Evaluation of Biological Control Agents for Verticillium Wilt in Peppermint

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Introduction

Results from the 2009 field trials showed that fungicides Headline®, Proline®, and Quadris® exhibited limited efficacy on Verticillium wilt (*Verticillium dahliae*) in peppermint. Because established peppermint (*Mentha piperita*) has a long window of susceptibility to Verticillium wilt, it may be unrealistic to find new chemicals for protection of peppermint roots for such a long period. On the other hand, because peppermint is a perennial crop, if biological control agents (BCAs) can survive through the winter, one application could potentially last for multiple years. This could make BCAs a more favorable management practice against Verticillium wilt in peppermint. Some biological control agents have showed promising results in controlling Verticillium wilt in a variety of crops. *Serratia plymuthica* is a ubiquitous bacterium in soils all over the world. In greenhouse trials, treatment with *S. plymuthica* reduced the percentage of Verticillium wilt in strawberry by 18.5 percent. Results were even better from dipping plants in a suspension of *S. plymuthica* prior to planting; this treatment reduced Verticillium wilt 24.2 percent, and increased yield 296 percent on average in field trials (Kurze et al. 2001). There is also ample evidence that vesicular arbuscular mycorrhizal (VAM) fungi can act as BCAs against a variety of soilborne plant diseases. Often the mechanisms include improved plant growth, direct competition, and/or inhibition.

Arbuscular mycorrhizal fungi were reported to be effective against Verticillium wilt in various crops (Liu 1995, Garmendia et al. 2004, Tahmatsidou et al. 2005). A previous study in India showed VAM fungi established symbiosis with roots and stolons of menthol mint (*Mentha arvensis*, Gupta et al. 2002). These results suggest that VAM fungi have a great potential to establish symbiosis and provide some benefits to peppermint plants. However, it is unclear which isolates provide the best benefit to peppermint, since it is generally understood that the benefits to plants depend heavily on the selection of VAM fungal isolates. It is also unknown if the increased vigor of peppermint resulting from VAM fungi can provide peppermint adequate protection when disease pressure from Verticillium wilt is relatively high. This aspect will be especially important to consider because of the high degree of sensitivity peppermint exhibits to Verticillium compared to other crop species. Therefore, our objectives were to evaluate the efficacy of different BCA treatments to control Verticillium wilt or mitigate its impacts on peppermint and to determine the effects of BCAs on growth of peppermint.

Materials and Methods

An experiment was conducted in 2010 at the Central Oregon Agricultural Research Center (COARC) in Madras, Oregon, to evaluate biological control agents for controlling Verticillium wilt in peppermint. RhizoStar obtained from E-nema GmbH (Raisdorf, Germany), and Myco®Apply Liquid Endo, containing four species of endomycorrhizae and a customized powdery product containing 9 species of endo-mycorrhizae provided by Mycorrhizal Application, Inc. (Grants Pass, Oregon) were used in the experiment. On May 24, Verticillium-free 'Black Mitcham' seedlings provided by Pioneer West, Inc. (La Grande, Oregon) were transplanted into 4-gal pots and buried in a plot without a history of Verticillium. The following are the eight treatments used in this study:

1) Noninoculated soil and no treatment;
2) Inoculated soil and no treatment;
3) Inoculated soil and seedling roots sprayed with MycoApply Liquid Endo at planting;
4) Inoculated soil and drenching with MycoApply Liquid Endo 10 days after transplanting;
5) Inoculated soil and seedling roots sprayed with customized 9-species VAM fungi at planting;
6) Inoculated soil and drenching with customized 9-species VAM fungi 10 days after transplanting;
7) Inoculated soil and seedling roots dipped in RhizoStar at planting;
8) Inoculated soil and seedling roots dipped in RhizoStar, with Headline sprayed at planting

For inoculation, microsclerotia of *Verticillium dahliae* were reproduced in laboratory, dried, and mixed into soil to achieve 1 microsclerotium per gram soil. The soil with microsclerotia was placed into a 4-gal pot and peppermint seedlings were transplanted into the soil and subjected to appropriate treatments. For the noninoculated control, clean soil without contamination by *V. dahliae* was used. The Rhizostar dipping treatment was done by dipping the seedling roots in the RhizoStar suspension (diluted with 1:1 water) prior to planting for 30 min. Spraying of Headline and mycorrhizal fungi was done after placing a seedling into each pot, and the roots of the seedlings were covered with soil immediately after spraying. Headline was diluted with (1:200) water and sprayed onto roots and surrounding soil at 20 ml/pot. MycoApply Liquid Endo (1:2 dilution with water) and water suspension of the 9-species powder (16 g/L) were sprayed at 20 ml/pot. Soil drenching was done 10 days after transplanting. MycoApply Liquid Endo (1:8 dilution with water) and water suspension of the 9-species powder (4 g/L) were drenched at 100 ml/pot. The pots were buried into a Verticillium-free field plot at COARC. The 8 treatments were arranged according to a randomized complete block design with 10 replicates (pots). The pots were fertilized, irrigated, and weeded as needed.

The weather in the early spring was mostly rainy and cold at Madras and the peppermint seedlings grew slowly until the end of June. The plants had had very limited growth until July 21, 2010. Due to the limited growth of plants, no root samples were taken for examining the colonization by mycorrhizal fungi until harvest. Prior to harvest on August 30, severity of Verticillium wilt was determined for each plant using the following scale (see Figure 1 for photo samples):

0	no disease
1	minor symptoms on a few leaves
2	symptoms on majority of leaves, minor dwarfing
3	significant dwarfing, yellowing, wilt, and defoliation
3.5	some shoots with severity 3, and some shoots dead
4	the whole plant dead

The plant height was measured for each pot, and then all the above-ground parts were cut off and the fresh plant weight was determined at harvest on August 31. Two stems were randomly selected, surface sterilized with 0.5 percent bleach, and rinsed twice in sterilized water. Five 1- to 2-mm samples were taken from each stem (apart from each other along the stem) and were plated onto NP-10 medium. The plates were examined for colonization of *V. dahliae* after 10 days of incubation at room temperature (about 68^{o}F). On September 1, about 40 percent of the roots were dug out from each pot. The root samples were cleaned, surface sterilized, and rinsed in sterilized water. Five small pieces from each of two randomly selected roots were plated onto NP-10 medium and the colonization of *V. dahliae* was determined as the above. The remaining roots were placed in a tea bag and cleaned with 10 percent KOH at 149^{o}F for 5 hours, rinsed with water 3 times and 1 percent HCl for 30 min, and stained in trypan blue (0.05% w/v in lactoglycerol) at 149^{o}F for 1 hour. The samples were kept at room temperature until the microscopy examination for colonization by mycorrhizal fungi.

Results and Discussion

In general, the inoculation of *V. dahliae* was successful; the average wilt incidence reached 66 percent at harvest for all different treatments with inoculated soil while 9 out of 10 of the noninoculated plants remained symptomless. It was interesting to note that the symptoms of wilt only became visible near the end of the season, when the plants started to flower. There were only 3 out of a total of 70 inoculated plants that exhibited wilt symptoms by August 6, but this number increased quickly, reached 19 on August 16, and 46 prior to harvest on August 31.

The results of the 2010 experiment showed that Verticillium wilt significantly affected the growth of peppermint plants. The wilt severity scales used in disease rating well represented the impacts of the disease on the growth of peppermint plants. The higher the severity, the lower the fresh plant weight (Figure 2). The plant height and fresh weight also were closely correlated (Figure 3). The disease severity was lower (average 1.25 and 1.30) and the fresh weight greater for treatments 6 and 8 than in other treatments (Figure 4). The disease incidence was 60 percent or higher for inoculated treatments with the exception of treatment 8 (roots dipped in Rhizostar and Headline sprayed at transplanting) (Table 1). The difference was not statistically significant because of the small number of plants used in the experiment. While the previous year's experimental design allowed us to compare multiple treatments and manipulate inoculum level accurately with limited cost, it offered us less power to differentiate treatment effects. It will be necessary to evaluate RhizoStar + Headline again in a field trial at an increased rate to confirm its effect in reducing wilt incidence and severity.

The plating tests revealed that disease reading (incidence and severity) and colonization of stems by *V. dahliae* matched very well (Table 2 and Figure 5). There were only 4 out of 46 symptomatic peppermint plants that tested negative (Table 2). However, the colonization of roots showed more mismatches with disease readings. Among the 46 symptomatic plants, only 22 plant roots were colonized by *V. dahliae* (Table 2). Microscopic examination of stained roots did not give any sign of colonization of roots by mycorrhizal fungi. Five root samples were sent to Mycorrhizal Applications, Inc. to confirm our findings; their results also showed low colonization by mycorrhizal fungi even when the roots were cleared and stained for a prolonged period of time.

In addition, the minimal effects of mycorrhizal fungi products on wilt incidence, wilt severity, and plant fresh weight might have been caused by low colonization of roots by mycorrhizal fungi. This was likely due to the low post-treatment soil temperature. A study on effects of root zone temperature demonstrated that colonization of sorghum roots by mycorrhizal fungus (*Glomus intraradices*) was significantly reduced at 59^{o}F compared with 73.4^{o}F and almost completely inhibited at 50^{o}F (Liu et al. 2004). It will be necessary to optimize the application techniques and timing for mycorrhizal fungi products for future studies.

Table 1. Average plant height, fresh weight, Verticillium wilt severity and incidence for potted peppermint plants subjected different treatments.

Treatment	Height (inch)	Fresh weight (g)	Wilt severity (0 = none to 4 = dead)	Wilt incidence
1	20.4	475.1	0.20	10%
6	18.7	381.7	1.25	70%
8	18.2	361.3	1.30	40%
3	18.1	354.6	1.65	60%
5	17.9	343.9	1.60	60%
4	18.1	334.1	1.60	60%
2	17.3	295.5	1.80	70%
7	17.0	230.5	2.50	90%

Table 2. Colonization of stems and roots by *Verticillium dahliae* for peppermint plants classified into symptomatic and symptomless groups.

Category	Number of plants	Stem colonization	Root colonization
Symptomatic	46	42	22
Symptomless	34	6	9

Figure 1. Photo examples of peppermint plants with different Verticillium wilt severity. From left to right and top to bottom were scaled as 0, 1.0, 2.0, 3.0, 3.5, and 4.0.

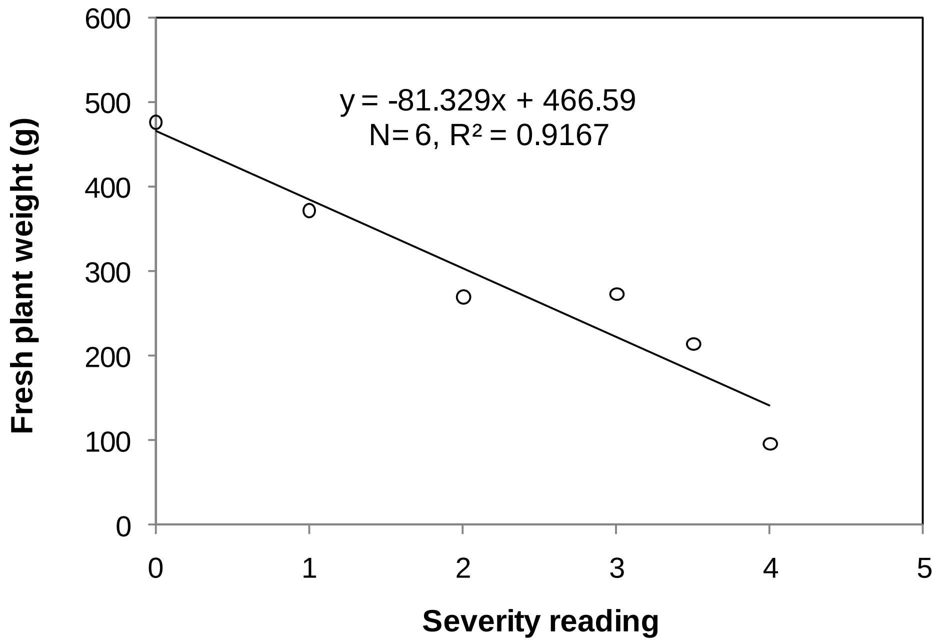


Figure 2. The relationship between Verticillium wilt severity reading and fresh peppermint plant weight at harvest, Central Oregon Agricultural Research Center, Madras, OR.

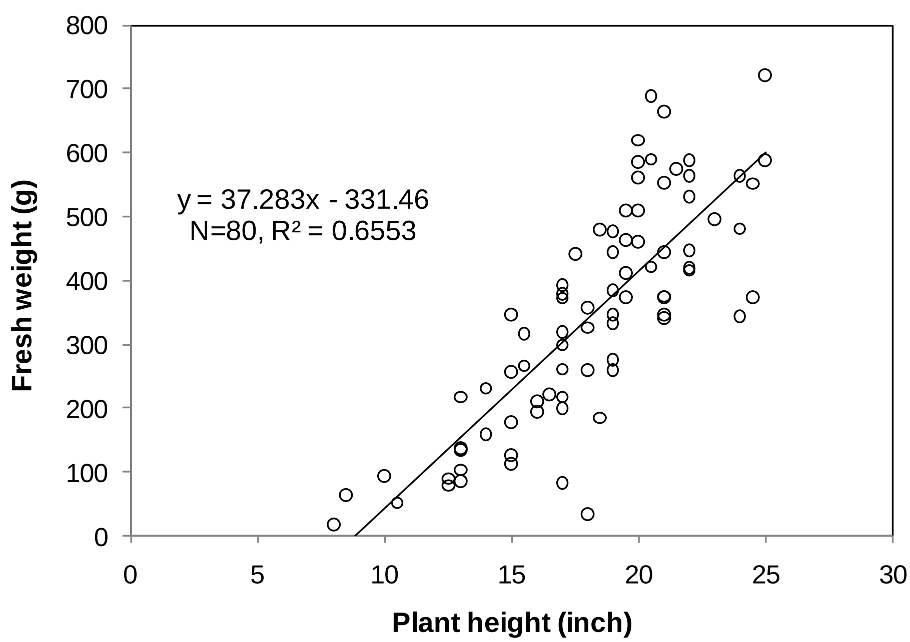


Figure 3. The relationship between the plant height and fresh weight of potted peppermint at harvest, Central Oregon Agricultural Research Center, Madras, OR.

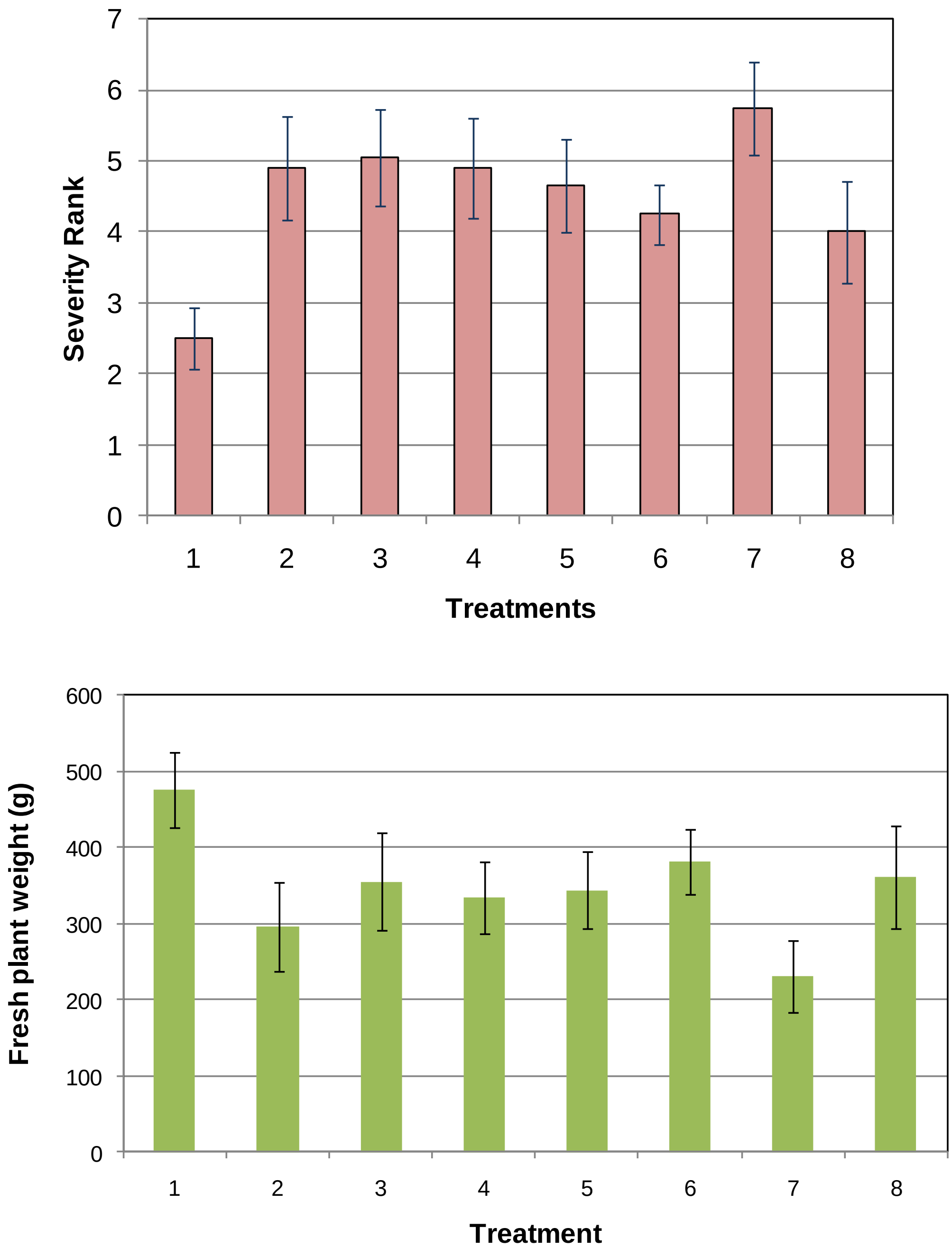
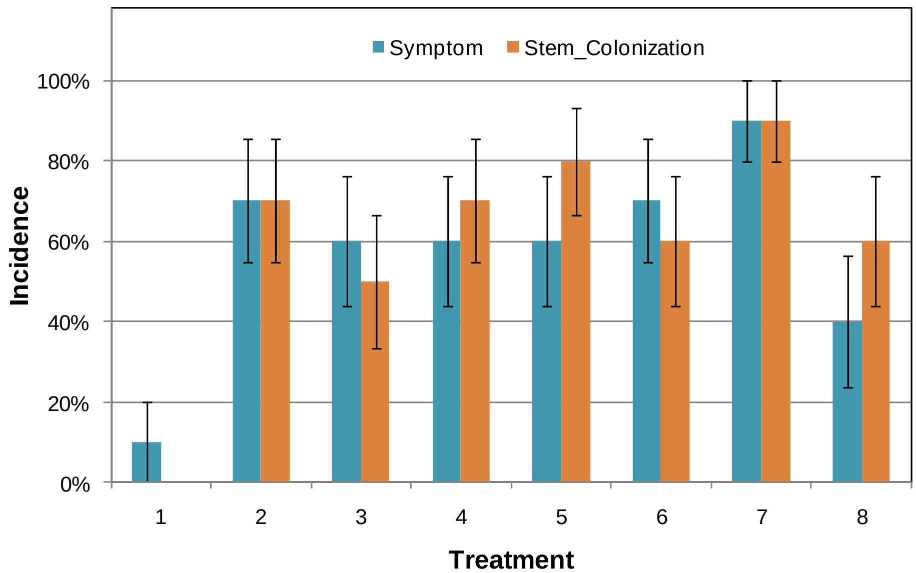


Figure 4. Average severity of Verticillium wilt (top) and fresh plant weight (bottom) of potted peppermint subjected to different treatments. The 8 treatments were: 1) Uninoculated control; 2) Inoculated control; 3) Inoculated soil and roots sprayed with MycoApply Liquid Endo; 4) Inoculated soil and soil drenched with MycoApply Liquid Endo; 5) Inoculated soil and roots sprayed with customized 9-species VAM fungi; 6) Inoculated soil and soil drenched with customized 9-species VAM fungi; 7) Inoculated soil and roots dipped in RhizoStar; 8) Inoculated soil, root stock dipped in RhizoStar, and sprayed with Headline. Central Oregon Agricultural Research Center, Madras, OR.



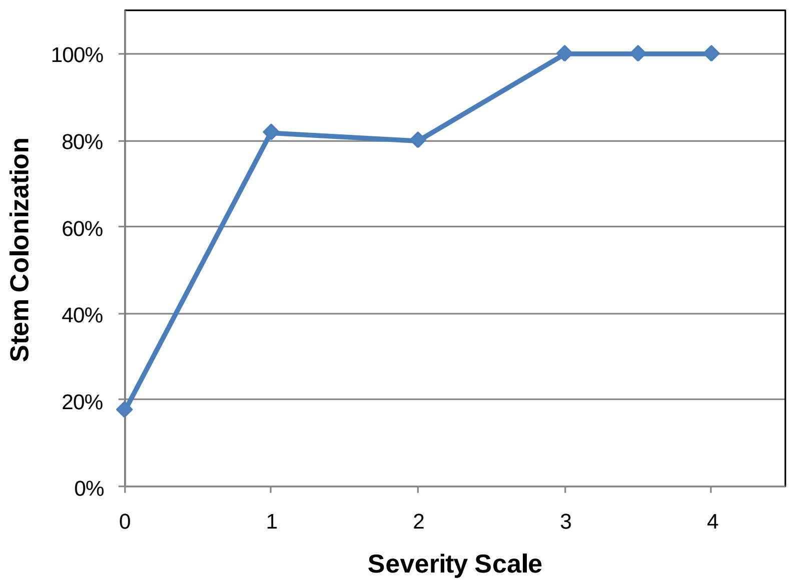


Figure 5. Relationship between colonization of peppermint stems by *Verticillium dahliae* and incidence of wilt symptom (top) / severity (bottom). COARC, Madras, OR.

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Acknowledgements

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