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Efficacy of *Trichoderma harzianum* as a biological control of *Fusarium oxysporum* in container-grown Douglas-fir seedlings

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Application. Growth of Douglas-fir seedlings successfully inoculated with *Trichoderma harzianum* was unaffected. Under seedling production conditions, root colonization by resident *Fusarium oxysporum* was reduced by *T. harzianum* inoculations, suggesting that *Trichoderma* may afford some protection to seedling crops.

Abstract. Inoculating a soilless medium with encapsulated *Trichoderma harzianum* did not affect any aspect of Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) seed germination or subsequent growth. Results of inoculating medium with a known pathogenic isolate of *Fusarium oxysporum* alone, or concurrently with *T. harzianum*, were the same: high levels of damping-off, low amounts of hypocotyl and root disease in midsummer, and significant reductions in height growth. When seedling roots grew through *T. harzianum*-inoculated medium before growing into a mixture of *T. harzianum-F. oxysporum*-inoculated medium, mortality was reduced about 50%. Although contamination by resident *Fusarium* occurred, subsequent root colonization was significantly reduced in *T. harzianum*-amended growing medium.

Introduction

Several *Fusarium* spp. damage Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) seedlings in nurseries (James et al. 1987, 1989, 1991). In particular, *F. oxysporum* Schlecht. causes damage through seed decay, hypocotyl rot, cotyledon blight, root disease, and damping-off (Bloomberg 1973; Brownell and Schneider 1983; Hansen et al. 1990). The traditional method of reducing *Fusarium*-caused diseases is repeated fungicide use (Landis et al. 1989), although on older seedlings, prevention and reduction of disease with fungicides are often unsuccessful (James et al. 1987; Dumroese et al. 1990). Fungicides can pose an immediate and/or long-term threat

to human health, water quality, and the overall environment (USDA 1987a, b). It is likely that future litigation and legislation will prohibit or restrict use of many fungicides. Successful biological control of *Fusarium*-caused disease would reduce nursery managers' reliance on chemical controls (James et al. 1993).

Particular *Trichoderma* strains were shown to protect seedlings from damping-off, root infection by pathogens, and enhance plant growth (Harman and Taylor 1990). The soil saprophyte *T. harzianum* Rifai effectively reduces populations of *F. oxysporum* and other soilborne fungal pathogens in many agricultural crops (Elad et al. 1982; Papavizas 1985; Campbell 1989). Damping-off caused by *Rhizoctonia solani* Kuhn was suppressed by *T. harzianum* on slash pine (*Pinus elliottii* Engelm. var. *elliottii*) (Huang and Kuhlman 1991). Because disease control alternatives to chemical fungicides are a high priority for nursery managers, and *T. harzianum* has efficacy on several agricultural and forest seedling crops, we examined the effectiveness of *T. harzianum* to reduce *Fusarium*-caused disease in container-grown Douglas-fir seedlings, along with its effects on seedling growth.

Materials and methods

Our *T. harzianum* isolate was obtained from Palouse silt loam soil on the University of Idaho Plant Science Farm in Moscow, Idaho. The isolate was multiplied, mixed with wheat bran, and added to aqueous $CaCl_2$ for encapsulation into polymerized alginate pellets following methods of Fravel et al. (1985) as modified by Knudsen and Bin (1990). Alginate-bran pellets lacking *Trichoderma* mycelium were used as a control.

Before planting on April 1, 1.7 kg of autoclaved growing medium (1:1 peat:vermiculite; W.R. Grace and Co., Portland, OR) (40% moisture content) was mixed with the appropriate treatment for each container tray (Table 1). We used the most aggressive *F. oxysporum* strain (43B) from an earlier pathogenicity test (Mousseaux 1992), grown on a mixture of potato dextrose agar (PDA), cornmeal and perlite, to inoculate the growing medium following methods of Miles and Wilcoxson (1984) as modified by James et al. (1989). An uninoculated cornmeal-perlite mixture served as control.

In new Ray Leach® trays (200 removable cells; 20 cells long × 10 cells wide), we filled 119 cells (2.5 cm wide × 16.5 cm deep; 66 ml) in the center of each tray (17 cells long × 7 cells wide) with one of seven treatments (Table 1). The 81 surrounding, untreated cells were filled with peat:vermiculite and sown with Douglas-fir to act as a buffer against intertray contamination. Each treatment was replicated three times. Six columns and rows of buffer trees were between each treatment section (three of each on

Table 1. Treatments and descriptions.

Treatment	Description
Tricho	*Trichoderma* inoculated pellets mixed with growing medium at 1:100 (dry) w/w basis.
Fus	*Fusarium* inoculated perlite beads mixed with growing medium at a 1:50 (dry) w/w basis.
Control	Control medium: no inoculum, alginate/bran or perlite.
Tricho-Fus	*Trichoderma* pellets and *Fusarium* beads mixed together with growing medium at 1:100 and 1:50 (dry) w/w basis, respectively.
Tricho-Fus Control	*Trichoderma* and *Fusarium* control containing non-inoculated perlite beads and non-inoculated alginate-bran pellets mixed with growing medium at 1:100 and 1:50 (dry) w/w basis, respectively.
Tricho over Tricho-Fus	*Trichoderma* and *Trichoderma* / *Fusarium* mix with the upper 50% of the tube containing *Trichoderma* pellets with growing medium at 1:100 (dry) w/w basis and *Trichoderma* pellets and *Fusarium* beads mixed with growing medium at 1:100 and 1:50 (dry) w/w basis, respectively, in the lower 50% of the tube.
Tricho over Tricho-Fus Control	*Trichoderma* and *Trichoderma* / *Fusarium* control with non-inoculated alginate-bran pellets with growing medium at 1:100 (dry) w/w basis in the upper 50% of the tube and non-inoculated alginate-bran pellets and perlite beads mixed with growing medium at 1:100 and 1:50 (dry) w/w basis, respectively, in the lower 50% of the tube.

each treatment tray). The most susceptible Douglas-fir seedlot (Bovill 3) from an earlier pathogenicity test (Mousseaux 1992) was treated 10 min with a 40% household bleach (5.25% aqueous sodium hypochlorite) solution to reduce seedborne fungi (Wenny and Dumroese 1987), rinsed in running water 48 h, cold-moist stratified 21 days at 5 °C, rinsed another 24 h in running water, and surface-dried. Two seeds were sown per cell and covered with a 1-cm layer of silica grit. We placed the trays in the University of Idaho Research Nursery, which is also used to produce commercial seedling crops, on a greenhouse bench in a seven (treatments) by three (blocks) randomized complete block design. We grew seedlings using the standard growing regime (Wenny and Dumroese 1992). Fungicides were not applied.

Once a week for one month, we counted seedlings with post-emergence damping-off. After one month, all ungerminated seeds were excavated and plated on a selective agar medium for *Fusarium* and closely related species (Komada 1975) to verify presence of *F. oxysporum*. For seeds infected with *F. oxysporum*, we attributed mortality to pre-emergence damping-off. Percent seedling mortality per treatment and block was recorded once per month thereafter for duration of the experiment. As seedlings died, they were removed to reduce incidence of secondary infection among treatments. Test seedlings were consolidated and surrounded by buffer trees to prevent possible edge

effects on growth. At least 50% of seedlings killed in each tray from mid-May to July had ten randomly selected root tips surface sterilized (1 min soak in 40% bleach, 10 sec soak in 70% ethanol, two sterile water rinses) and plated (five tips each on Komada's and PDA) to verify presence of *F. oxysporum* and *T. harzianum* and to isolate any other fungi associated with mortality. We replaced dead or diseased buffer trees to reduce contamination between treatments. *Trichoderma* and *Fusarium* were identified to species level using a key by Rifai (1969), and manuals by Barnett and Hunter (1972) and Nelson et al. (1983).

In November, we randomly sampled 20 seedlings from each treatment and block (420 seedlings total) and estimated *Fusarium* and *Trichoderma* population densities in the growing medium, expressed as average colony forming units (cfu). A composite 10 g growing medium sample (dry weight basis) was collected from around the 20 root systems, followed by a 10^{-1} – 10^{-5} standard soil dilution series and plating on Komada's medium and 2% malt agar (Johnson et al. 1959). Roots were gently washed to remove adhering medium particles and volumes determined using Burdett's (1979) water displacement technique. We measured shoot height, root collar diameter (RCD), and after drying 24 h at 70 °C, oven-dry shoot and root weights. Ten root tips from each of six additional seedlings lacking above ground disease symptoms (non-diseased) from each treatment were plated on Komada's medium and PDA to determine *Fusarium* and *Trichoderma* colonization rates among non-diseased seedlings.

Statistics

Mean treatment height, RCD, root volume, dry shoot and root weights, and growing medium propagule levels were transformed by natural log to satisfy the homogeneity of variance assumption of the analysis of variance procedure (Kirk 1982). Mortality and infection percentages for treatments and blocks were transformed with $2 \cdot \sqrt{Y + 1}$ to satisfy the homogeneity of variance assumption of the analysis of variance procedure (Bartlett 1947). We tested for treatment and block differences using analysis of variance, a standard F-test procedure, and a pairwise comparison of means using Tukey's Honestly Significant Difference (HSD) procedure (Ott 1988) at $p = 0.05$.

Results and discussion

The disease threshold of conifer seedlings in nurseries, defined in terms of inoculum levels, host susceptibility, and biotic and abiotic factors pertaining to growth, survival and reproduction of the pathogen or its isolates, including

antagonism by other fungi and bacteria, is poorly understood. This hampers experimentation to elucidate epidemiology of Fusarium root disease. In this study, the problem with disease threshold was compounded since *Fusarium* and *Trichoderma* spp. are ubiquitous fungi in container nurseries, and may be disseminated throughout the growing area by water splash, air movement, insects, and workers, as well as arriving within the growing medium or on seeds (James et al. 1991, 1994). Although we used new containers, autoclaved growing medium, and surface sterilized seeds, growing test seedlings within an operational nursery allowed *Fusarium* and *Trichoderma* spp. to colonize seedling roots in all treatments. Because high levels of *T. aureoviride* Rifai, *T. pseudokoningii* Rifai and *T. harzianum* were isolated from growing medium ($16.6 \times 10^4 - 180 \times 10^4$ cfu/g) and from roots of dead and non-diseased seedlings, we could not determine if the *T. harzianum* subsequently isolated originated in our pellets or was a chance contaminant. For the remainder of this discussion, all *Trichoderma* spp. are subsequently tabulated together. However, we believe our *F. oxysporum* isolate and pelletized *T. harzianum* inoculated into autoclaved growing medium, in the absence of immediate competition, were the primary organisms involved in this study.

Inoculating growing medium with *T. harzianum* and *F. oxysporum* together (Tricho-Fus) failed to protect seedlings against *Fusarium*-caused disease, especially when seedlings were most susceptible to damping-off. This contrasts work that found incorporating *T. harzianum* and *Phytopthora cinnamomi* Rands simultaneously provided the most effective suppression of the pathogen on shortleaf pine (*Pinus echinata* Mill.) (Kelley 1976). Damping-off in Fus and Tricho-Fus was mostly pre-emergent (69%) compared with post-emergent (31%), and was significantly higher than all other treatments (Table 2). Damped-off seeds from all treatments were collected and plated, and all were infected by *F. oxysporum*. Average *Trichoderma* spp. colonization of damped-off seed was 7% for Fus and 6% for Tricho-Fus (data not shown). Many surviving Tricho-Fus and Fus seedlings had poor root development (less branching, more necrosis, and high levels of root tip *Fusarium* infection). Cumulative mortality was also highest in Fus and Tricho-Fus (Table 2).

Fusarium inoculum in container nurseries is usually concentrated at the bottom of containers (James et al. 1988). We simulated this condition by adding *T. harzianum* to the upper 50% of seedling containers, with *T. harzianum* and *F. oxysporum* mixed in the lower 50% (Tricho over Tricho-Fus). This combination also failed to provide complete protection against *Fusarium*-caused disease throughout the growing season, although damping-off levels were similar to Tricho and Control. Mortality was lower than Fus, but it is unclear if this was afforded by *Trichoderma* or if developing roots

Table 2. Damping-off, cumulative mortality and mean morphological measures of surviving Douglas-fir seedlings by treatment, and root colonization by *Fusarium* on non-diseased seedlings. Morphology values are the mean measure of 60 seedlings per treatment, and colonization percentages are for 60 root tips per treatment.

Treatment[1]	Damping-off (%)	Cumulative mortality (%)	Root colonization by *Fusarium* (%)	Height (cm)	RCD (mm)	Root volume (cm^3)	Root weight (g)	Shoot weight (g)
Tricho	0 a[2]	6 a	23 a	17.6 a	2.21 a	4.0 a	0.60 a	0.72 a
Fus	48 b	70 c	85 b	13.7 b	2.14 a	3.6 ab	0.55 ab	0.57 cd
Control	1 a	5 a	58 b	17.5 a	2.06 ab	3.6 ab	0.57 ab	0.69 ab
Tricho-Fus	67 b	80 c	95 b	14.1 b	2.17 a	3.1 bcd	0.56 ab	0.60 bc
Tricho-Fus Control	1 a	31 b	93 b	14.0 b	2.03 ab	3.3 bc	0.59 a	0.56 cd
Tricho over Tricho-Fus	3 a	34 b	87 b	14.6 b	1.88 bc	2.8 cd	0.51 ab	0.55 cd
Tricho over Tricho-Fus Control	3 a	39 b	72 b	13.3 b	1.80 c	2.5 d	0.47 b	0.49 d

[1] See Table 1 for treatment descriptions.
[2] Means with different letters are significantly different at p = 0.05 using Tukey's HSD.

were not exposed to *F. oxysporum* inoculum until older and less susceptible; both *Fusarium* and *Trichoderma* spp. were isolated at similarly high levels. Compared with other *F. oxysporum*-inoculated treatments, total mortality in Tricho over Tricho-Fus was reduced nearly 50% (Table 2).

Adding *T. harzianum* to growing medium (Tricho) did not affect seed germination; mean total germination among treatments was similar at 93% (data not shown). In Tricho, root colonization by contaminating *Fusarium* spp., mostly *F. oxysporum* and rarely *F. tricinctum* (Corda) Sacc., was significantly reduced when compared to Control (Table 2). Cumulative mortality in Tricho was similar to Control; both had the lowest total mean mortality (Table 2). Largest heights and dry shoot weights were in Control and Tricho (Table 2). This contrasts research that *T. harzianum* added to autoclaved soil significantly improved germination and dry weight of roots and shoots of tobacco and tomato (Windham et al. 1986) and pea (Dandurand and Knudsen 1993) seedlings.

Inoculating *F. oxysporum* 43B at 1:50 (w/w) was based on earlier studies (James and Gilligan 1984; James et al. 1989). While our goal was to exceed the threshold and induce disease, we may have greatly exceeded the threshold, resulting in high mortality rates, extensive root colonization of non-diseased seedlings, and high populations of *F. oxysporum* detected within the growing medium ($3.3 \times 10^4 - 10.0 \times 10^4$ cfu/g). High *F. oxysporum* levels may have masked any possible biological control exerted by the inoculated *Trichoderma* strain since *Trichoderma* root tip colonization is dependent on concentration of *F. oxysporum* inoculum; higher *F. oxysporum* levels result in lower colonization by *T. harzianum* (Sivan and Chet 1989).

In container nurseries in the northern Rocky Mountains, we found that by the end of the growth cycle the roots of a large percentage of Douglas-fir seedlings were colonized with *Fusarium*, although this did not necessarily result in disease (James et al. 1987, 1988). Likewise, in this study, colonization and mortality were apparently unrelated as colonization levels were similar for all treatments except Tricho, but mortality varied widely. Without both uninoculated alginate pellet and cornmeal-perlite controls to discern separate effects of the additives, interpretation of these results is difficult. Work with shortleaf pine (Kelley 1976), peas (Campbell 1989), and cucumbers (Wolffhechel and Jensen 1992) indicates *Trichoderma* inoculum preparations with an organic substrate could provide a food source for competing pathogens like *Phytopthora, Pythium*, and *Rhizoctonia*, causing increased disease. Cornmeal used in the *F. oxysporum*-inoculant is an unlikely food source since most is colonized by *Fusarium* prior to inoculation into growing medium, and an earlier pathogenicity study with several *F. oxysporum* isolates and young Douglas-fir seedlings indicated an uninoculated perlite-cornmeal

amendment did not cause seedling mortality (James et al. 1989). Except for Fus and Tricho-Fus, most mortality in perlite-amended treatments (Tricho-Fus Control, Tricho over Tricho-Fus, Tricho over Tricho-Fus Control) occurred in July and August. Perlite amendment was added at low amounts but may have reduced water retention of the growing medium during summer sufficiently to induce stress and increase mortality counts.

Regardless of an organic amendment's role, moderate levels of residential *Trichoderma* spp. were detected in the Control indicating *Trichoderma* could establish on seedling roots without an extra carbohydrate source. Coating melon, tomato, and cotton seeds with *T. harzianum* before sowing effectively inhibits *F. oxysporum* chlamydospore germination in rhizospheres (Sivan and Chet 1989). Instead of an artificial carbohydrate source (bran), *T. harzianum* uses seed and root exudates to proliferate along the developing seedling root and inhibit root pathogens. Two seed-coating methods, solid matrix priming and liquid coating, are effective means of inoculating seeds with *T. harzianum* (Taylor et al. 1994).

Management implications

Little work has been done with biological control of root disease in container nurseries producing conifer seedlings. Concurrently adding *T. harzianum* with a known pathogenic *F. oxysporum* to growing medium at the beginning of the growing season failed to protect Douglas-fir seeds and seedlings from disease. However, when subjected to resident amounts of *Fusarium* spp., additions of *T. harzianum* did significantly reduce subsequent colonization by potentially-pathogenic *Fusarium*. Lower colonization may reduce disease occurrence. When compared with biological controls developed for disease in other agricultural systems but tested on Douglas-fir seedlings, *T. harzianum* had a benign effect on seedling emergence, survival and growth. This contrasts with some deleterious effects we have found associated with *Gliocladium virens* Miller, Giddens & Foster (GL-21) (Dumroese et al. 1996), Mycostop biofungicide (*Streptomyces griseoviridis* L. Anderson (Strain K61)) (James et al., unpublished data) and non-pathogenic *F. oxysporum* (Strain Fo-47) (James et al., unpublished data). Of organisms we have tested thus far for biological control of root diseases in container nurseries (*Gliocladium, Streptomyces, F. oxysporum* and *Trichoderma*), *T. harzianum* seems best suited.

Askew and Laing (1994) found the most aggressive *T. harzianum* isolates for control of *Pythium* resided in soil beneath benches in greenhouses. An evaluation of *T. harzianum* isolates from within container conifer nurseries may yield more aggressive isolates against *Fusarium*. Such isolates, delivered

on seed rather than bran, may yield the level of biological control necessary for satisfactory suppression of *Fusarium* root disease in container nurseries.

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