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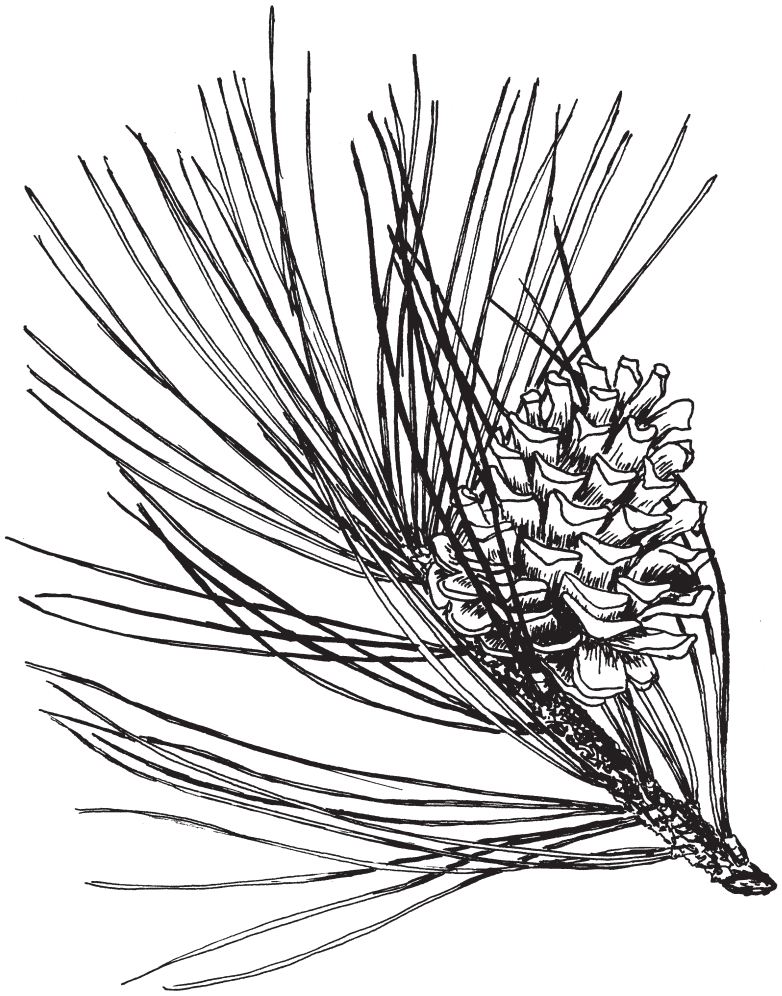

Ponderosa pine drawing by Lorraine Ashland, College of Natural Resources, University of Idaho.

Detection and Control of *Fusarium oxysporum* and *Cylindrocarpon destructans* in Forest Nursery Soils

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Abstract: *Fusarium oxysporum* and *Cylindrocarpon destructans* cause root disease that leads to significant crop losses in forest nurseries when not treated. Treatment currently relies on methyl bromide fumigation to eradicate soil pathogens. New environmental protection laws, however, are phasing out methyl bromide. Alternative chemical treatments are being tested, as well as biological fumigants such as seed meals and cover crops of *Brassica* spp. In this study, several different *Brassica*-based biofumigation treatments are being tested at Washington Department of Natural Resources Webster Forest Nursery in Olympia, WA. Fungal populations are being traced using molecular techniques such as PCR-ELISA and Real Time PCR. Use of molecular techniques to quantify the fungal pathogens should increase pathogen detection sensitivity and accuracy over the traditional dilution plating method.

Keywords: *Fusarium commune*, *Brassica* spp., seed meals, biofumigation, PCR-ELISA, Real Time PCR

Introduction

Conifer seedling production is plagued by soilborne fungal pathogens. The costs of chemical controls, both monetary and environmental, are rising, and seedling producers are finding new interest in alternative methods for disease control. In order to quickly and accurately assess both pathogen pressure in soils and the effectiveness of alternative treatments, new methods of detection and quantification are needed.

Biofumigation with *Brassica* spp. and other mustard species has been successful in some production systems (Larkin and Griffin 2006). Green manures of *Brassica* spp. are used by both organic and conventional potato growers in Central Washington to control scab. Brassicaceous seed meal and green manure soil amendments release glucosinolates, including volatile methyl isothiocyanate (sold commercially as MiTC). The glucosinolates released by *Brassica* spp. have been shown to be fungitoxic (Fan and others 2008). As with most biological treatments, timing and application method are critical to success. Methods used in one system are not directly transferable to another system. If timing and application rates can be determined, Brassica biofumigants show promise in reducing soil populations of fungal pathogens on conifer roots.

In conifer seedling production, the major pathogens include *Fusarium commune*, *Cylindrocarpon destructans*, and *Pythium ultimum*. Quantification of *Fusarium* spp. pathogens has been only marginally successful because traditional plating methods cannot separate pathogenic *Fusarium commune* from non-pathogenic *Fusarium oxysporum*. In order to accurately quantify the soil pathogen load before and after traditional or alternative treatment, molecular methods are being developed. Real Time PCR protocols (Schroeder 2008) are also being developed for pathogen quantification.

Methods

Greenhouse

Three brassicaceous seed meals, *Brassica juncea*, *Sinapus alba*, and *B. carinata*, and green manures of *B. juncea* and *S. alba* were used in a greenhouse trial to assess application rate and timing for biofumigation in nursery soil at Washington Department of Natural Resources Webster Forest Nursery (Olympia, WA). Potting mixes were made using 10% contaminated soil, perlite, vermiculite, and the biofumigant. Two rates of seed meals were tested for each species, that is, 2.2 tonne/ha and 4.4 tonne/ha (1 ton/ac and 2 ton/ac). Potting mixes were incubated in semi-sealed plastic bags to simulate tarping for 1 week or 4 weeks before one-year old Douglas-fir (*Pseudotsuga menziesii*) seedlings were planted

into the mixes. Plantings were done in parallel at both Washington State University (Pullman, WA) and Webster Nursery. Trees were assessed for height and stem diameter at 5 weeks and 12 weeks, and destructively sampled at 12 weeks to assess root and shoot growth as well as root pathogen populations. Root pathogen populations were assessed by the standard plating method (James 2008). Samples were saved to be assessed using PCR-ELISA and Real Time PCR (RT-PCR).

Field

Field scale trials of the most promising greenhouse treatments used four replications of *B. juncea*, *B. carinata* and *S. alba* seed meals, a methyl bromide-fumigated control, and an untreated control in a randomized complete block design in 1.2 x 9 m (4 x 30 ft) beds. Trees were assessed for height, caliper, root and shoot mass, and root pathogen populations at 6 and 12 weeks, and will be assessed again at harvest.

Pathogen Detection

Isolates of *Fusarium* species from seedling roots were used to generate sequence from the ITS1 region of the genome. Both *F. commune* and *F. oxysporum* were found, with high homology to samples sequenced from other conifer nurseries (Stewart and others 2006). Sequence alignments provided 4 regions suitable for PCR-ELISA probes.

Table 1. Treatment codes.

Treatment code	Treatment
Ctl	Control
AutoClv	Autoclaved
BcSM1t	*Brassica carinata* at 1 ton/ac
BcSM2t	*Brassica carinata* at 2 ton/ac
BjSM2t	*Brassica juncea* at 2 ton/ac
SaSM2t	*Sinapus alba* at 2 ton/ac

1 ton/ac = 2.2 tonne/ha

Results

Greenhouse

In the greenhouse trial, differences in visual scoring of root infection were found between the brassicaceous seed meal treatments and the untreated control potting mix (Figure 1). Differences were also observed in the pathogen population counts (Figures 2 and 3). In general, *S. alba* increased root pathogens, while *B. juncea* reduced pathogen populations. *Trichoderma* spp., a beneficial fungus, was also found to be elevated by the seed meal treatments, with *B. juncea* being the most effective at increasing Trichoderma populations. Only *S. alba* significantly reduced tree height relative to the control (Figure 4).

Field

Field trials are still in progress.

Pathogen Detection

Testing of the four probes yielded two probes with strong, specific binding properties needed for detection and discrimination of *F. commune*. Testing of the PCR-ELISA protocol is currently underway.

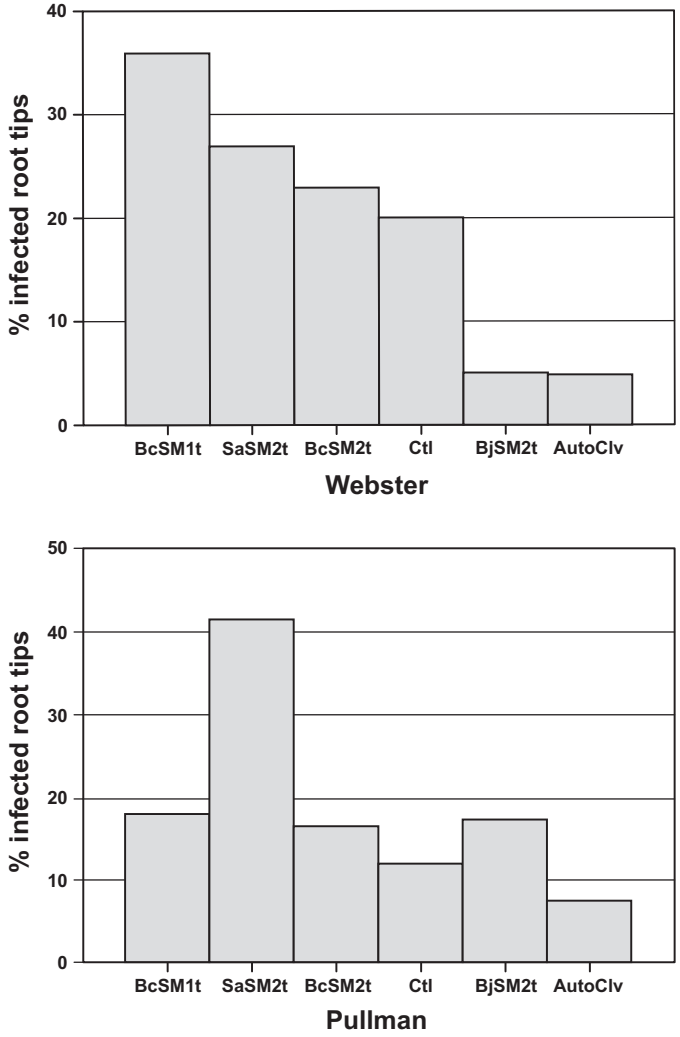

Figure 1. Visual root infection scores after 12 weeks in potting mixes amended with brassicaceous seed meal.

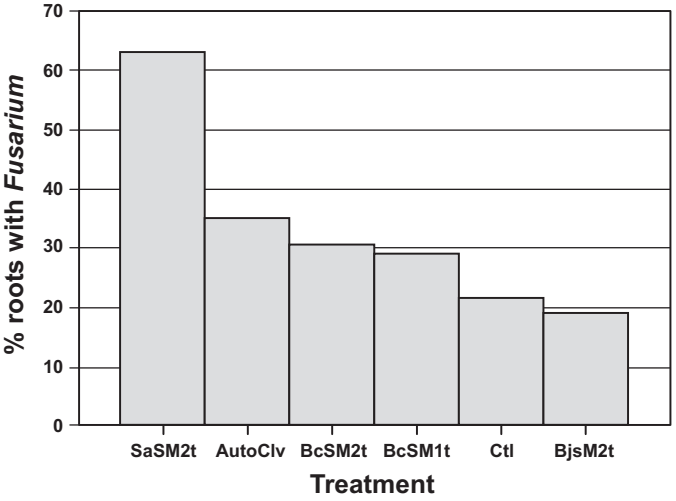

Figure 2. *Fusarium* spp. counts on seedling roots after 12 weeks of growth. No significant differences were observed for location; data presented are combined counts from Washington State University and Washington Department of Natural Resources Webster Nursery.

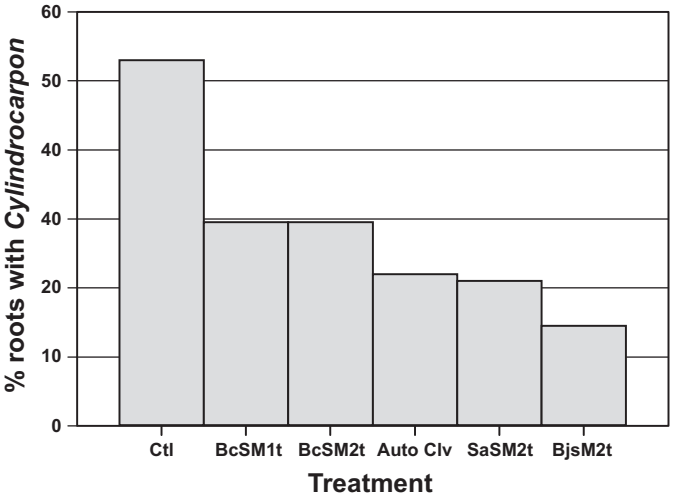

Figure 3. *Cylindrocarpon* spp. counts on seedling roots after 12 weeks of growth. No significant differences were observed for location; data presented are combined counts from Washington State University and Washington Department of Natural Resources Webster Nursery.

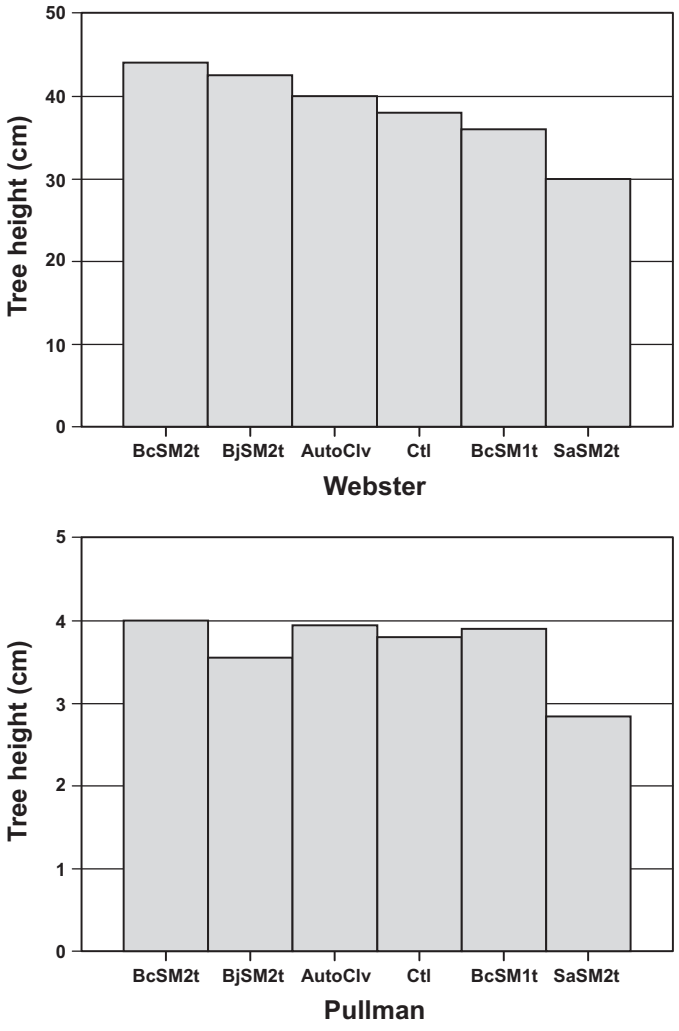

Figure 4. Tree height measurements after 5 weeks of growth in potting mixes amended with brassicaceous seed meal.

Experimental parameters to maximize detection of F. commune, as well as calibrations to make the reactions semi-quantitative, are currently being developed.

Discussion

From the greenhouse trial, several potential field scale treatments were determined. Field trials are currently running. *B. juncea* and *B. carinata* (available commercially) appeared to reduce *Fusarium* spp. populations and increase *Trichoderma* spp. populations. Molecular probes have been developed for *F. commune*, and PCR-ELISA methods (Grimm and Geisen 1998) can now be used to discriminate between *F. commune* and *F. oxysporum*. The next step will allow detection of *F. commune* in soils. With the recent advances in molecular methods to quantify *F. commune*, the major soil pathogen in this system, the greenhouse trial samples will yield even more data on the effectiveness of brassicaceous seed meal treatments. Data from the field will also be valuable in determining whether biofumigation will provide adequate pathogen control for Webster Nursery.

References

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