

Observations on
Root Disease *of*
Container
Whitebark
Pine Seedlings
treated with
Biological
Controls

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ABSTRACT

I observed that whitebark pine (*Pinus albicaulis* Engelm. [Pinaceae]) germinants treated with biological controls, one commercially available (*Trichoderma harzianum* strain T-22), and the other being studied for potential efficacy (*Fusarium oxysporum* isolate Q12), experienced less seedling mortality caused by root disease than did a non-treated control. Seedlings treated with the biological controls and non-symptomatic seedlings in the control treatment had similar morphology. The simple use of biological controls may be useful to nursery managers looking to reduce incidence of root disease in their container crops.

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KEY WORDS

Pinus albicaulis, whitebark pine, *Fusarium oxysporum*, *Trichoderma harzianum*, *Cylindrocarpon*

NOMENCLATURE

USDA NRCS (2008)

Root diseases caused by fungi in the genera *Fusarium* and *Cylindrocarpon* can limit production of conifer seedlings in container nurseries (James and others 1991, 1994). In the Pacific Northwest US, all conifer species are hosts of *Fusarium* (James and others 1991), while *Cylindrocarpon* favors five-needled pines (James and others 1994). In container nurseries, the most prevalent *Fusaria* are grouped within the *Fusarium oxysporum* taxon (species complex), including *F. oxysporum* (Dumroese and James 2005). Root diseases caused by *Cylindrocarpon* are very similar to those caused by pathogens in the *Fusarium oxysporum* taxon—these organisms have similar etiology and prefer similar edaphic conditions (Dumroese and James 2005). These root diseases can reduce seedling quality by compromising the root system (and thereby hampering plantation establishment) or, if severe, cause seedling mortality in the nursery.

Because controlling *Fusarium* root disease with fungicides is usually ineffective (James and others 1988; Dumroese and others 1990a), effective integrated pest management (IPM) strategies that reduce reliance on chemical controls while maintaining seedling production have been suggested (Dumroese and others 1990b; Shrimpton 1992), and the topic thoroughly reviewed (James and others 1990). Recently, more emphasis has been placed on incorporating biological control of *Fusarium* root disease into the IPM toolbox (James and Dumroese 2007).

Whitebark pine (*Pinus albicaulis* Engelm. [Pinaceae]) communities occur at high elevations in the northern Rocky Mountains of the US and adjacent Canada. They are threatened by insects (bark beetles), disease (blister rust), and an imbalance of natural disturbance (fire) (see Tomback and others 2001). Because whitebark pine is a keystone species, providing valuable forage (pine nuts) for endangered grizzly bears, much emphasis is being placed on restoring these communities. Therefore, demand for whitebark pine seedlings is high. Because of the slow growth characteristic of this high-elevation species, whitebark pine seedlings require 2 growing seasons at the nursery before they are large enough for outplanting (Eggleston 2007; Wick and others 2008). During this prolonged production cycle, fungi in the genera *Fusarium* and *Cylindrocarpon* often cause serious root disease problems, with *Cylindrocarpon* the more serious pest during the second season (James 1991, 2004; James and Burr 2000). During the first season, mortality caused primarily by seedborne *Fusarium* can be up to 25% in vigorous seedlots, and two or three times that in poorer quality seedlots (Burr and others 2001).

METHODS AND MATERIALS

One thought for reducing the impact of root disease is to simply shorten the production cycle. In spring 2007 I installed a

controlled-release fertilizer study to examine this. The whitebark pine seeds were collected southwest of Cascade, Idaho, in the vicinity of Snowbank Mountain, along Forest Service Road #446 on the Boise National Forest (Lat 44°24'56"N, Long 116°6'45"W) at an elevation of 2240 m (7350 ft). In this test, I noted increased damping-off in treatments with higher rates of controlled-release fertilizer. As it turned out, because of the long duration of whitebark pine seed germination, I had 191 seeds that germinated after commencement of the controlled-release fertilizer study. I decided to evaluate them under 4 different, potential, biological control scenarios to see if any could mitigate the mortality I was noting.

I decided to evaluate 4 treatments: 1) commercially available PlantShield HC® (BioWorks®, Victor, New York), which contains *Trichoderma harzianum* strain T-22, applied at the label rate (see below); 2) *Fusarium oxysporum* Q12; 3) a combination of PlantShield and Q12; and 4) a non-inoculated control. *Fusarium oxysporum* Q12 is an isolate that we discovered that appears to have good biological control potential activity against pathogenic *Fusarium* in nursery trials (unpublished data; see James and Dumroese 2007).

Fortunately, I also had a pathogenicity test evaluating Q12 underway. I therefore was able to use inoculum from that pathogenicity test in this biological control study. Briefly, the inoculum was made by growing Q12 on potato dextrose agar and then adding it to an autoclaved mixture of cornmeal, potato dextrose agar, and perlite. The inoculated mixture was incubated in the dark at about 24 °C (75 °F) for 24 d and then air-dried on a tabletop for 3 d before storage in plastic bags at about 8 °C (46 °F) until needed. This technique produces true-to-type inoculum that can be stored this way for several months without loss of viability. For complete details, see James and others (1989) who based their technique on that of Miles and Wilcoxson (1984).

Inside a greenhouse at the USDA Forest Service laboratory in Moscow, Idaho (Lat 46°43'24"N, Long 117°0'7"W), I filled enough 164 ml (10 in³) Ray Leach Cone-tainers® (Stuewe and Sons Inc, Corvallis, Oregon) with a 1:1 (v:v) medium of *Sphagnum* peat moss and vermiculite for the PlantShield and control treatments. For the Q12 and PlantShield + Q12 treatments, I used the same medium, but amended it with 1 part inoculum to 50 parts medium on a weight-to-weight (w:w) basis (the medium had a moisture content of 40% ± 5 percentage points) before filling additional Ray Leach Cone-tainers. The 191 germinants were then randomly assigned and carefully transplanted into the 4 treatments (n = 48 germinants per treatment with the exception of n = 47 for the control). Photoperiod was extended to 20 h. About 4 wk after transplanting, germinants in the PlantShield and PlantShield + Q12 treatments were drenched (solution was dripping from the bottom of the containers) with PlantShield at the label rate (0.3 ml/l [4 oz/100 gal]). At this time, all transplanted seedlings lacked foliar symptoms of root disease.

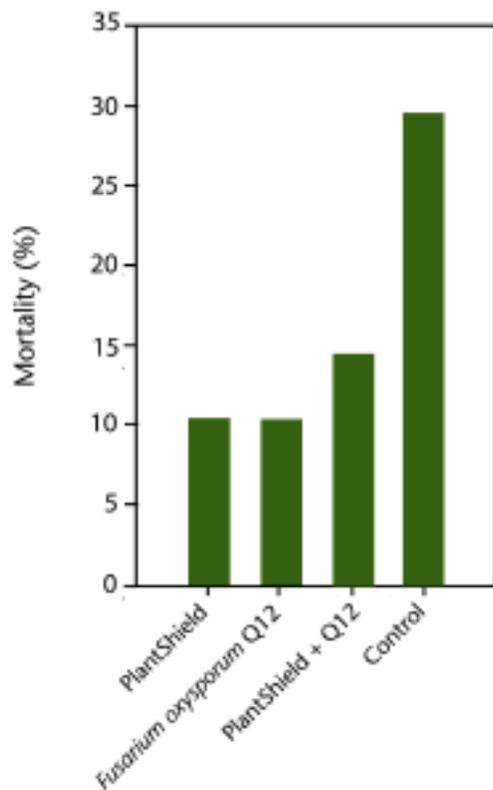


Figure 1. Mortality of whitebark pine seedlings treated with Plant Shield (*Trichoderma harzianum* strain T-22), *Fusarium oxysporum* isolate Q12, a combination of PlantShield and Q12, or left untreated (control).

After drenching, seedling survival was checked weekly. Seedlings that died were collected for analysis following the protocol described in James and others (1989): roots were thoroughly washed, surface sterilized with bleach, plated on a selective agar medium (Komada 1975), and subsequently identified based on morphology according to Nelson and others (1983). Beginning 2 wk after transplanting, seedlings were fertigated (irrigated with a soluble fertilizer containing these parts per million [ppm] of these nutrients: 100 nitrogen [25 NO_3^- and 75 NH_4^+], 50 phosphorus, 170 potassium, 45 calcium, 20 magnesium, 62 sulfur, 20 iron, and 15 STEM® [micronutrients; The Scotts Company, Marysville, Ohio]) about every 3 d for a total of 7 fertigations. Then, the rate of nitrogen was increased to 225 ppm (75 NO_3^- and 150 NH_4^+) and applied once each week for the remainder of the trial (20 fertigations); however, seedlings were irrigated whenever the container weights reached 85% of field capacity weight.

Six months after transplanting, 10 seedlings from each treatment were removed from the containers and their root systems gently cleaned of medium. Seedlings were evaluated

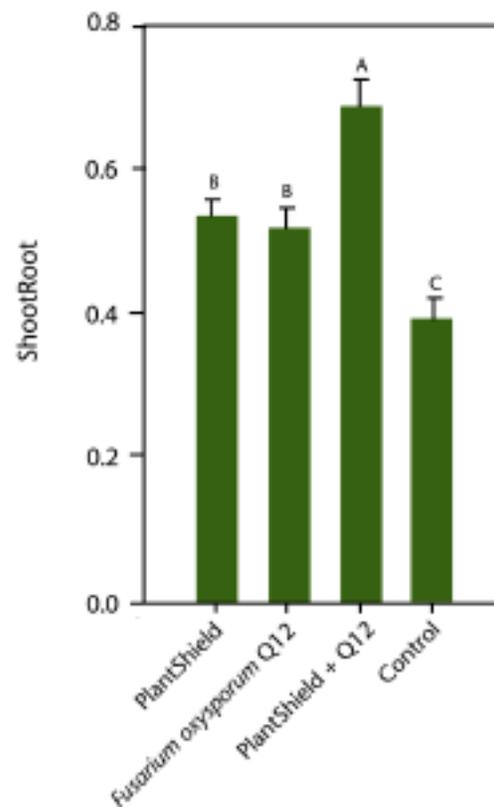


Figure 2. Shoot-to-root ratios of whitebark pine seedlings treated with PlantShield (*Trichoderma harzianum* strain T-22), *Fusarium oxysporum* isolate Q12, a combination of PlantShield and Q12, or left untreated (control). Different letters indicate significant treatment differences at $\alpha = 0.05$.

for height (groundline to tip of most acropetal bud), root-collar diameter, longest needle length, number of fascicles, root volume using water displacement (Burdett 1979), and dry weights of buds, needles, stems (collectively “shoots”), and roots after drying at 60 °C (140 °F) for 72 h.

Analysis of variance (ANOVA) was used to examine the main effect of biological controls on seedling height, root-collar diameter, longest needle length, number of fascicles, root volume, shoot dry weight, and root dry weight using PROC GLM ($\alpha = 0.05$) in SAS version 9 (SAS Inc, Cary, North Carolina). Pair-wise comparisons were made using Bonferroni groupings.

OBSERVATIONS

Amending the medium with any of the biological control agents increased survival compared with the non-inoculated control (Figure 1). The PlantShield, Q12, and PlantShield + Q12 combination yielded similar survival rates (between 85 and 90%), whereas survival was only 70% in the control.

Nearly all of the mortality occurred early in the growing cycle. Assays of dead seedlings revealed infection by fungi within the *Fusarium oxysporum* taxon, and most likely these were *Fusarium commune* (Stewart and others 2006), although we did not complete the requisite DNA analysis to confirm this. My result with PlantShield compares favorably with operational applications at the USDA Forest Service's Coeur d'Alene nursery (Eggleston 2007).

Seedling growth was quite variable across all treatments—for all of the morphological variables analyzed, only S:R was significant among treatments. The PlantShield + Q12 treatment had a significantly greater S:R than either PlantShield or Q12 applied singly, which were not significantly different, but significantly greater than the control (Figure 2). All S:R were, however, much less than 1. Seedlings in this trial had smaller root-collar diameters (1.75 mm versus 3.5 mm) than reported by Burr and others (2001), but terminal bud lengths were similar (10 mm); seedlings in this trial were started later and given less intensive fertilizer (Eggleston 2007) than those of Burr and others (2001). Interesting, however, is that the control tended to have the smallest mean values for root volume, needle dry weight, shoot dry weight, needle length, and number of fascicles per seedling (data not shown). Future studies engaging larger sample sizes and better replication may be more able to discern other benefits (for example, improved shoot growth and root volume) of using biological controls, rather than simply improved survival.

Fusarium oxysporum Q12 yielded similar seedling survival and growth rates as that of the commercially available PlantShield, indicating its potential as a biological control. Further trials are currently underway to validate the efficacy of this isolate against pathogenic *Fusarium* spp.

POTENTIAL IMPLICATIONS

From this small, non-replicated observation, it appears that applying PlantShield to recently germinated whitebark pine seeds may have potential to reduce losses from root disease caused by *Fusarium* spp. This simple-to-apply treatment may reduce losses to root disease and thereby improve the seed-to-seedling ratio, which would help meet the demand for whitebark pine seedlings for restoration. Nursery managers may wish to install their own field trials with the commercially available PlantShield, especially for species in which damping-off and (or) root disease is a problem.

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REFERENCES

- Burdett AN. 1979. A nondestructive method for measuring the volume of intact plant parts. *Canadian Journal of Forest Research* 9:120–122.
- Burr KE, Eramian A, Eggleston K. 2001. Growing whitebark pine seedlings for restoration. In: Tomback DF, Arno SF, Keane RE, editors. *Whitebark pine communities: ecology and restoration*. Washington (DC): Island Press. p 325–345.
- Dumroese RK, James RL. 2005. Root diseases in bareroot and container nurseries of the Pacific Northwest: epidemiology, management, and effects on outplanting performance. *New Forests* 30:185–202.
- Dumroese RK, James RL, Wenny DL. 1990a. Trial of a granular etridiazole and thiophanate-methyl fungicide to control *Fusarium* root disease of container-grown Douglas-fir seedlings. *New Forests* 4:231–236.
- Dumroese RK, Wenny DL, Quick KE. 1990b. Reducing pesticide use without reducing yield. *Tree Planters' Notes* 41(4):28–32.
- Eggleston KL. 2007. Personal communication. Coeur d'Alene (ID): USDA Forest Service, Coeur d'Alene Nursery. Horticulturist.
- James RL. 1991. *Cylindrocarpon* root disease of container-grown whitebark pine seedlings—USDA Forest Service Nursery, Coeur d'Alene, Idaho. Missoula (MT): USDA Forest Service, Northern Region, Forest Pest Management. Report 91-8. 10 p.
- James RL. 2004. Pathogen infection and colonization of container-grown whitebark pine seedlings—USDA Forest Service Nursery, Coeur d'Alene, Idaho. Missoula (MT): USDA Forest Service, Northern Region, Forest Health Protection. Nursery Disease Note #154. 10 p.
- James RL, Burr KE. 2000. Diseases associated with whitebark pine seedling production—USDA Forest Service Nursery, Coeur d'Alene, Idaho. Missoula (MT): USDA Forest Service, Northern Region, Forest Pest Management. Report 00-8. 11 p.
- James RL, Dumroese RK. 2007. Potential for using *Fusarium* to control *Fusarium* diseases in forest nurseries. In: Riley LE, Dumroese RK, Landis TD, technical coordinators. *National proceedings, forest and conservation nursery associations—2006*. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. Proceedings. RMRS-P-50. p 54–60.
- James RL, Dumroese RK, Wenny DL. 1988. Occurrence and persistence of *Fusarium* within styroblock and Ray Leach containers. In: Landis TD, technical coordinator. *Proceedings: combined meeting of the western forest nursery associations*. Fort Collins (CO): USDA Forest Service, Rocky Mountain Forest and Range Experiment Station. General Technical Report RM-167. p 145–148.

- James RL, Dumroese RK, Gilligan CJ, Wenny DL. 1989. Pathogenicity of *Fusarium* isolates from Douglas-fir seed and container-grown seedlings. Moscow (ID): University of Idaho, Idaho Forest, Wildlife and Range Experiment Station. Bulletin 52. 10 p.
- James RL, Dumroese RK, Wenny DL. 1990. Approaches to integrated pest management of *Fusarium* root disease in container-grown seedlings. In: Rose R, Campbell SJ, Landis TD, editors. Target seedling symposium: proceedings, combined meeting of the Western Forest Nursery Association and Intermountain Nursery Association. Fort Collins (CO): USDA Forest Service, Rocky Mountain Forest and Range Experiment Station. General Technical Report RM-200. p 240–246.
- James RL, Dumroese RK, Wenny DL. 1991. *Fusarium* diseases of conifer seedlings. In: Sutherland JR, Glover SG, editors. Proceedings of the first meeting of IUFRO Working Party S2.07-09 (Diseases and Insects in Forest Nurseries). Victoria (BC): Forestry Canada, Pacific Forestry Centre. Information Report BC-X-331. p 181–190.
- James RL, Dumroese RK, Wenny DL. 1994. Observations on the association of *Cylindrocarpon* spp. with diseases of container-grown conifer seedlings in the inland Pacific Northwest of the United States. In: Perrin R, Sutherland JR, editors. Diseases and Insects in Forest Nurseries; 1993 Oct 3–10; Dijon, France. Paris, France: Institut National de la Recherche Agronomique. Les Colloques 68. p 65–78.
- Komada H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Review Plant Protection Research 8:114–125.
- Miles MR, Wilcoxson RD. 1984. Production of fungal inoculum using a substrate of perlite, cornmeal, and potato dextrose agar. Plant Disease 68:310.
- Nelson PE, Toussoun TA, Marasas WFO. 1983. *Fusarium* species: an illustrated manual for identification. University Park (PA): The Pennsylvania State University Press. 193 p.
- Shrimpton G. 1992. Sanitation methods and monitoring progress reduce disease in British Columbia container nurseries. In: Landis TD, technical coordinator. Proceedings: Intermountain Forest Nursery Association meeting. Fort Collins (CO): USDA Forest Service, Rocky Mountain Forest and Range Experiment Station. General Technical Report RM-211. p 100–102.
- Stewart JE, Kim M-S, James RL, Dumroese RK, Klopfenstein NB. 2006. Molecular characterization of *Fusarium oxysporum* and *Fusarium commune* isolates from a conifer nursery. Phytopathology 96:1124–1133.
- Tomback DF, Arno SF, Keane RE, editors. 2001. Whitebark pine communities: ecology and restoration. Washington (DC): Island Press. 440 p.
- [USDA NRCS] USDA Natural Resources Conservation Service. 2008. The PLANTS database. URL: <http://plants.usda.gov> (accessed 15 Jan 2008). Baton Rouge (LA): National Plant Data Center.
- Wick D, Luna T, Evans J, Hosokawa J. 2008. Propagation protocol for production of container *Pinus albicaulis* Engelm. plants (172 ml conetainers); USDI NPS Glacier National Park, West Glacier, Montana. In: Native Plant Network. URL: <http://www.nativeplantnetwork.org> (accessed 7 May 2008). Moscow (ID): University of Idaho, College of Natural Resources, Forest Research Nursery.

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