.

V. dahliae is a slow growing fungal pathogen, its colony can extend only 3.5 cm in two weeks when cultured on plates (Wu et al. 2008). Its long distance movement in plants must be mainly via its propagules moving with the xylem fluid. Normally xylem fluid moves from the roots up toward stems and leaves; therefore, the lower parts of stems contain a higher level of viable propagules of *V. dahliae* than the upper parts of stems in this study. However, the movement of xylem fluid in stolons may be different because new roots form at nodes of stolons of peppermint when they touch the soil. As more and more new functional roots form along the stolon, the xylem fluid moves from the roots of the mother plant to the stolon tips reducing as the stolon extends because the new roots can provide the majority or even 100% water to support the growth of the stolon tips. This may be a reason for the very low inoculum levels detected in the stolon collected from microplots because formation of new roots usually starts from the beginning of stolon extension. This also explains why there were fewer symptomatic and *V. dahliae* positive shoots at the far end of grafted stolons than at the near end in the greenhouse experiment. Different from the microplot trial, to have adequate stolon length so they could reach another pot, we waited more than two months after inoculation to graft, when most of the mother plants had already shown wilt symptoms. This may have allowed some propagules of *V. dahliae* to move into the stolons even before grafting. In the future, it would be interesting to test how grafting at different times after inoculation affects the movement of *V. dahliae* in stolons.

In summary, this study demonstrated that flaming individual diseased plants and soil surfaces for 10 seconds each can kill almost 100% propagules of *V. dahliae* in the stems and stolons (above or near soil surface). Because the results of this study also suggest that propagules in underground stolons and soil may not be important to the spread of Verticillium wilt in peppermint, we deduced that this new strategy may be effective in slowing down the spread of this disease in commercial field. Meanwhile, flaming only individual diseased plants dramatically reduced the fuel cost compared with flaming the whole field. Using the handheld torch, the consumption of propane was only about 2.0 gallons for 45 minutes of flaming, which is enough to cover flaming 90 diseased plants if flaming 30 seconds at each plant (15 seconds plant parts, 15 seconds on soil surface). It is also obvious that this strategy must be used in combination with other practices including long term rotation, planting Verticillium-free roots and no tillage or other practice that moves roots, plant residues and soil in established mint. If we can achieve an initial disease level of 1.3 diseased plants in 2000 square feet via other disease management practices, the average level observed in our 2011 field trial (Uppala et al. 2011), 2.0 gallons of propane would be enough for flaming more than 3 acres. At this disease level, the labor cost for flaming would also be limited, approximately 30 minutes per acre [(30 s flaming per plant + 30 s moving between diseased plants with a 12 ft bandwidth) × 30 diseased plants/acre]. In the light of the findings gained this year, the authors strongly propose further testing to determine how effective this novel strategy is in slowing the spread of Verticillium wilt in large plot trials and in commercial fields over multiple cropping seasons.

References

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Fig. 1. A photo of the handheld propane flamer used for flaming peppermint plants in the microplot trial. An extended torch was attached to a 5-gallon propane tank.

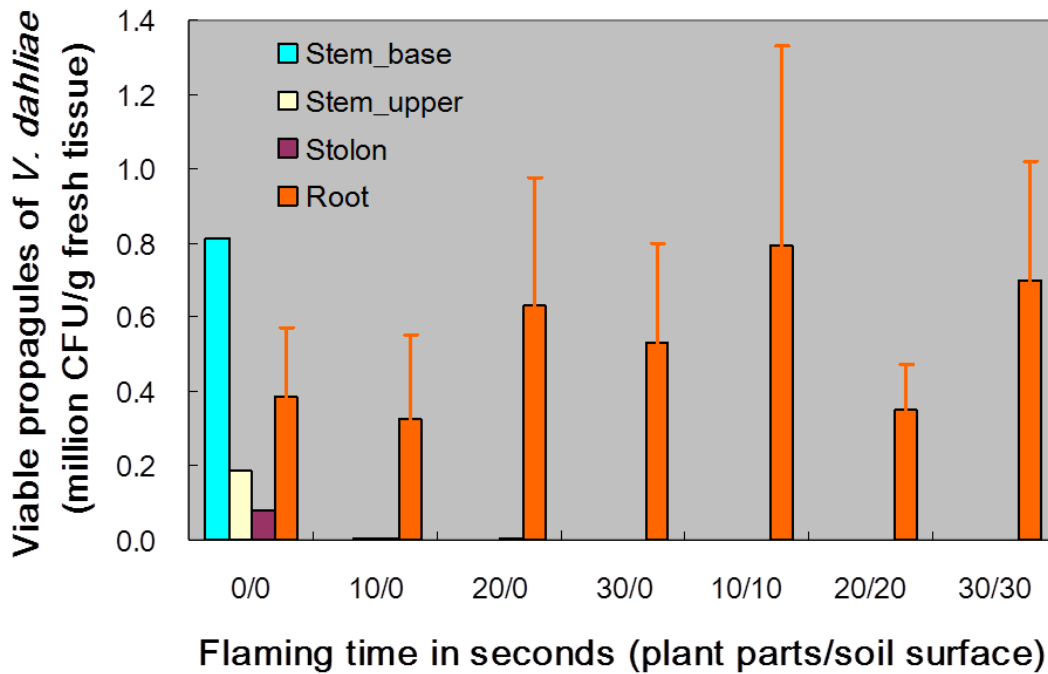


Fig. 2. Effects of propane flaming on viability of *Verticillium dahliae* propagules in different peppermint tissues as affected by the length of flaming time (seconds for plant parts/ seconds for soil surface).

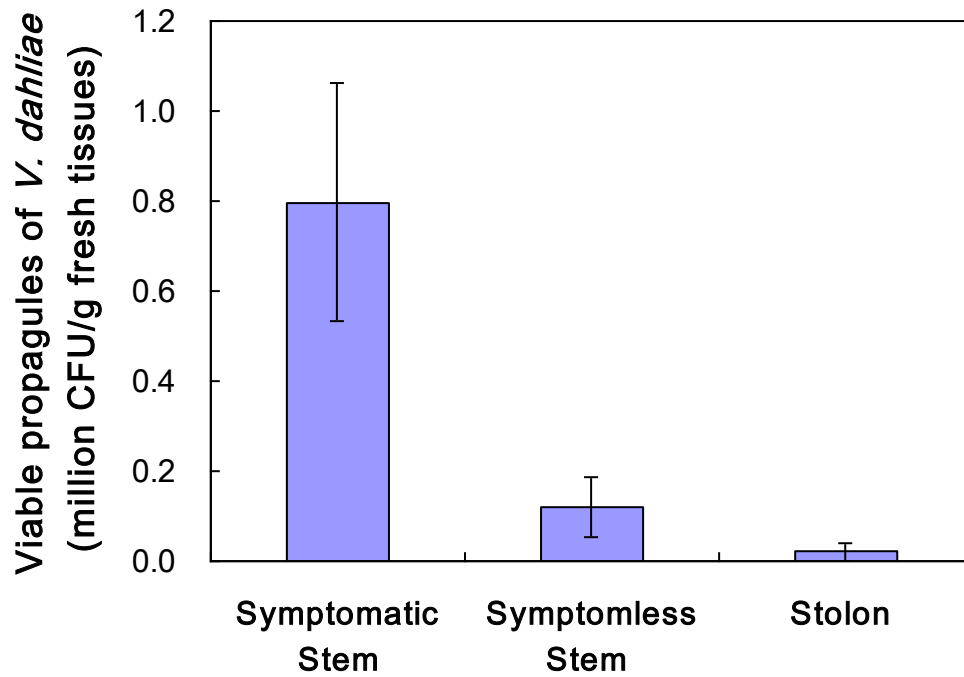


Fig. 3. Density of viable propagules of *Verticillium dahliae* in peppermint stolons, symptomatic and symptomless stems.

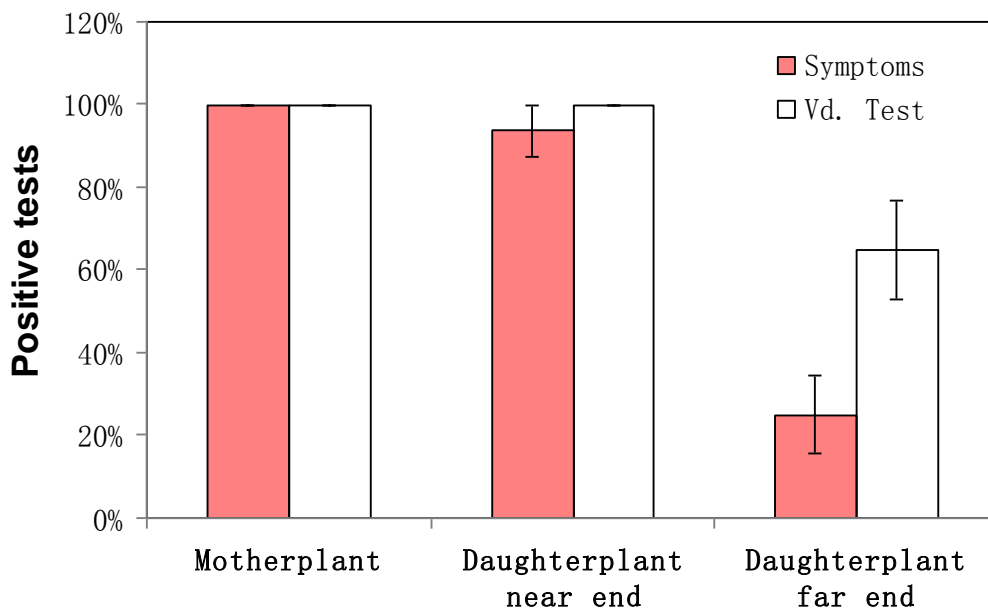


Fig. 4. Symptoms of *Verticillium* wilt and results of tests for *Verticillium dahliae* for the samples collected from mother plants inoculated with microsclerotia of *V. dahliae*, the near end and the far end of the stolons initiated from the diseased mother plants.