The Effect of *Trichoderma harzianum* and Arbuscular Mycorrhizae on Fusarium Root Rot in Asparagus

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SUMMARY. Commercially available biocontrol agents *Trichoderma harzianum* Rifai and the arbuscular mycorrhizal fungus *Glomus intraradices* Schenck and Smith were tested for their efficacy in controlling fusarium root rot in potted asparagus (*Asparagus officinalis* L.) seedlings. High and low concentrations of *Fusarium oxysporum* (Schlect.) emend. Snyd. & Hans. f. sp. *asparagi* Cohen & Heald (FOA) were combined with *G. intraradices* and / or *T. harzianum* treatments. In both experiments included in this study, *T. harzianum* and *G. intraradices* alone and in combination effectively reduced root rot caused by FOA when asparagus seedlings were grown in low levels of FOA-infested medium. When seedlings were grown in high levels of FOA-infested medium, the combination of *T. harzianum* + *G. intraradices* significantly increased dry shoot mass and limited root rot compared to the control.

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In Michigan, crown and root rot of asparagus (*Asparagus officinalis*) are caused by *Fusarium oxysporum* f. sp. *asparagi* (FOA) and *F. proliferatum* (T. Matsushima) Nirenberg. Damping-off of seedlings in crown nurseries, poor plant stands in newly established fields, and a slow decline of productivity in mature fields are typically attributed to FOA. *Fusarium oxysporum* f. sp. *asparagi* is ubiquitous in Michigan and may be found in soil with no history of asparagus culture (Hartung and Stephens, 1983). Cultural strategies to reduce fusarium crown and root rot include maintaining a pH of 7.0 to 7.5 (Hodupp, 1983), controlling weeds and insects to promote a vigorous crown and root system (Damicone and Manning, 1987), and reduced or no tillage (Putnam and Lacy, 1977). Currently, chemical-based strategies to manage fusarium crown and root rot are not recommended in Michigan (Lacy, 1979).

Inoculation with arbuscular mycorrhizal (AM) fungi decreases disease incidence caused by *Fusarium* sp. in asparagus (Wacker et al., 1990), tomato (*Lycopersicon esculentum* Mill.) (Caron et al., 1986; Datnoff et al., 1995), and potato (*Solanum tuberosum* L.) (Niemira et al., 1996). A commercially available peat mix containing propagules of the AM fungus *Glomus intraradices* effectively colonizes asparagus (Pederson et al., 1991). Soilborne AM fungi form mutualistic associations with many plants in native and agricultural ecosystems including asparagus (Bagyaraj, 1984; Gerdemann, 1967; Linderman, 1994; Safir, 1994; Wacker et al., 1990), and benefit their hosts primarily by facilitating increased nutrient (especially phosphorus) uptake (Gerdemann, 1967) and conferring disease resistance (Caron et al., 1986; Datnoff et al., 1995; Linderman, 1994; Niemira et al., 1996; Wacker et al., 1990).

Trichoderma harzianum is a naturally occurring soil fungus (Chet, 1987). When used as a biocontrol agent, *T. harzianum* controls *Fusarium* sp. in a number of crops, including tomato, cotton (*Gossypium barbardense* L.), muskmelon (*Cucumis melo* L.), and wheat (*Triticum aestivum* L.) (Datnoff

et al., 1995; Sivan, 1987; Sivan and Chet, 1986). A commercially produced form of *T. harzianum* strain KRL-AG2 (T-220) (Bioworks Inc., Geneva, N.Y.) is available.

The objective of this research was to determine the potential of commercially available formulations of *T. harzianum* and *G. intraradices* in reducing crown and root rot on asparagus seedlings.

Materials and methods

A virulent FOA isolate previously cultured from Michigan soils was grown on millet using the protocol of Wacker et al. (1990). The FOA-infested millet was incorporated into peat at high or low [1.00 $g \cdot L^{-1}$ or 0.05 $g \cdot L^{-1}$ (0.134 or 0.007 oz / gal)] rates providing 1×10^6 and 2×10^4 colony-forming units (cfu) / L peat, respectively, verified by plating aliquots of serial dilutions of the FOA-infested millet in distilled water onto Komada's medium (Komada, 1975).

A commercial granular clay preparation of *T. harzianum* strain KRL-AG2 (T-220) (Bioworks, Inc. Geneva, N.Y.) containing $\approx 10^6$ cfu / g was applied to treatments at the rate of 0.25 g of *T. harzianum* per 250-mL (0.5-pt) pot. For the AM treatments, the FOA millet inoculum was incorporated into the commercial mycopeat mix (MycoRise) from Premier Tech Ltd. (Montreal, Que., Canada) containing *G. intraradices* at ≈2 propagules / g peat. Mycorrhizal spore and propagule concentrations were verified routinely using a centrifugation flotation procedure (Caveness and Jensen, 1955; Elias and Safir, 1987). Peat mix (Premier Tech, Montreal, Que., Canada) without *T. harzianum* and / or *G. intraridices* was used for the controls.

Seeds of 'Martha Washington' asparagus (Abbot and Cobb, Feasterville, Pa.) were surface sterilized according to the protocol of Damicone et al. (1981), and germinated on filter paper (no. 1, Whatman International Ltd., Maidstone, England) overlaying 10% water agar. Two germinated seeds were sown into 250-mL pots containing media infested with low or high levels of FOA with the following treatments: unamended, *T. harzianum*, *G. intraradices*, and *T. harzianum* + *G. intraradices*. Germlings were also planted in peat (Premier Tech Ltd., Montreal, Que., Canada) uninfested by FOA as a control. Treatments and control were placed in a 25 °C (77 °F) growth chamber (Sherer, Marshall, Mich.) with a 12 h light / 12 h dark cycle and watered with distilled water as needed. Seven days after sowing, seedlings were thinned to one plant per pot. This study was arranged in a randomized complete block design with five blocks and two replications per block for a total of 10 plants per treatment. This experiment was repeated.

Plant death was monitored daily. Root rot was assessed 38 d after planting based on the percentage root area exhibiting lesions or discoloration (reddening) as follows: 1 = 0% to 10%, 2 = 11% to 20%, 3 = 21% to 30%, 4 = 31% to 40%, and 5 = more than 40% (Wacker et al., 1990). Dry mass was measured on shoots harvested when root rot was assessed, that had been dried for 5 d at 30 °C (86 °F).

To confirm the presence of *T. harzianum*, root segments from five plants were chosen arbitrarily from each *T. harzianum* treatment and plated onto *Trichoderma*-selective medium (Elad et al., 1981). Root colonization by *G. intraradices* was determined using the method of Phillips and Hayman (1970), with the following modifications: the roots were washed, cleared, and stained with trypan blue. About 1/3 to 1/2 of each plant's root system was cut into 0.5- to 1.0-cm (0.2- to 0.4-inch) segments, placed in a petri plate, and suspended in a lactoglycerol destaining solution. Percentage of *G. intraradices* root colonization was determined using the gridline intersect method (Kormanik and McGraw, 1982), whereby root segments were placed on the stage of a Wild stereo dissecting microscope (Heerbrugg, Switzerland) overlaid with a t-grid and viewed at ×250 magnification. The total number of root segments which came in contact with the grid were counted, as were the number of root segments with vesicles and associated hyphae indicating *G. intraradices* colonization. The ratio of colonized root segments to total root segments was used to determine the percentage of *G. intraradices* root colonization.

All data were subjected to an analysis of variance (ANOVA) using SigmaStat for Windows 1.0 (Jandel Corp., San Rafael, Calif.).

Results

LOW FOA. FOA-inoculated control plants had an average root rot rating of 4.6 (≈31% to 40% of roots affected) and 2.6 (≈11% to 20% of roots affected) for Expts. 1 and 2, respectively (Table 1). In both experiments, all treatments amended with *G. intraradices* and / or *T. harzianum* had significantly less root rot than the inoculated control, with a maximum root rot rating of 2.9 (≈11% to 20% of roots affected) in Expt. 1 and 1.3 (≈0% to 10% of roots affected) in Expt. 2. Treatments did not differ significantly from each other in either experiment. Dry shoot mass did not differ significantly between the inoculated control and

Table 1. Dry shoot mass (g) and root rot rating when asparagus seedlings were grown in media infested with low or high levels of *Fusarium oxysporum* f. sp. *asparagi* (FOA) and treated with *Trichoderma harzianum*, *Glomus intraradices*, *T. harzianum* + *G. intraradices*, or not treated (infested control).

	Low FOA				High FOA			
	Dry shoot mass (g)[z]		Root rot rating[y]		Dry shoot mass (g)		Root rot rating	
Treatment	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
FOA-infested control	0.09 ab[x]	0.04 a	4.6 a	2.6 a	0.02 a	0.02 a	5.0 a	3.6 a
T. harzianum	0.06 b	0.06 a	2.9 b	1.1 b	0.05 b	0.05 b	3.7 b	1.8 ab
G. intraradices	0.08 ab	0.06 a	1.8 b	1.3 b	0.04 b	0.06 b	3.7 b	2.0 ab
T. harzianum + *G. intraradices*	0.11 a	0.05 a	1.6 b	0.9 b	0.07 b	0.06 b	3.0 b	1.3 b

[z]28.35 g = 1.0 oz.
[y]Root rot rating based on the percentage of roots exhibiting lesions as follows: 1 = 0% to 10%, 2 = 11% to 20%, 3 = 21% to 30%, 4 = 31% to 40%, and 5 = >40%.
[x]Numbers in a column with a letter in common are not significantly different (ANOVA; $P = 0.05$).

the treatments in either experiment (Table 1).

HIGH FOA. Inoculated control plants had an average root rot rating of 5.0 (>40% of roots affected) and 3.6 (≈21% to 30% of roots affected) for Expts. 1 and 2, respectively (Table 1). In Expt. 1, root rot ratings differed significantly between the inoculated control and all treatments. In Expt. 2, the *T. harzianum* + *G. intraradices* treatment limited root rot to a mean rating of 1.3 (≈0% to 10% of roots affected), which was significantly less than the inoculated control. Dry shoot mass was significantly greater for all treatments compared with the inoculated control in both experiments.

All AM-inoculated plants were colonized by *G. intraradices*. Average colonization of plants by *G. intraradices* was 55.3% (control), 50.2% (low FOA), or 49.6% (high FOA) for the treatment in which *G. intraradices* was amended. For the *T. harzianum* + *G. intraradices* treatments, average colonization of plants by *G. intraradices* was 35.4% (low FOA) or 39.0% (high FOA). *Trichoderma harzianum* was isolated from all sampled roots.

Discussion

Biological control of fusarium crown and root rot has been investigated and improved the growth of asparagus transplants in fusarium-infested soil. Studies demonstrating long-term disease suppression have not been attempted. In both experiments included in this study, *T. harzianum* and *G. intraradices* alone and in combination effectively reduced root rot caused by FOA when asparagus seedlings were grown in low levels of FOA-infested medium. When seedlings were grown in high levels of FOA-infested medium, the combination of *T. harzianum* + *G. intraradices* significantly increased dry shoot mass and limited root rot compared to the control.

Our results confirm observations by Wacker et al. (1990) in which inoculation of asparagus with AM fungi had significantly lower disease incidence in the greenhouse and in the field. Datnoff et al. (1995) found that both *T. harzianum* and AM fungal inoculum reduced crown and root rot disease (determined by percent of plants having necrosis of the stem and root) caused by *F. oxysporum* f. sp. *radicis-lycopersici* in tomatoes. Similarly, Sivan and Chet (1986) found that application of *T. harzianum* reduced disease caused by *Fusarium* sp. in cotton, wheat and muskmelon.

In our study, a decrease in AM root colonization was found in plants also inoculated with *T. harzianum*. However, there were no significant changes in disease incidence associated with inoculation of both biocontrol agents compared with single inoculations. Rousseau et al. (1996) observed *T. harzianum* parasitizing the AM fungus *G. intraradices* in vitro which suggests that these fungi could act as antagonists when coinoculated. McAllister et al. (1994) also found a reduction in AM root colonization when corn was inoculated with AM fungi simultaneously with *T. harzianum*, but not when *T. harzianum* was applied 2 weeks following AM fungal inoculations. More research is needed to determine whether timing of inoculations of *T. harzianum* and AM fungi can avoid potential antagonism between these biocontrol organisms.

The investigation of biocontrol treatments is particularly important to the asparagus industry because chemical controls have been found to be largely ineffective in controlling fusarium root rot (Elmer, 1992; Lacy, 1979). The results of this study suggest that the commercially available forms of *T. harzianum* and the AM fungus *G. intraradices* may have potential as biocontrol treatments of FOA in the field. The low concentration of FOA (2.5×10^6 cfu / g soil) in this study was similar to the highest reported concentration of *Fusarium* sp. occurring naturally in Michigan soils (Hartung et al., 1990). If the decrease in root rot and increase in shoot production that we have observed is maintained in the field, the application of these biocontrol products may be helpful in disease management.

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