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

Laura L. Arriola,1 Mary K. Hausbeck,2 John Rogers,3 and Gene R. Safir4

ADDITIONAL INDEX WORDS. Asparagus officinalis, Fusarium oxysporum f. sp. asparagi, Glomus intraradices

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 (Schlecht.) 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.

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 (Bagyara, 1984; Gerderman, 1967; Linderman, 1994; Safir, 1994; Wacker et al., 1990), and benefit their hosts primarily by facilitating increased nutrient (especially phosphorus) uptake (Gerderman, 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, muskmelon (Cucumis melo L.), and wheat (Triticum aestivum L.) (Datnoff...

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 L⁻¹ or 0.05 g L⁻¹ (0.134 or 0.007 oz/gal)] rates providing 1×10⁴ and 2×10⁴ colony-forming units (cfu)/L peat, respectively. Fifty percent of the FOA-infested millet was incorporated into peat (Premier Tech Ltd., Montreal, Que., Canada) uninfested by FOA as a control. Treatments and controls were placed in a 25 °C (77 °F) growth chamber (Sherer, M. arshall, 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 lactic acid destaining solution. Percentage of G. intraradices root colonization was determined using the gridline intersect method (Kornamik and M cGraw, 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 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. Treatment 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).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Low FOA</th>
<th>High FOA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry shoot mass (g)</td>
<td>Root rot rating</td>
</tr>
<tr>
<td></td>
<td>Expt. 1 Expt. 2</td>
<td>Expt. 1 Expt. 2</td>
</tr>
<tr>
<td>FOA-infested control</td>
<td>0.09 ab* 0.04 a</td>
<td>4.6 a 2.6 a</td>
</tr>
<tr>
<td>T. harzianum</td>
<td>0.06 b 0.06 a</td>
<td>2.9 b 1.1 b</td>
</tr>
<tr>
<td>G. intraradices</td>
<td>0.08 ab 0.06 a</td>
<td>1.8 b 1.3 b</td>
</tr>
<tr>
<td>T. harzianum + G. intraradices</td>
<td>0.11 a 0.05 a</td>
<td>1.6 b 0.9 b</td>
</tr>
</tbody>
</table>

28.35 g = 1 oz.

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%.

A number 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). Tricho- 
derma 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 each 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 plant 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 that T. harzianum parasitizing the AM fungus G. intraradices in vitro which suggests that these fungi could act as antagonists when coinculated. McCaullister 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 x 10⁶ cfu/g soil) in this study was similar to the highest reported concentrations of Fusarium sp. occurring naturally in M. ichigan 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.

**Literature cited**


Scheneck (ed.). Methods and principles of mycorrhizal research. APS Press, St. Paul, Minn.


