Microbial Mixtures for Biological Control of Fusarium Diseases of Tree Seedlings

Cynthia M. Ocamb[1], Cynthia A. Buschena[2] , and Joseph O'Brien[1]

Ocamb, C. M.; Buschena, C.A.; O'Brien, J. 1996. Microbial Mixtures for Biological Control of Fusarium Diseases of Tree Seedlings. In: Landis, T.D.; South, D. B., tech. coords. National Proceedings, Forest and Conservation Nursery Associations. Gen. Tech. Rep. PNW-GTR-389. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station: 159-166. Available at: http://www.fcanet.org/proceedings/1996/ocamb.pdf

Abstract-Alternatives to soil fumigation with methyl bromide are needed for controlling Fusarium diseases in tree nurseries. Studies are under way for developing microbial mixtures (bacteria & ectomycorrhizal fungi) that control Fusarium diseases of eastern white pine (*Pinus strobus* L.) seedlings. Greenhouse studies in containerized production have shown that the application of rhizosphere bacteria to conifer seed, coupled with ectomycorrhizal fungi application at sowing, can protect seedlings against Fusarium root rot. Not only do seedlings have a reduced incidence and severity of root rot, they also have greater levels of ectomycorrhizal roots. When applied to seeds for field (bareroot) production, the bacterial strains are associated with increased stand numbers.

INTRODUCTION

Mortality of field-grown (bareroot) *Pinus strobus* L. (eastern white pine) seedlings from Fusarium root rot (17) causes serious economic losses in the Lake States region (12,15,16). Infected seedlings suffer reduced vigor and growth, if not killed by root rot. In one survey, up to 75 % of inspected seedlings had root rot (13). Moreover, 68 % of the seedlings intended for sale from one nursery were culled due to root rot (14). In addition to causing root rot, *Fusarium* spp. are known to incite damping-off of *P. strobus* (6,7). Containerized seedlings are also susceptible to Fusarium diseases. Current control measures include soil fumigation with methyl bromide-chloropicrin (bareroot production) and biweekly applications of fungicides (containerized production), but root rot of white pine is still a serious problem in nurseries. Disease problems will undoubtedly increase when methyl bromide is no longer available for soil fumigation(19). Alternatives are needed for controlling Fusarium diseases in tree nurseries and use of biological control agents is an important method of alternative pest control.

Use of microorganisms as biological control agents has been studied with agronomic and horticultural plant diseases but limited information is available for diseases of conifer seedlings. Microorganisms antagonistic to *Fusarium spp*. can be applied to conifer seed. Upon successful colonization of the rhizosphere, conifer seedling roots can be protected Fusarium against root rot. Biological control microbes may also protect germinates from damping-off (1). Successful use of ectomycorrhizal fungi for suppressing pathogenic *Fusarium* spp. has been reported with *Laccaria laccata* on *Pinus banksiana* (1) and *Pseudotsuga menziesii* (18); as well as *Paxillus involutus* on *Pinus resinosa* (4,5). Abundant ectomycorrhizal root formation enables nursery managers to significantly decrease fertilization rates; generating savings above those from minimized seedling losses due to death and culling. In addition, biological control agents may preclude other soilborne pathogens from causing disease. Biological control microbes will probably benefit other Lake States conifer species, as well as conifer seedlings grown in other geographic areas. By

introducing rhizosphere colonists that are beneficial to conifer seedlings and antagonistic to soilborne pathogens, growth and spread of indigenous or exotic pathogens may be minimized through protection of susceptible host tissue.

TESTING IN CONTAINERIZED PRODUCTION

Representative isolates of *F. oxysporum*, *F. oxysporum* var. *redolens* (W. L. Gordon), *F proliferatum* (T. Matsushima) Nirenberg, and *F solani* (Mart.) Sacc. were collected from necrotic P. strobus roots or nursery soil, purified by the single-spore method, and stored on silica gel at 5 C (20). Inoculum was increased by transferring 5-mm agar plugs from carnation leaf agar (10) cultures to sterile cornmeal-sand medium (97 g sand, 3 g cornmeal, 40 ml distilled water). Each pathogenic *Fusarium* isolate was added to a growing medium (Fafard #2) at a rate of 0.005 g/cc soil.

Bacteria (Table 1) were isolated from white pine rhizosphere soil (11), stored in sterile water at 24 C, and increased in oatmeal broth (2). The mycorrhizal fungi, *Hebeloma* sp. and *Laccaria* sp., were stored as outlined in Doudrick and Anderson (3) and grown in modified Melin-Norkrans' nutrient solution (9). White pine seeds (lot A05884, courtesy of G. Dinkel, USDA Forest Service) were surface-disinfested by agitation in 3 % H_2O_2 for 2 hr, rinsed four times in sterile water, wrapped in moist cheesecloth, and placed in cold storage (5 C) for eight wk. After stratification, seeds were soaked in bacterial cultures for 60 min then air-dried.

Table 1. Bacteria used as biological control agents for suppression of Fusarium root rot in containerized or field production

Acquisition #	Identification via Fatty Acid Analyses
BCT5a	*Streptomyces violaceusniger* subsp. *violaceusniger*
BCT19b	*Streptomyces rochei* subsp. *rochei*
BCB175	*Bacillus megaterium*
BCB176	*Streptomyces lavendulae*
BC19	*Methylobacterium mesophilicum*
BC20	*Rhodococcus erythropolis/Kocuria varians/Pseudomonas diminuta*

Pine cell cone-tainers (Stuewe & Sons, Corvallis, OR), 17 cm in length and 24 mm in diameter, were plugged with 5 cc of Fafard #2, then 5 cc of *Fusarium* infested medium was added. The pine cells were filled the rest of the way with Fafard #2. Two white pine seeds were placed atop soil and 1 ml of ectomycorrhizal slurry was pipetted into the soil. Seeds were covered with 2.5 cc of Fafard #2 and perlite was spread over the top of each pine cell container. Treatments are listed in Table 2. Mycostop® (8), a commercial formulation of *Streptomyces* sp., was included in this study. Mycostop® was reapplied every 4-6 wk

according to label guidelines. Seedlings were grown in a greenhouse according to standard nursery practices.

Table 2. Treatments included in study on the microbial suppression of Fusarium root rot in containerized production

Ectomycorrhizal soil drench	**Seed-application of biological control bacterium**
Laccaria sp.	BCT5a
Laccaria sp.	BCT19b
Laccaria sp.	BCB176
Hebeloma sp.	BCT5a
Hebeloma sp.	BCT19b
Hebeloma sp.	BCB176
Laccaria sp.	BCT5a, BCT19b, BCB176
Hebeloma sp.	BCT5a, BCT19b, BCB176
Laccaria sp.	Mycostop®
Hebeloma sp.	Mycostop®
---	Mycostop®
---	BCT5a
---	BCT19b
---	BCB176
---	BCT5a, BCT19b, BCB176
Laccaria sp.	---
Hebeloma sp.	---
---	BC20
water	---

Eleven months after sowing, shoot height, root volume, root rot, and percentage of root system with ectoinycorrhizal roots were recorded for each seedling. Rot root ratings are based on a 1 to 5 rating system: 1 = apparently healthy, 2 = over 50 % length of one lateral root exhibiting rot, 3 = lower 1/3 of tap root is symptomatic or greater than 50 % of two or more lateral roots is necrotic, 4 = lower 2/3 of tap root is rotted (with or without lateral root injury), and 5 = upper third of tap root is rotted or entire root system is affected.

Applications of the *Hebeloma* sp. mixed with a rhizosphere-derived bacteria were associated with a significant (P=0.05) decrease in root rot severity compared to the water-disease control

(Figure 1).

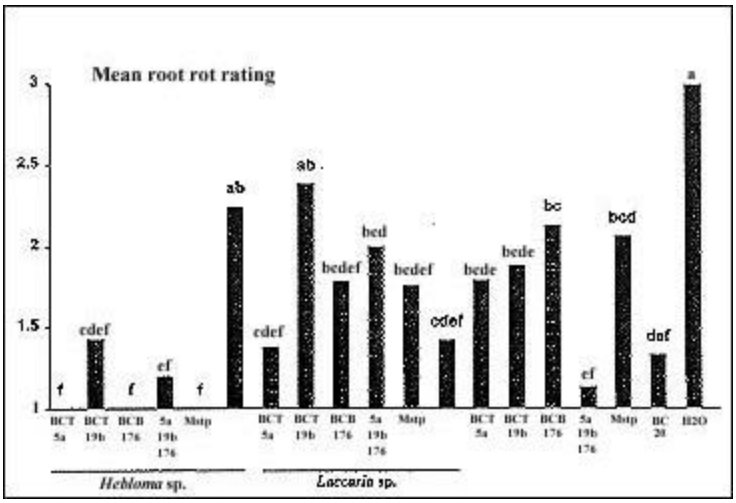

Figure 1. Mean root rot ratings of eastern white pine containerized seedlings grown in *Fusarium*-infested growing medium for 11 months. Seeds were coated with bacterial biological control agents (Table 1). Mycostop® (Mstp) was included. Two ectomycorrhizal fungi, *Hebeloma* sp. and *Laccaria* sp., were drenched over white pine seeds. Sterile water (H_2O) was used as a water disease control. Disease severity rating classes included: RR1=apparently healthy, RR2=over 50% length of one lateral root exhibiting root rot, and RR3=lower 1/3 of tap root is symptomatic or greater than 50% of two or more lateral roots is necrotic.

Bars represent means based on 20 seedlings in each of two replicates (40 total). Bars labeled with the same letters are not significantly different (P=0.05) according to Tukey's W statistic.

Similarly, significantly greater levels of ectomycorrhizal roots were observed in these same microbial mixtures (Figure 2). In contrast, *Laccaria* sp. applications yielded fewer ectomycorrhizal roots compared to *Hebeloma* applications, though microbial mixtures that included the *Laccaria* isolate generally offered significant disease suppression relative to the water-disease control. When the rhizosphere bacteria were applied without an accompanying ectomycorrhizal fungus, the result was a significant decrease in root rot severity compared to the water-disease control. Root volume was significantly increased with mixtures of the *Hebloma* sp. with BCT19b, BCB176, or Mycostop® compared to the water-disease control (Figure 3). Generally, no increase in seedling height was associated with any of the biocontrol applications (Figure 4).

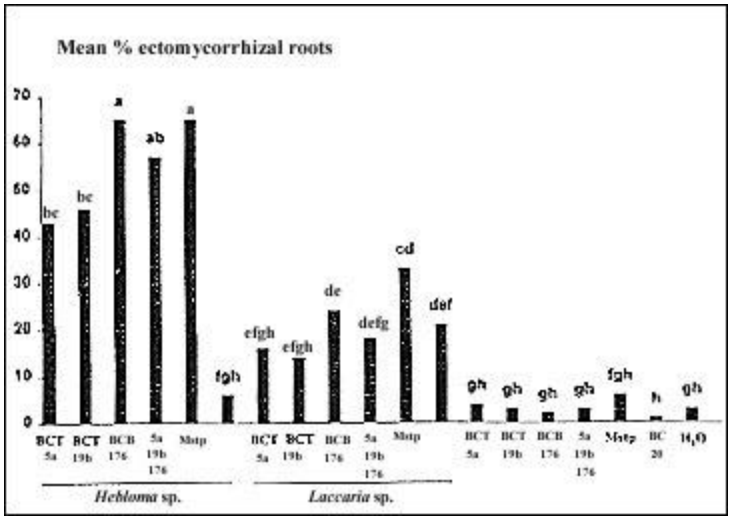

Figure 2. Mean percentages of ectomycorrhizal roots of eastern white pine containerized seedlings grown in *Fusarium*-infested growing medium for 11 months. Seeds were coated with bacterial biological control agents (Table 1). Mycostop® (Mstp) was included. Two ectomycorrhizal fungi, *Hebeloma* sp. and *Laccaria* sp., were drenched over white pine seeds. Sterile water (H_2O) was used as a water-disease control. Bars represent means based on 20 seedlings in each of two replicates (40 total). Bars labeled with the same letters are not significantly different (P=0.05) according to Tukey's W statistic.

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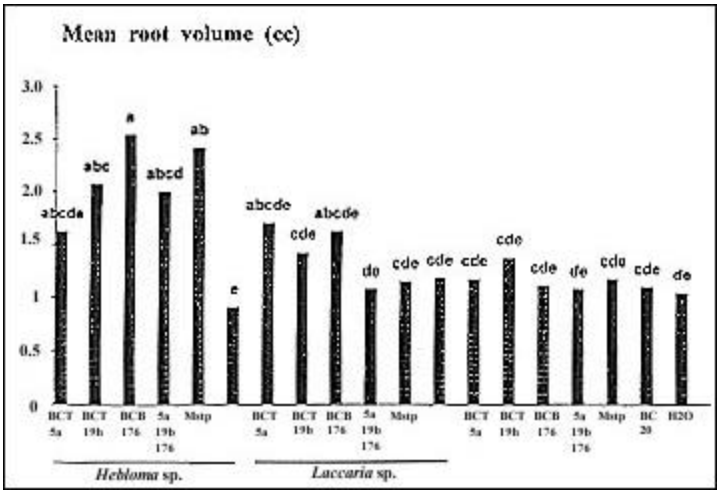


Figure 3. Mean root volume of eastern white pine containerized seedlings grown in *Fusarium*-infested growing medium for 11 months. Seeds were coated with bacterial biological control agents (Table 1). Mycostop® (Mstp) was included. Two ectomycorrhizal fungi, *Hebeloma* sp. and *Laccaria* sp., were drenched over white pine seeds. Sterile water (H_2O) was used as a water-disease control. Bars represent means based on 20 seedlings in each of two replicates (40 total). Bars labeled with the same letters are not significantly different (P=0.05) according to Tukey's W statistic.

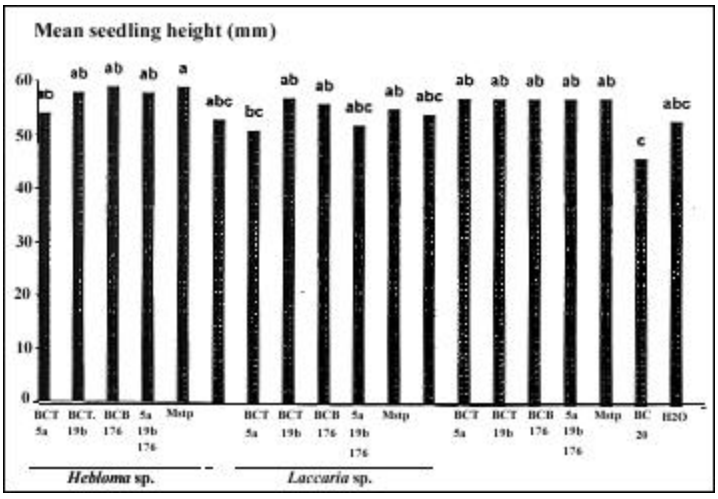


Figure 4. Mean height of eastern white pine containerized seedlings grown in *Fusarium*-infested growing medium for 11 months. Seeds were coated with bacterial biological control agents (Table 1). Mycostop® (Mstp) was included. Two ectomycorrhizal fungi, *Hebeloma* sp. and *Laccaria* sp., were drenched over white pine seeds. Sterile water (H_2O) was used as a water-disease control. Bars represent means based on 20 seedlings in each of two replicates (40 total). Bars labeled with the same letters are not significantly different (P=0.05) according to Tukey's W statistic.

FIELD TESTING (BAREROOT PRODUCTION)

Operational fields in two nurseries: Wilson State Forest Nursery, Boscobel (WSFN), Wisconsin, and J. W. Tourney Nursery (TN), Watersmeet, Michigan, were used as study sites. Fields were fumigated with dazomet at a rate of 570 kg/ha. White pine seeds of lots H-167B (courtesy of T. Marty, Wisconsin Department of Natural Resources) and A0588-4 were surface-disinfested by agitation in 3 % H_20_2 on a orbital shaker at 120 rpm for 2 hr then rinsed four times in sterile, distilled water. Seeds of lot A0588-4 underwent stratification similar to the method described above. Prior to sowing, seeds were soaked in bacterial cultures for 60 min then air-dried. Seedlot H-167B was fallsown in WSFN and A0588-4 was spring-sown in TN. Ectomycorrhizal fungi were applied by drenching slurries along side of emerging seedlings. Each field was maintained according to standard nursery practices.

Each field was blocked four areas, across the width of the field. At TN, one of seven seed/ ectomycorrhizal treatments (Tables 1 & 3) were randomly assigned to the bed row within

each block. At WSFN, one of four seed treatments (Tables 1 & 4) were randomly assigned to each row and three plots of each of the three ectomycorrhizal treatments were randomly assigned to each bed row within each block. Stand counts were made during late-summer. At TN, seed disinfestation alone or accompanied by the *Hebeloma* sp. only slightly improved stand counts whereas the presence of the biocontrol bacteria was associated with at least a 10 % improvement in stand relative to the untreated, nursery standard (Figure 5). In WSFN, BCB 175 appeared to improve stands by 3545 % relative to the untreated, nursery standard (Figure 6). Disinfestation of seeds alone was associated with approximately a 10 % stand improvement.

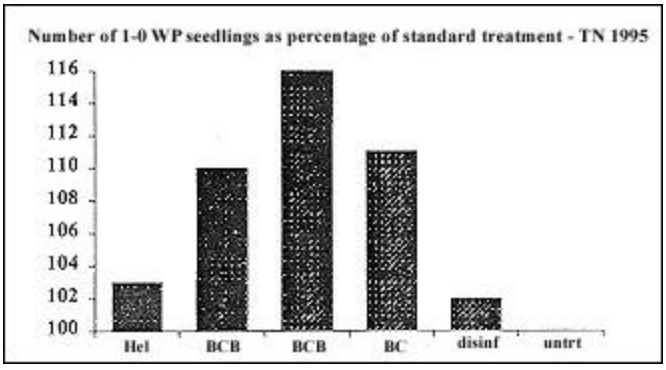
Table 3. Treatments included in study on the microbial suppression of damping-off and Fusarium root rot in field (bareroot) production in Toumey Nursery.

Seeds disinfested	Ectomycorrhizal inoculation	Seed-application of biological control bacteria
-	-	-
+	-	-
+	Hebeloma sp.	-
+	-	BCB 176
+	Hebeloma sp.	BCB 176
+	-	BCB 175
+	-	BC 19

Table 4. Treatments included in study on the microbial suppression of damping-off and Fusarium root rot in field (bareroot) production in Wilson Nursery.

Seeds disinfested	Seed-application of biological control bacteria	Ectomycorrhizal inoculation
-	-	-
-	-	*Hebeloma* sp.
-	-	*Laccaria* sp.
+	-	-
+	-	*Hebeloma* sp.
+	-	*Laccaria* sp.
+	BCB 175	-
+	BCB 175	Hebeloma sp.
+	BCB 175	*Laccaria* sp.
+	BCB 176	-
+	BCB 176	*Hebeloma* sp.
+	BCB 176	*Laccaria* sp.

Figure 5. Mean percentage stand number improvement of 1-0 eastern white pine seedlings in the field relative to stand nursery practice (untrt) in Toumey Nursery. Disinfested seeds (disinf) were coated with *Streptomyces lavendulae* (BCB176), *Bacillus megaterium* (BCB175), or *Methylobacterium mesophilicum* (BC19), The ectomycorrhizal fungus, *Hebeloma* sp. (Hel), was applied as a soil drench next to emerging seedlings. Counts are based on three plots per treatment combination.



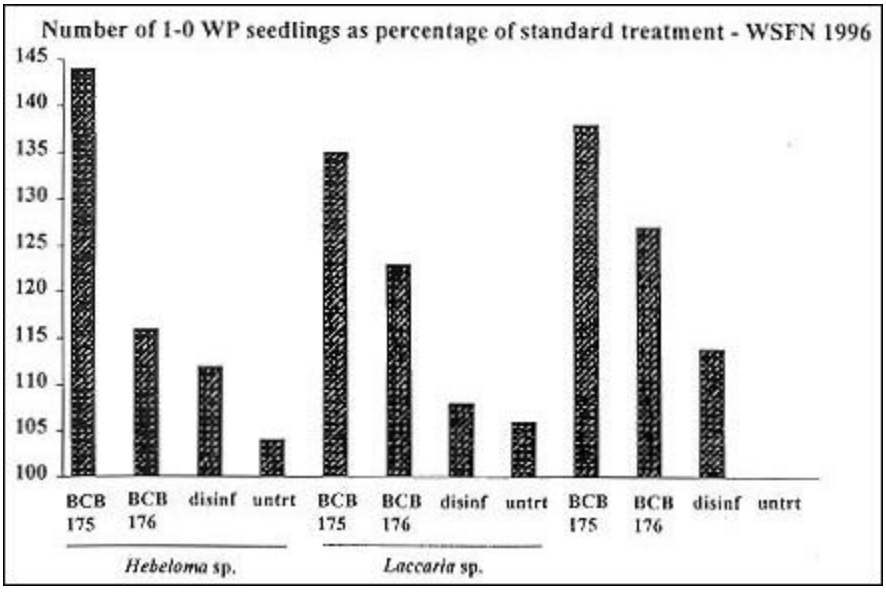


Figure 6. Mean percentage stand number improvement of 1-0 eastern white pine seedlings in the field relative to stand nursery practice (untrt) in Wilson Nursery. Disinfested seeds (disinf) were coated with *Bacillus megaterium* (BCB175) or *Streptomyces lavendulae* (BCB176). The mycorrhizal fungi, *Hebeloma* sp. and *Laccaria* sp., were applied as a soil drench next to emerging seedlings. Counts are based on nine plots per treatment combination.

CONCLUSION

Microbial mixtures, integrated with seed disinfestation, look favorable for root rot control in containerized tree seedling production. In addition, the *Hebeloma* isolate apparently needs rhizosphere bacteria for ectomycorrhizal root formations in our study. Seed disinfestation slightly improved stand counts in the field, but application of our rhizosphere bacteria coupled with disinfestation and dazomet fumigation greatly enhanced field stand numbers. Root systems will be examined during the second growing season, and microbial efficacy for root rot control can then be determined. Current research efforts include improvement in delivery of ectomycorrhizal fungi, increased component numbers in microbial mixtures, and evaluations with *Pinus resinosa* Aiton (red pine), *Pseudotsuga menziesii* (Mirb.) Franco (Douglas-fir), and *Pinus patula* Schlechtend. & Cham. (Mexican weeping pine) seedlings.

ACKNOWLEDGMENTS

The authors wish to thank Wilson State Forest Nursery, Boscobel, Wisconsin, J. W. Tourney Nursery, Watersineet, Michigan, and the Department of Plant Pathology of the University of Minnesota for providing assistance, equipment, and supplies. The technical assistance of J. Bitz, P. Castillo, K. Cease, A. Dejarlais, D. Gardner, L. Haugen, A. Hilmonowski, M. Jones, M. Labalan, T. Lewis, D. McDougall, S. Meyer, J. Paul, A. Reyes, and K. Weinke is gratefully acknowledged. Operating funds for a portion of this study was provided by a Technological Development grant (USDA-FS 24-42-TN-031114), Challenge Cost Share grant (USDA-FS 152103), and USDA-FAS-RSED grant (SF08) to the first author.

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[1]*U. S. Department of Agriculture, Forest Service, North Central Forest Experiment Station, 1992 Folwell Avenue, St. Paul, Minnesota 55108.*

[2]*Department of Forest Resources, 1530 N. Cleveland Ave., University of Minnesota, St. Paul,*

Minnesota 55108.

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