Evaluation of Biofumigation with Allyl Isothiocyanate to Reduce Verticillium Wilt in Mint

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Abstract

Verticillium wilt, caused by the fungus *Verticillium dahliae*, is the most important disease of commercial mint production in the Pacific Northwest. Green manure crops with biofumigant properties, specifically those which produce compounds such as allyl isothiocyanate (AITC), can suppress Verticillium wilt. The objective of this research was to determine the efficacy of different rates of the synthetic AITC to reduce *V. dahliae* inoculum and wilt symptoms in peppermint in the greenhouse. A greenhouse bioassay and a growth chamber soil tube assay were conducted to determine the efficacy of AITC treatment to reduce wilt symptoms and reduce inoculum in field soils. Although significant effects of AITC treatment on wilt symptoms were not observed, AITC treatments resulted in lower area under disease progress curve values in Black Mitcham peppermint compared to inoculated, non-treated controls. *Verticillium dahliae* was not recovered from any soils treated with AITC at 10, 20, 30, and 40 gal/acre, suggesting that AITC can reduce *V. dahliae* populations in field soils under laboratory conditions.

Introduction

Verticillium wilt is the major disease impacting mint production in Oregon and the Pacific Northwest. Black Mitcham peppermint and Scotch spearmint are very susceptible to Verticillium wilt while other mints, including native spearmint and some peppermint varieties, exhibit varying degrees of resistance to the disease. Verticillium wilt is caused by *Verticillium dahliae*, a soilborne fungus which has a wide host range and can survive for ten years or more in field soils. Although the fungus can colonize and infect a wide range of crops, a mint pathotype exists which is highly aggressive on mint.

Existing practices to reduce Verticillium wilt in mint include the use of certified, disease-free planting materials, long rotations, propane flaming, and pre-plant soil fumigation. Alternatives to chemical fumigants are needed due to increasing regulatory mandates and restrictions associated with their use, their high economic cost, unforeseen availability in the future, and potential environmental and human health risks. Green manure crops with biofumigant properties, specifically those which produce glucosinolate-derived compounds such as isothiocyanates, can suppress the growth of a broad range of weeds, bacteria, nematodes, and fungi, including *V. dahliae*. However, removing a field from production in order to grow a green manure crop is not always economically feasible. A broad-spectrum biopesticide (Dominus®; Isagro USA, Morrisville, NC) containing 96.3% allyl isothiocyanate (AITC) was recently approved by the U.S. Environmental Protection Agency and offers the potential benefits of green manure crops without taking land out of production. In addition, the product is based on natural materials and is expected to be approved for both conventional and organic farm systems. The objective of this research project was to determine the efficacy of different rates of AITC to reduce *V. dahliae* inoculum and Verticillium wilt symptoms in peppermint in the greenhouse. Two experiments, a greenhouse bioassay and a growth chamber soil tube assay, were conducted

to address this objective. The greenhouse bioassay was used to determine the potential of AITC treatment to reduce Verticillium wilt symptoms. The soil tube assay was performed to quantify the ability of AITC to reduce inoculum in field soils.

Materials and Methods

Greenhouse bioassay

Tissue-cultured plantlets of *Mentha* x *piperita* "Black Mitcham" (susceptible to *V. dahliae*) and *M*. x *piperita* M-83-7 (moderately resistant to *V. dahliae*) were rooted in propagation flats (4 cm wide by 6 cm deep) filled with peat-based potting mix for 5 weeks. Pots (10 cm wide) were filled with potting mix and infested with sand inoculated with *V. dahliae* isolate MT-96-1-4, which was shown to be aggressive on mint in a previous study. Pots were infested with *V. dahliae* at rates of 10 or 20 CFU/cm^3 of potting mix or were left as non-infested controls. Biofumigant treatments (95% AITC at 10, 20 and 40 gal/acre) or sterile distilled water controls were then applied as a drench in a total of 50 ml of water/pot. Pots were kept slightly moist and incubated in the greenhouse. After 3 weeks, rooted plants were transplanted to pots and maintained in the greenhouse. Verticillium wilt was evaluated at the onset of symptoms and approximately weekly thereafter using the following disease severity index (DSI): 0 = no symptoms; 1 = mild (apical) chlorosis or wilt on <10% of the plant; 2 = distinct chlorosis/wilt on 10 to 25% of the plant; 3 = asymmetrical apical growth and/or chlorosis/wilt on 25 to 50% of the plant; 4 = chlorosis/wilt on ≥50% of the plant or severe stunting (<50% the height of non-inoculated control plants); 5 = necrosis of ≥50% of the plant; and 6 = dead/nearly dead plant. Weekly ratings were converted to area under disease progress curve (AUDPC) values. The experiment consisted of four replications and was arranged in a randomized complete block design, with plants blocked by initial size at transplanting. The number of CFUs per plant was determined by extracting sap from stems and plating onto semi-selective NP-10 medium. Dried aboveground plant mass was measured at the completion of the trial. Yield ratios were calculated by dividing the dry aboveground plant mass by the mean of the non-inoculated and non-treated control treatment. A yield ratio < 1 indicates reduced yield relative to the control treatment.

Soil tube assay

Test tubes (2.5 by 25 cm) were filled with field soil collected from central Oregon and autoclaved. Soils were then artificially infested with *V. dahliae*-infested sand at rates of 10, 50, and 100 CFU/g or left non-infested. Infested soils were treated with 95% AITC at a rate of 0, 10, 20, 30, or 40 gal/acre in a total volume of 9 ml of sterile distilled water. Test tubes were capped to simulate tarping and incubated in a growth chamber at 26°C day/10°C night. The number of *V. dahliae* CFU was determined for each soil tube after two weeks of incubation. Soil from each tube was then dried for two weeks and plated onto semi-selective NP-10 medium.

Results and Discussion

Bioassay

Verticillium wilt symptoms were first observed between 4 and 5 weeks after transplanting peppermint into infested and treated soils. Symptoms were relatively mild but AUDPC values were significantly different among cultivars ($P < 0.0001$), with Black Mitcham peppermint exhibiting greater AUDPC values than the moderately resistant M-83-7 variety

(Tables 1 and 2). Although significant effects or interactions of *V. dahliae* inoculum level and AITC treatment on Verticillium wilt symptoms were not observed, non-treated Black Mitcham peppermint plants exposed to *V. dahliae* at either 10 or 20 CFU/cm^3 exhibited greater AUDPC values than all other treatment combinations (Tables 1 and 2). *Verticillium* was not recovered from the stem sap of any plants, indicating low disease pressure overall. Yield ratios were only significantly different among cultivars ($P = 0.0009$) but significant effects or interactions of AITC treatments and *V. dahliae* inoculum levels were not observed ($P > 0.05$).

Soil tube assay

Results from the soil tube assay experiment indicate that AITC is effective at reducing *V. dahliae* populations in field soils under laboratory conditions. *Verticillium dahliae* was not recovered from any soils treated with AITC at 10, 20, 30, and 40 gal/acre, but the pathogen was recovered from soils that were not treated with AITC at levels comparable to or greater than the initial infestation rate (*data not shown*).

Based on the low disease pressure in the initial bioassay but the promising results obtained in the soil tube assay, an additional bioassay was initiated in the growth chamber using higher rates of *V. dahliae* inoculum. Soils were infested with *V. dahliae* at a rate of 60 CFU/g soil and treated with AITC at a rate of 40 gal/acre. Non-infested and non-treated controls are included for comparison. This study is currently in progress.

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Tables

Table 1. Area under disease progress curve (AUDPC) and yield ratio values of Black Mitcham peppermint after treatment with allyl isothiocyanate (AITC) in soils infested with *V. dahliae* at 0, 10, and 20 CFU/cm^3 soil

***V. dahliae* (CFU)**	**AITC (gal/acre)**	**AUDPC**	**Yield ratio[1]**
0	**0**	0.0	1.00
10	**0**	71.8	0.79
20	**0**	82.3	0.78
0	**10**	0.0	0.92
10	**10**	20.1	0.89
20	**10**	44.6	0.83
0	**20**	0.0	0.96
10	**20**	22.8	1.08
20	**20**	28.0	0.86
0	**40**	0.0	0.91
10	**40**	34.1	0.91
20	**40**	30.6	0.92

[1] A yield ratio < 1 indicates reduced yield compared to the non-inoculated control and a yield ratio > 1 indicates a higher yield relative to the non-inoculated control.

Table 2. Area under disease progress curve (AUDPC) and yield ratio values of M-83-7 peppermint after treatment with allyl isothiocyanate (AITC) in soils infested with *V. dahliae* at 0, 10, and 20 CFU/cm^3 soil

***V. dahliae* (CFU)**	**AITC (gal/acre)**	**AUDPC**	**Yield ratio[1]**
0	**0**	0.0	1.00
10	**0**	6.1	0.88
20	**0**	17.5	0.87
0	**10**	0.0	1.07
10	**10**	17.5	1.19
20	**10**	6.1	1.23
0	**20**	0.0	1.23
10	**20**	30.6	0.90
20	**20**	12.3	1.00
0	**40**	0.0	1.16
10	**40**	13.1	1.08
20	**40**	8.8	1.09

[1] A yield ratio < 1 indicates reduced yield compared to the non-inoculated control and a yield ratio > 1 indicates a higher yield relative to the non-inoculated control.