Biological Control of Fusarium Diseases of Conifer Seedlings

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Abstract-An alternative to soil fumigation with methyl bromide is needed for control of Fusariurn root rot and damping-off of conifer seedlings. Studies with eastern white pine *(Pinus strobus, L.)* and red pine (P. *resinosa,* Ait.) are underway to develop biological control microorganisms for application to conifer seed. Preliminary results show an ectomycorrhizal fungus, as well as bacteria derived from the rhizosphere, suppress Fusariurn root rot of eastern white pine.

INTRODUCTION

With the loss, in the year 2001, of methyl bromide as a soil fumigant, reduction of seedling growth and vigor, as well as increased seedling mortality, is anticipated. Because conifer seedlings are susceptible to root rot and damping-off, caused by *Fusarium* species, an alternative is needed to control *Fusarium* in bareroot nurseries. The application of a mixture of biological control agents is a promising and necessary alternative.

Many, but not all *Fusarium* species are pathogenic. *Fusariurn* species may cause seed decay and damping-off early in the growing season. Later damage usually consists of root necrosis and some chlorosis. The damage is usually clustered in the nursery beds as evidenced by brown patches of seedlings. Fusarium root rot and damping-off are among the most important soilborne diseases of eastern white pine (Pinus strobus, L.) in bareroot nurseries.

Seedborne Fusarium Species

Fusarium may be introduced into nursery beds on seed. These *Fusarium* species can decrease seed germination or seedling emergence (James et al. 199 1, Kelley& Oak 1989). Nine seedlots of eastern white pine, collected from the Lake States, were tested for the presence of *Fusarium* species. The proportion of seeds infested with *Fusarium* ranged from 40 - 100 % of seeds tested, with the majority of seed lots being nearly 100% infested (Figure 1). To try to reduce seedborne *Fusarium,* various methods were evaluated for effective seed disinfestation. Washing eastern white pine seeds for 48 hours or treating seed in 0.05% NaOCI for 40 minutes decreased the percentage of seeds with *Fusarium* species. Immersing seed in hydrogen peroxide for three hours resulted in the greatest reduction in percent seed with *Fusarium* species (Figure 2).

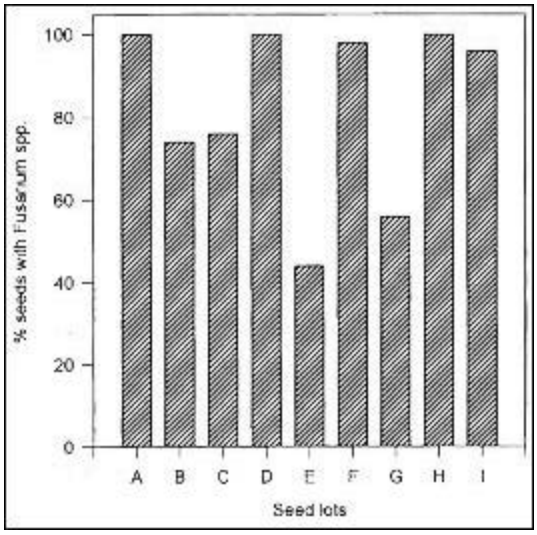

Figure 1. Percent of eastern white pine seeds from which Fusarium spp. were isolated. Nine seed lots (A-1) collected from the Lake States region were examined.

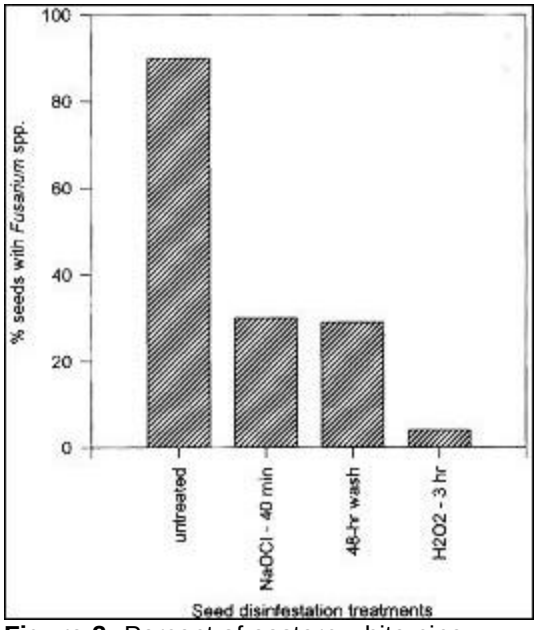

Figure 2. Percent of eastern white pine seeds from which Fusarium spp. were Isolated. Seeds were disinfested in 0.05% sodium hypochlorlte (NaOCI) for 40 min, hydrogen peroxided (H_2 0_2 for 3 hr, running tap water (48-hr wash), or left untreated.

Soilborne Fusarium species

Even when soil is fumigated *Fusarium* species still can be present in nursery soil, perhaps surviving in plant debris (Juzwik and Ocamb, unpublished). An analysis of plant debris from different soil depths revealed that *Fusarium* species were found on the majority of plant debris, greater than I mm in diameter in soil, one month after fumigation with methyl bromide--chloropicrin (MC-33) (Ocamb, unpublished). Clearly, even when methyl bromide is used, Fusarium diseases can be a present in nursery beds.

Rhizosphere-derived biological control candidates

Numerous soil organisms have an inhibitory effect on soilborne pathogenic *Fusarium* species. A technique was used to isolate microbes to be used as biological control candidates (Ocamb 1994). Rhizosphere soil from a variety of sources was mixed with media and incubated in petri plates for 48 hours. An isolate of *Fusarium* was minced with agar, then layered on top of the rhizosphere soil dilution plates. Microorganisms were then isolated from the center of zones free of growth by *Fusarium* species. Using this technique, more than 500 candidate organisms were obtained. These biological control candidates were challenged with combinations of pathogenic *Fusarium* species and candidates that were judged to be the most suppressive to *Fusarium* growth were further tested in greenhouse evaluations.

Mycorrhizal fungi

We wanted to include ectomycorrhizal fungi in our mix of biological control agents. Ectomycorrhizae are not only necessary for conifer survival and health but have been shown to

inhibit development of some soilborne pathogens (Chakravarty & Hwang 199 1, Sinclair et al. 1975, Duchesne et al. 1988, Farquhar & Peterson 1991). Isolates of two species of ectomycorrhizal fungi obtained from sporocarps associated with white pine nursery seedlings, were tested separately with and without *Fusarium* present in the soil in a series of growth chamber experiments. On average, seedlings inoculated with isolate #2 had 70% mycorrhizal roots and isolate #1 had approximately 45% mycorrhizal roots, without *Fusarium* present in the soil (Figure 3). With *Fusarium* present, mycorrhizal colonization by isolate #1 dropped off to approximately 10%, while mycorrhizal colonization of seedlings inoculated with isolate #2 did not greatly drop off. In addition, root rot was reduced when isolate #2 was present (Figure 4). Isolate #2, which forms a thick mantle around each short root, appears promising as a biological control agent against pathogenic *Fusarium* species.

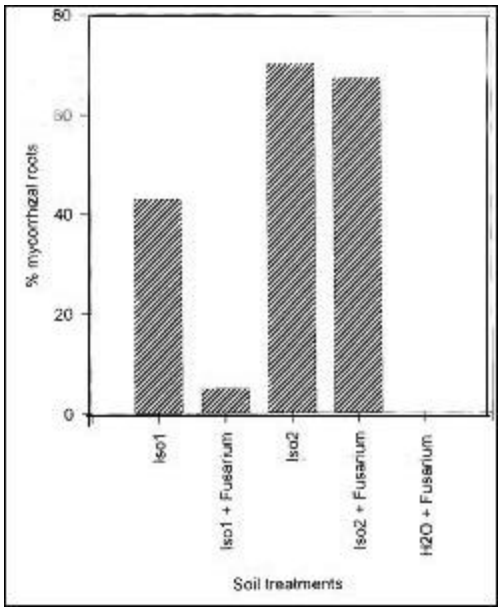

Figure 3. Percent of eastern white pine seeds with mycorrhizal associations. Two mycorrhizal fungi, lsol and lso2, were applied to seedlings growing in pasteurized field soil with and without artificial infestation of pathogenic Fusarium spp.

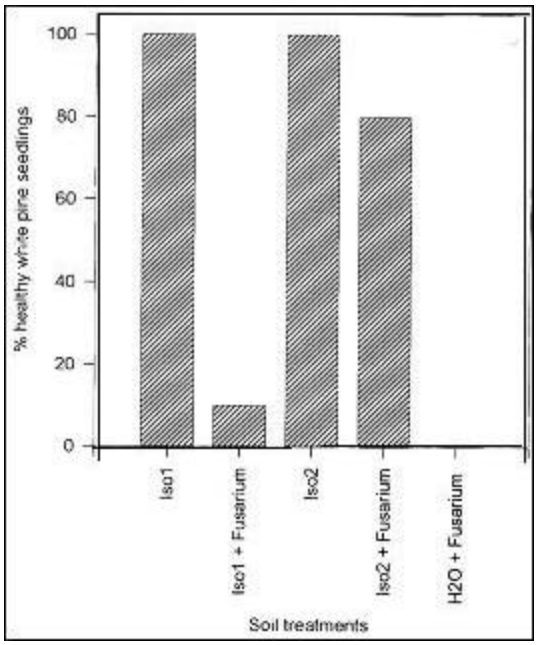

Figure 4. Percent healthy eastern white pine seedlings when two mycorrhizal fungi, lsol and lso2, were applied to seedlings growing in pasteurized field soil with and without artificial infestation of pathogenic Fusarium spp.

The growth chamber trials were conducted with a relatively small number of seedlings. In January 1995, 480 containers were inoculated in a greenhouse at George W. Tourney Nursery in Watersmeet, Michigan with two ectomycorrhizal fungi, other biocontrol candidates collected from the rhizosphere of white pine, and Mycostop", a commercial formulation. The seedlings at Tourney were treated the same as other seedlings grown for production except they did not receive any fungicide applications. Trees treated with the mycorrhizal fungi showed evidence of sporocarp production by seven months of age. Seedlings will be evaluated for percent mycorrhizal colonization, size and root health.

In addition to the greenhouse study, three field studies have been installed at Tourney Nursery, testing the rhizosphere-derived biological control candidates and ectomycorrhizal fungi. Two studies are on eastern white pine and the other is red pine. The experimental designs are a randomized complete blocks. The treatments were blocked over the length of the nursery bed. Seed treatments included non-treated seeds and seeds disinfested with hydrogen peroxide. Disinfested seeds were coated with the biological control candidates. Ectomycorrhizal fungi inoculations were coupled with all seed treatments.

Approximately 450 white pine seeds were sown in each plot. Stand counts were made approximately 10 weeks after sowing. Seedling numbers were greater in plots treated with some biological control agents than in the control plots. Figure 5 illustrates some of the preliminary results with eastern white pine. In general, dazomet-treated beds had a greater seedling stand count than that found in MC-33 treated plots. Disinfesting seed alone did not improve the stand count relative to that found with untreated seeds. Application of BCA I and Mycostop8 increased the stand counts. An evaluation of ectomycorrhizal colonization is underway.

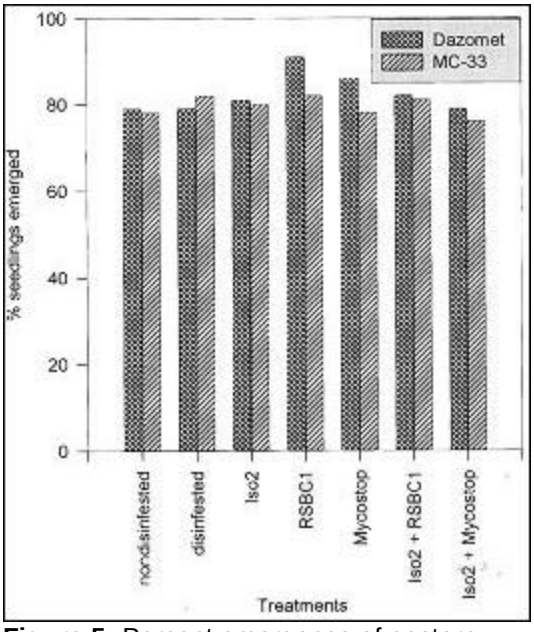

Figure 5. Percent emergence of eastern white pine seedlings when a mycorrhizal fungus (Iso2), a rhizosphere-derived biocontrol agent (RSBC1), or Mycostop were applied to disinfested seeds sown in the field.

CONCLUSION

Our research with biological control agents looks very promising. Preliminary data suggests that by integrating three components: (1) seed disinfestation, (2) soil fumigation with dazomet, and (3) treatment with a mix of biological control candidates, we can control Fusarium root rot and damping-off of conifer seedlings in the nursery. Future direction includes looking for a practical and economical delivery system, application rates, and secondary effects

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