Further Evaluation of Biological Control Agents for Verticillium Wilt in Peppermint

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Introduction

Results of our 2010 study with potted peppermint suggested that the incidence of Verticillium wilt was lower in the treatment of RhizoStar+Headline than that in other inoculated treatments, but the difference was not statistically significant. To test if significant differences would result if a greater number of plants were assessed, the RhizoStar+Headline treatment was evaluated in a plot trial at an increased scale during 2011.

Other published studies suggested that mycorrhizal fungi could colonize peppermint roots, improve the growth of peppermint and reduce yield losses due to Verticillium wilt. However, colonization of potted peppermint roots by mycorrhizal fungi was very low in our 2010 study. Two greenhouse experiments were conducted in 2011 to determine if the low colonization in our previous study was due to low soil temperature or high phosphorus levels. We also conducted a plot trial at COARC and a field trial in Culver, Oregon to further evaluate mycorrhizae products for controlling Verticillium wilt in peppermint. Two other biological control agents, Actinovate and Tenet, were also added in the evaluation.

Materials and Methods

Greenhouse Studies

Two greenhouse experiments were conducted in 2011 to determine the effects of soil temperature and phosphorus level on the colonization of peppermint roots by mycorrhizal fungi. In the first experiment, 'Black Mitcham' seedlings growing in 0.5-gal pots with potting mix were drenched with 0.423 cup of mycorrhizae products, either Ultrafine Endo Powder water suspension (0.534 oz/gal) or Liquid Endo dilution (1.28 fl oz/gal) (manufactured by Mycorrhizae Application Inc., Grants Pass, OR). Plants were then grown at two different temperatures: "low" and "high" temperature regime. The plants in the low temperature treatment were placed directly on a bench in the greenhouse while the plants in high temperature treatment were placed on an electric heating-pad. The soil temperature was monitored using soil temperature probes in 3 pots for each temperature treatment. After growing for 40 days, roots of the peppermint plants were collected, rinsed with tap water, cleared by boiling in 10% potassium hydroxide, stained in 0.05 % w/v trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water), and then examined under a microscope to determine the colonization by vesicular arbuscular mycorrhizae (VAM). Twenty hairy roots per plant were examined and the percentage of root length where either spores or mycelium of mycorrhizae were present was estimated and an average percentage used in analyses.

In the second experiment, 'Black Mitcham' seedlings were planted in 1-gal pots using a mixture of field soil and river sand (phosphorus level was as low as 12 ppm) and were treated to represent low, moderate, and high phosphorus levels, with and without mycorrhizae. The following seven treatments were arranged in a randomized complete block design with 10 potted-plants per treatment: 1) Low phosphorus, Liquid Endo 1.28 fl oz/gal at 0.845 cup/pot on Aug 1 and 30; 2) Low phosphorus, Ultrafine Endo Powder 0.534 oz/gal at 0.845 cup/pot; 3) Low phosphorus, no mycorrhizae check; 4)Moderate phosphorus, Ultrafine Endo Powder 0.534 oz/gal at 0.845 cup/pot; 5) Moderate phosphorus, no mycorrhizae check; 6) High phosphorus, Ultrafine Endo Powder 0.534 oz/gal at 0.845 cup/pot; and 7) High phosphorus, no mycorrhizae check. The low, moderate and high phosphorus levels were achieved by drenching the soil with a liquid fertilizer containing N at 0.04%, and P at 0%, 0.012%, and 0.036%, respectively. On August 23 and September 5, each pot was drenched with 0.845 cup of the liquid fertilizer. Root samples were collected on December 12 from each pot and assayed for colonization of VAM as described above.

Plot Trial at COARC

Eight treatments were included in the plot trial at COARC: 1) Untreated check; 2) Seedling drench with Ultrafine Endo Powder water suspension (0.534 oz/gal, 0.845 fl. oz/plant) before transplanting on April 20, 25 and 29; 3) In-furrow spray with Ultrafine Endo Powder water suspension (0.534 oz/gal, 0.845 fl. oz/plant) at transplanting on May 27; 4) Seedling drench with Ultrafine Endo Powder water suspension (0.534 oz/gal, 0.845 gal per plot) on June 13 when soil temperature reached 59^{o}F; 5) In-furrow spray with Actinovate (0.21 oz/gallon, 0.845 fl. oz/plant) at transplanting; 6) Seedling soak in RhizoStar water suspension (1:1) for 1 hour immediately prior to transplanting; 7) RhizoStar+Headline: seedling soak in 1:1 RhizoStar water suspension prior to transplanting and in-furrow spray with Headline (0.67 fl. oz/gal H_2O, 0.845 fl. oz/plant) at transplanting; and 8) In-furrow spray with Tenet (0.32 oz/gallon, 0.845 fl. oz/plant) at transplanting.

The treatments were arranged in a randomized complete block design with 5 replicates. Each plot was 5 ft × 5 ft in size with a 5 ft buffer between adjacent plots. Laboratory-produced microsclerotia of *Verticillium dahliae* were used to infest the top 6 inches of soil in all plots, resulting in one microsclerotia per gram soil in the top 6 inches. Twenty-four peppermint seedlings (cv. Black Mitcham) were planted in each plot. Disease incidence was recorded on August 30, September 7 and September 14. Plant height and fresh weight were determined at harvest on September 14. Stems sections assayed for *V. dahliae* on NP-10 medium. Root samples were collected, cleared, stained and examined for colonization by VAM under a microscope as described above.

Trial in a Commercial Field

A commercial field was planted with certified peppermint roots during the spring of 2011, after rotation with other crops for about 25 years. The following 5 treatments were arranged according to a randomized complete block design with 5 replicates: 1) Untreated check; 2) Tenet at 6 oz/acre; 3) Liquid Endo at 3.4 oz/acre; 4) Ultrafine Endo Powder at 12 oz/acre; and 5) Actinovate at 6 oz/acre. Each plot was 1200 ft × 27.5 ft in size. All treatments were done by band spraying in 20 gallon water /acre on June 3 when soil temperature reached 55^{o}F, immediately followed by a 2-hr sprinkler irrigation. Early in May, 5 soil cores (1 inch diameter, 6 inch deep) were taken and pooled as one soil sample from each plot. Each soil sample was assayed for *V. dahliae* via plating on NP-10 medium plates (0.1 g pulverized dry soil per plate). Prior to harvest, peppermint plants in an area of 2000 ft^2 were evaluated for Verticillium wilt.

Results and Discussions

Greenhouse Studies

The average soil temperature recorded over the study period was 72.8^{o}F and 61.0^{o}F, respectively, for high and low temperature treatments. Because the temperature started to warm up during our study period, the low temperature in the greenhouse during March was already higher than the soil temperature in commercial fields in May. Therefore, we didn't observed the limiting effects of low temperature (<50^{o}F). There was no significant difference between temperature treatments in colonization of peppermint roots by VAM (Figure 1). Roots treated with Liquid Endo showed higher VAM colonization than roots treated with Ultrafine Endo Powder, but even the Liquid Endo treatment resulted in relatively low colonization rates (Figure 1). The higher VAM colonization in Liquid Endo treatment than Ultrafine Endo Powder treatment was probably due to a greater number of VAM propagules contained in Liquid Endo (36718 propagules/gal in the diluted Liquid Endo vs. 5663 propagules/gal in the Ultrafine Endo Powder suspension). The potting mix used in this first study had a high phosphorus level (test results ranged from 79 to 268 ppm) which may account for the low mycorrhizal colonization rate found with both products. Our 2011 Greenhouse study # 2 is still underway, and this investigation into the effects of phosphorus level shall hopefully be able to answer this question.

Plot Trial at COARC

No significant difference was detected in incidence of Verticillium wilt among the 8 treatments tested (Figure 2). The average incidence varied in a narrow range from 24.5% to 32.5%. Differences in plant height and plant fresh weight were also insignificant among the 8 treatment (Figs. 3 and 4). The percentage of root length colonized by VAM was significantly higher in plots treated with mycorrhizae products than in plots without mycorrhizae treatment (Figure 5). However, considerable amounts of roots were also colonized by VAM in plots without any mycorrhizae treatment. These results are very different from our results obtained in when using potting mix. This might be partially attributed to natural mycorrhizae inoculum in the field soil

and the relatively low phosphorus level in the soil (24 ppm). The colonization of peppermint roots, based on the presences of spores and/or mycelium of VAM, was generally high in this study (Figure 6), but no effects of mycorrhizae on disease incidence, plant height or fresh weight were detected. The reasons why high mycorrhizae colonization did not provide protection to peppermint roots against infection by *V. dahliae* are unclear, but uneven coverage of roots by VAM may be one major reason. An overwhelming disease pressure due to a great number of pathogen propagules in the soil might be another reason. Interestingly, peppermint roots treated with Headline showed the lowest colonization by VAM, which is consistent with reports that fungicides negatively affect the colonization of roots by VAM.

Trial in a Commercial Field

For all 25 soil samples, no *V. dahliae* was detected by plating 0.5 gram soil (Table 1). This suggested the soil-borne *V. dahliae* inoculum level was very low after rotation with other crops for 25 years. A method assaying a larger volume of soil is needed for detecting such a low level of *V. dahliae*. As the result of low inoculum level, incidence of Verticillium wilt was also very low in the field regardless of treatments. The highest incidence of Verticillium wilt was 4 diseased plants in 2000 square feet (Table 1), and the difference among treatments were statistically insignificant (data not shown). Therefore, no conclusion can be drawn on the effects of treatments.

Table 1. Inoculum density of *Verticillium dahliae* in the top 6 inch soil and number of diseased plants within 2000 square ft in a commercial peppermint field.

Treatments	No. of Diseased Plants		Microsclerotia in 0.5 g soil
	Mean	Range	
Untreated check	1.8	1-3	0
Actinovate	0.8	0-3	0
Tenet	1.2	1-2	0
Mycorrhizae--Liquid Endo	1.4	0-2	0
Mycorrhizae--Endo Powder	1.4	0-4	0

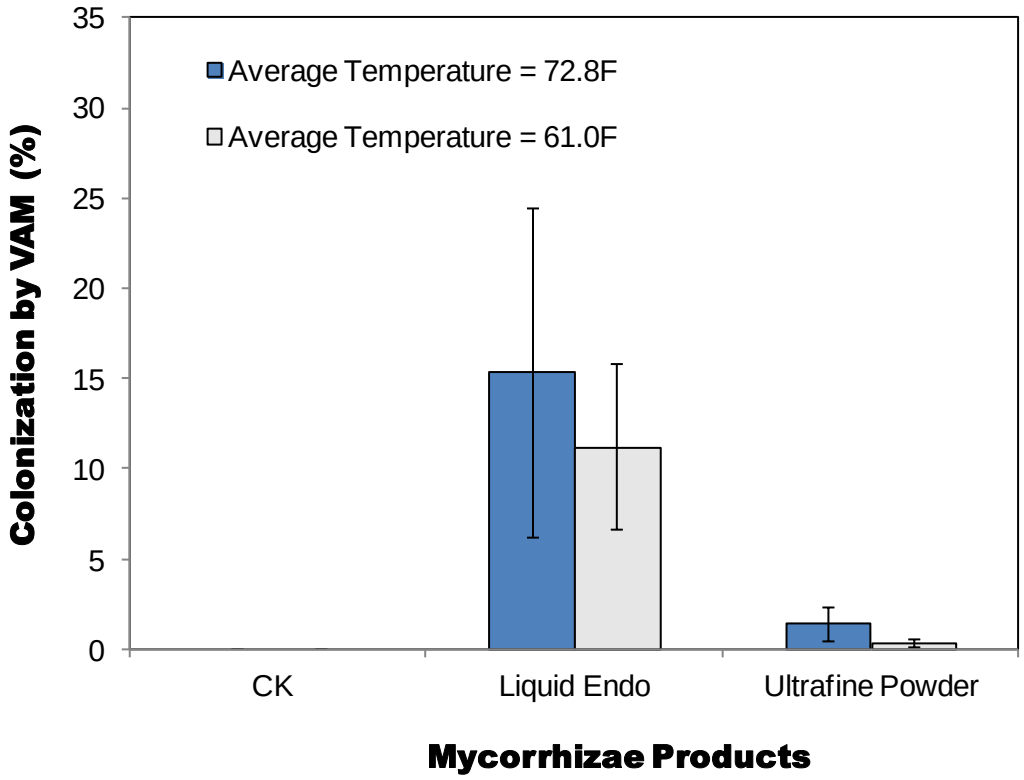

Figure 1. Rate of VAM colonization for peppermint roots subjected to different soil temperature treatments in a greenhouse experiment at Central Oregon Agricultural Research Center, Madras, Oregon

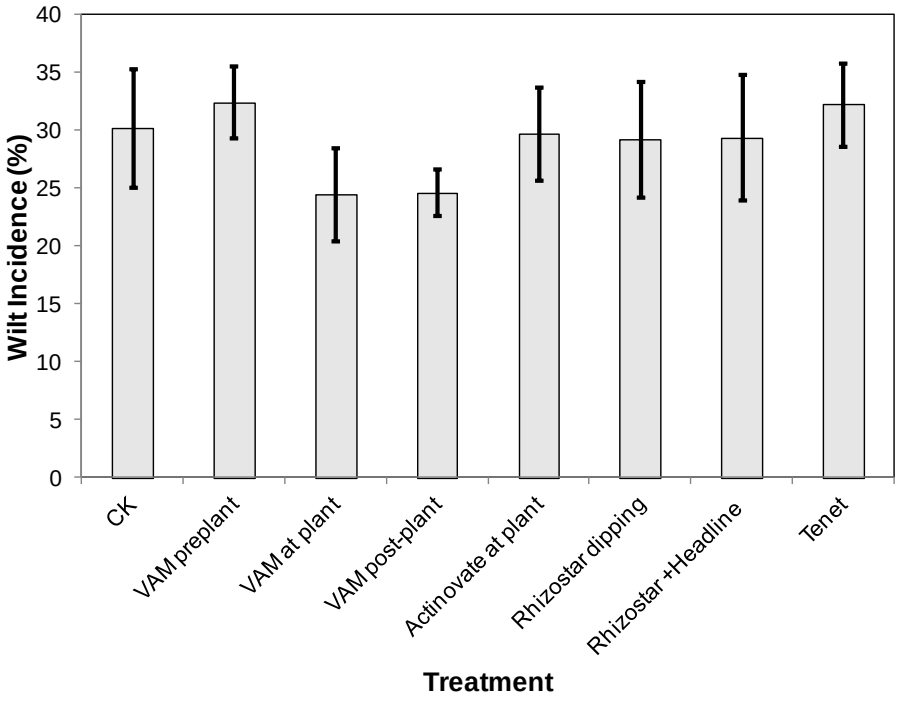

Figure 2. Incidence of Verticillium wilt in peppermint plots with different treatments in a plot trial at COARC, Madras, Oregon, 2011.

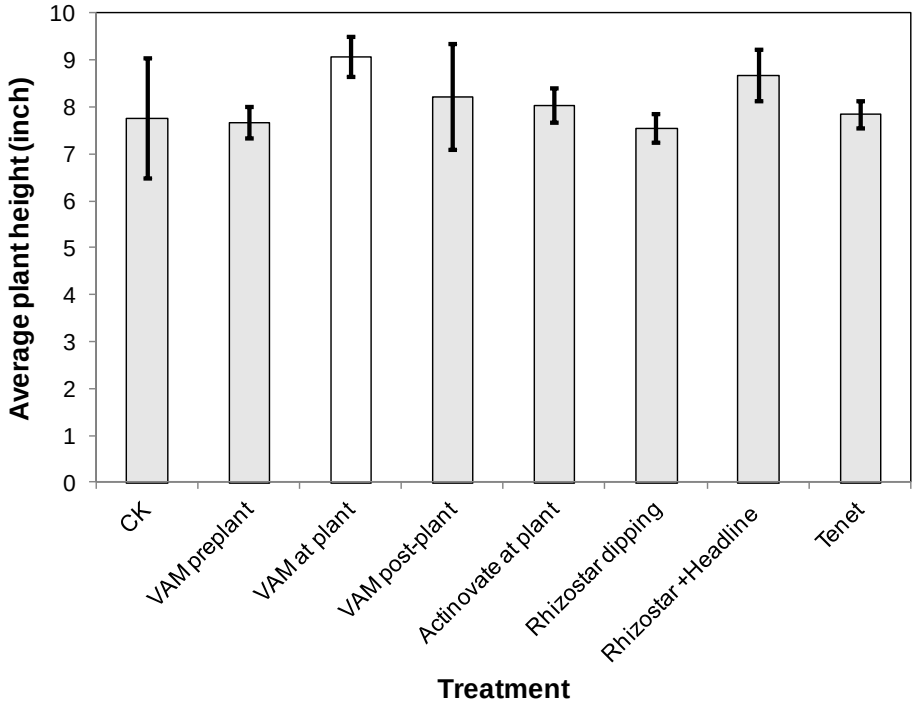

Figure 3. Average heights (inch) of peppermint plants in plots with different treatments in a plot trial at Central Oregon Agricultural Research Center, Madras, Oregon, 2011.

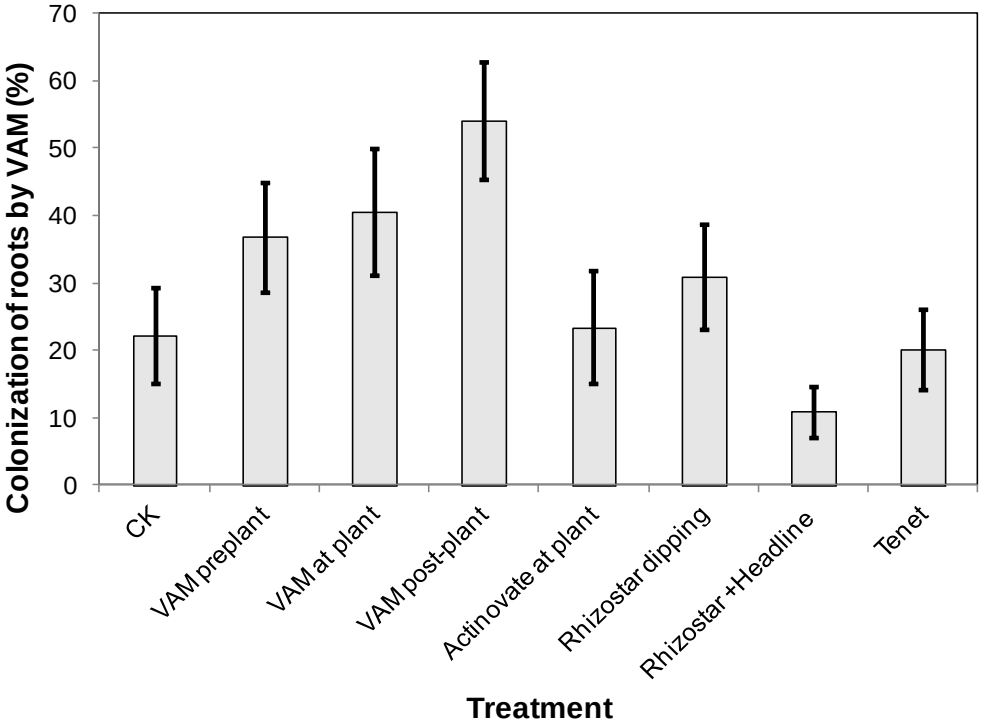

Figure 4. Average fresh weight (gram) of peppermint plants in plots with different treatments in a plot trial at Central Oregon Agricultural Research Center, Madras, Oregon, 2011.

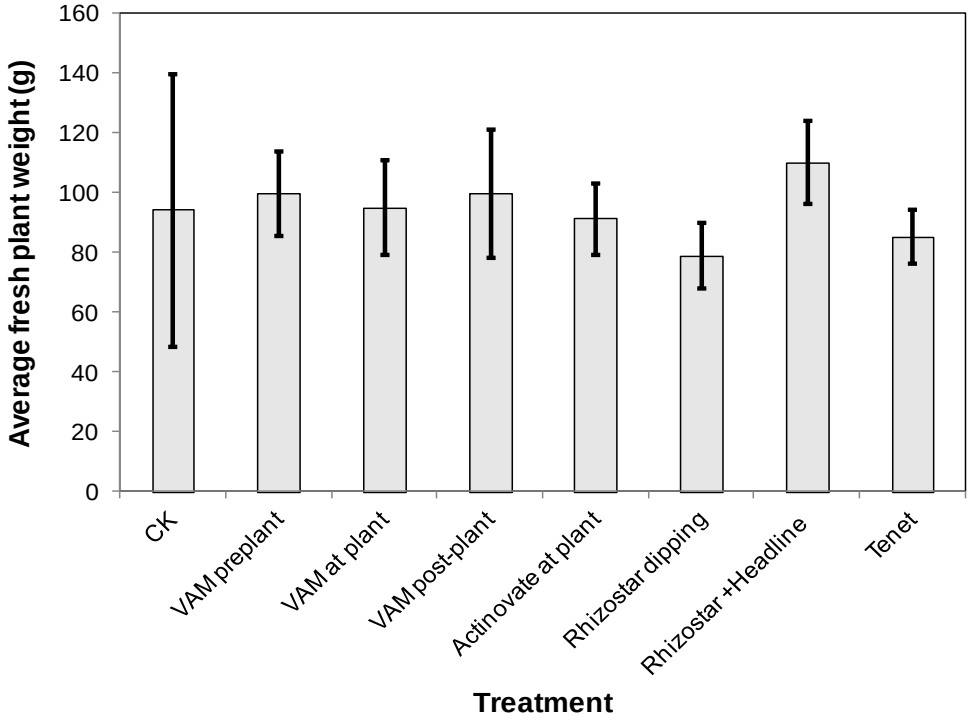

Figure 5. Average percent root length that was colonized by vesicular arbuscular mycorrhizae for peppermint plants subjected to different treatments in a plot trial at Central Oregon Agricultural Research Center, Madras, Oregon, 2011.

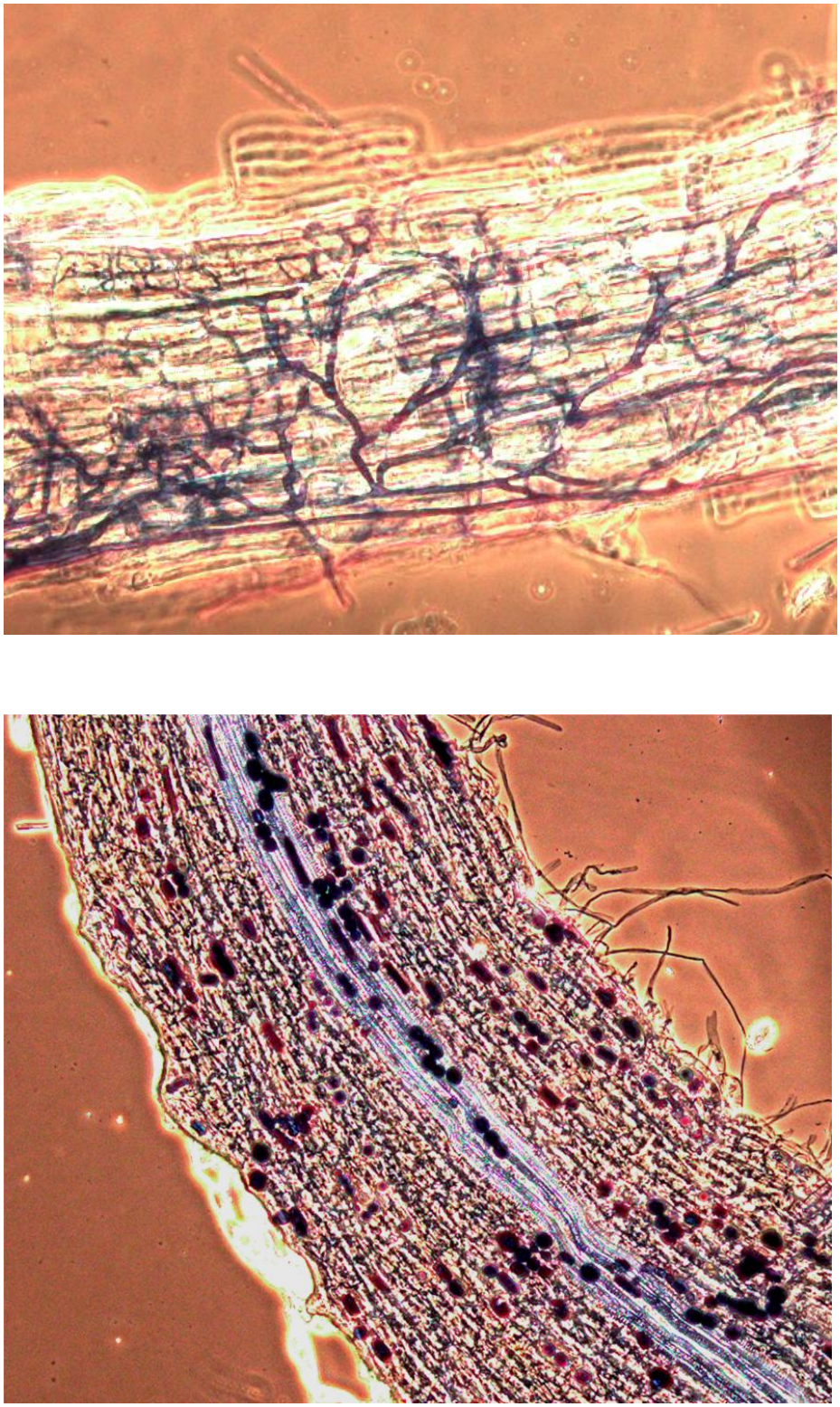

Figure 6. Microscopic photos of peppermint roots colonized by vesicular arbuscular mycorrhizae (VAM) showing spores (top) and mycelium (bottom) of VAM in the root tissues.