APPLICATION OF BIOCONTROL AGENTS IN FOREST NURSERIES

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Introduction.

Bare-root conifer seedling culture consists of growing seedlings (sown or transplanted) in soil, and is the predominant method for supplying Americaôs need for healthy regeneration stock to produce and sustain forests, wildlife food sources, fiber, wood products, paper, bio-pharmaceuticals and now potential biofuels. Each year well over 300 million seedlings are grown for regeneration purposes in the Southeastern and Western United States. Soil fumigation with methyl bromide (MB) and other chemical agents has been the operational means to assure that soils are free of weeds and pathogens. Recent EPA RED decisions on re-registration of soil fumigants impact this critical aspect in the successful culture and regeneration of Americaôs forests and woodlands. It is therefore important to understand how new fumigation application rates and cultural regimes will fit into the overall seedling production operation. Furthermore it is imperative to design and test new pest control strategies that can consistently deliver cost-effective and environmentally acceptable integrated pest management (IPM) alternatives for disease and weed control, enhance seedling yields, quality, and field performance. The development of alternative best agricultural practices for soil disease control in conifer nurseries is needed by this industry. Specifically, conifer bare-root nurseries require effective soil fumigation coupled with alternative disease control methodology (i.e. biocontrol agents) to mitigate a root pathogen complex against falling fumigant rates and loss of chemical fumigant registration. Here we report on biocontrol applications that were performed at two nurseries where nonfumigant and reduced-rate fumigant treatments were applied.

Approach. Four fumigation treatments (Table 1) were applied according to a randomized complete block design with four replicate blocks at two conifer nurseries in western Oregon. Root and soil samples were collected 2 weeks before and 6 weeks after fumigation in mid-August 2010 in order to assess soil populations of Cylindrocarpon, Fusarium, and Pythium species. One-year-old Douglas-fir seedlings were planted into each treatmnet plot in May 2011 and they were assessed for root colonization by Cylindrocarpon, Fusarium, and Pythium species. Four biocontrol treatment subplots (Table 3) were established within each soil fumigation treatment plot according to a randomized design in early June 2011 and four biocontrol agents (BCAs) (Table 2) were applied according to a schedule (Table 3). The first biocontrol treatment application occurred in early June 2011 in order to colonize and protect newly transplanted Douglas-fir roots. Subsequent applications in July and October 2011 coincided with Summer and

Fall flushes of root growth, respectively. All BCAs were applied as a drench according to label instructions. Biocontrol treatment 1 evaluates all four BCAs because published literature indicates that multiple BCAs can be more effective for disease control than reliance on a single BCA. Biocontrol treatments 2 and 3 test evaluate whether bacterial BCAs are more effective when applied in Spring than fungal BCAs. Treatment 4 is an untreated control.

Results.

Preliminary results for *Fusarium* indicate that each fumigant was effective in reducing both soil and root pathogen populations (Figs. 1 and 2). Nonfumigated plots, on the other hand, had much greater pathogen populations in both root and soil samples. Results were similar for the other two pathogenic genera (data not shown).

Each of the biocontrol agents selected for use in this study (Table 2) were tested for activity against *Cylindrocarpon*, *Fusarium*, and *Pythium* species *in vitro* before field application into fumigated and nonfumigated plots as described under Plans and Procedures. Preliminary results indicate that RootShield and SoilGard (Fig. 3) and Companion and Actinovate (data not shown) are capable of inhibiting the growth of *Fusarium*. However, only Actinovate has shown inhibitory activity against isolates of *P. irregulare*, *P. sylvaticum*, and *P. ultimum*.

Preliminary analyses of 122 root isolates of *Cylindrocarpon* indicate that at least three species are present: *Cylindrocarpon destructans*, *C. liriodendri*, and *C. pauciseptatum*. Isolates were identified based on growth and colony morphology data. Work is continuing to further identify these isolates based on DNA fingerprinting and to evaluate their pathogenicity to Douglas-fir seedlings in a greenhouse study.

Table 1. Fumigant treatments.

Treatment	Rate of Application	Film Type
Untreated Control		TIF
Methyl Bromide + Chloropicrin	250 lbs/A (50:50)	TIF
Metam Sodium + Chloropicrin	27 gal/A + 150 lb/A	TIF
Pic-Chlor	285 lbs/A (60:40)	TIF

Table 2. Biocontrol agents to be applied at each nursery.

Product Manufacturer	Main Biological Agent	Primary Target Pathogen Complex
A. Companion *Growth Products Ltd.*	*Bacillus subtilis GB03*	*Rhizoctonia, Pythium, Fusarium, Phytophthora*
B. Actinovate *Natural Industries Inc.*	*Streptomyces lydicus*	*Soil-borne disease (Pythium)*
C. SoilGard *Certis USA LLC*	*Gliocladium virens*	*Rhizoctonia and Pythium*
D. RootShield *Bioworks Inc.*	*Trichoderma harzianum*	*Pythium, Rhizoctonia and Fusarium*

Table 3. Biocontrol treatments to be applied within each fumigation treatment plot at each nursery.

Treatment	early June 2011	Late July 2011	October 2011
1. All biocontrol agents	A + B + C + D	A + B + C + D	A + B + C + D
2. Bacteria then fungi	A + B	A + B	A + B + C + D
3. Fungi then bacteria	C + D	C + D	A + B + C + D
4. Untreated control	water drench only	water drench only	water drench only

A = Companion, B = Actinovate, C = SoilGard, D = RootShield

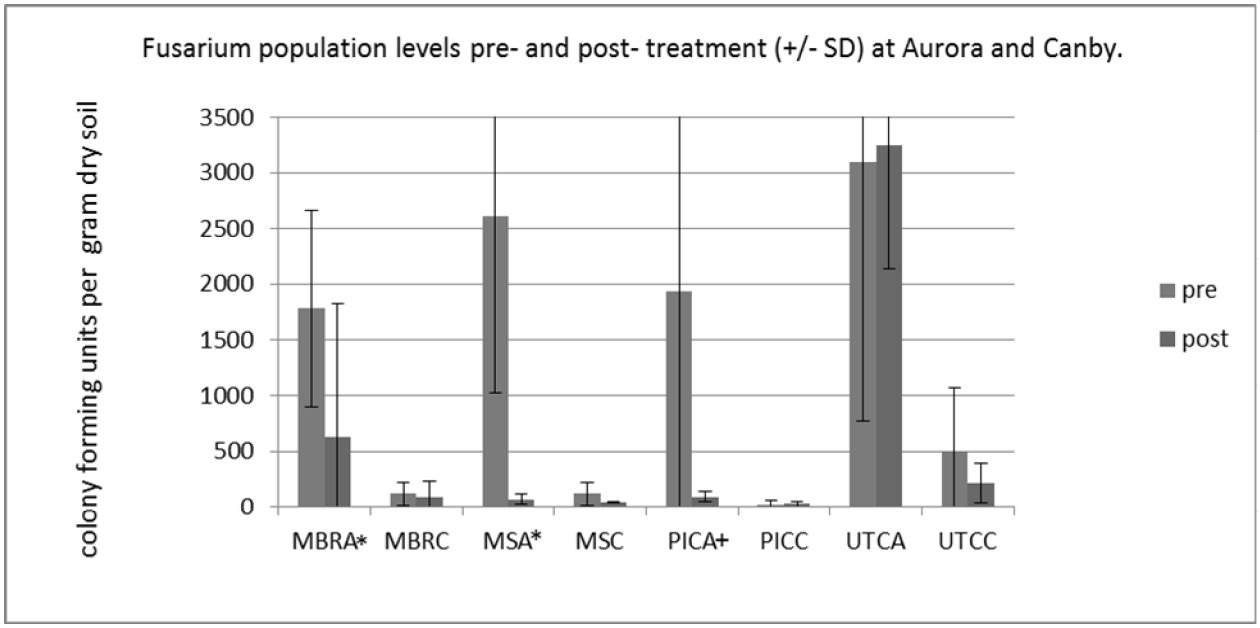

Figure 1. Fusarium population levels pre- and post- treatment at Aurora and Canby Nurseries. MBR -methyl bromide; MS- metam sodium; PIC- Chloropicrin; UTC- solarization only. Suffixes A and C refer to Aurora and Canby nurseries respectively. * post fumigation fusarium levels are significantly lower than pre-treatment levels at alpha=.05; + at alpha =.1. Error bars are standard deviations of the mean.

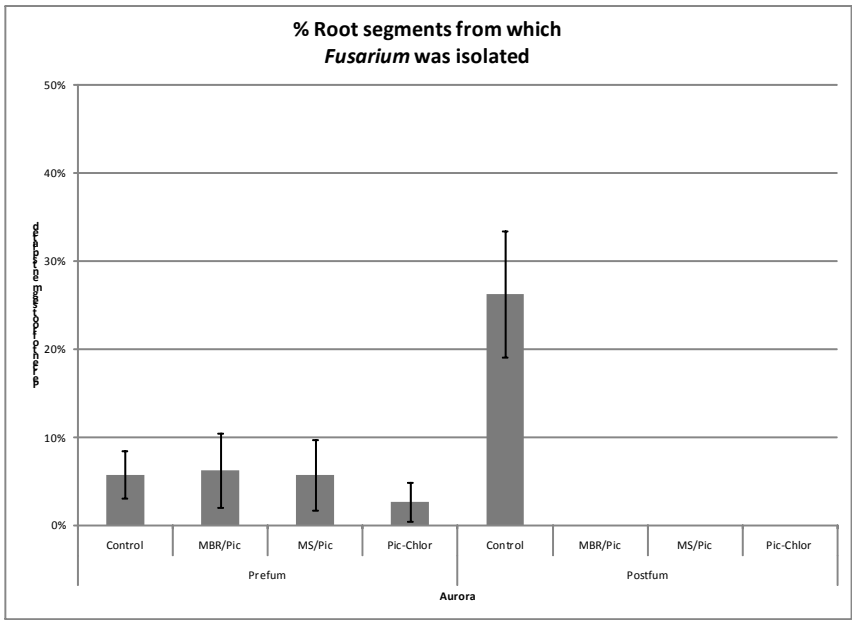

Fig. 2. Percentage of root segments colonized by Fusarium pre- and post-treatment at Aurora Nursery.
MBR/Pic -methyl bromide; MS/Pic- metam sodium; PIC-Chlor Chloropicrin; Control- solarization only

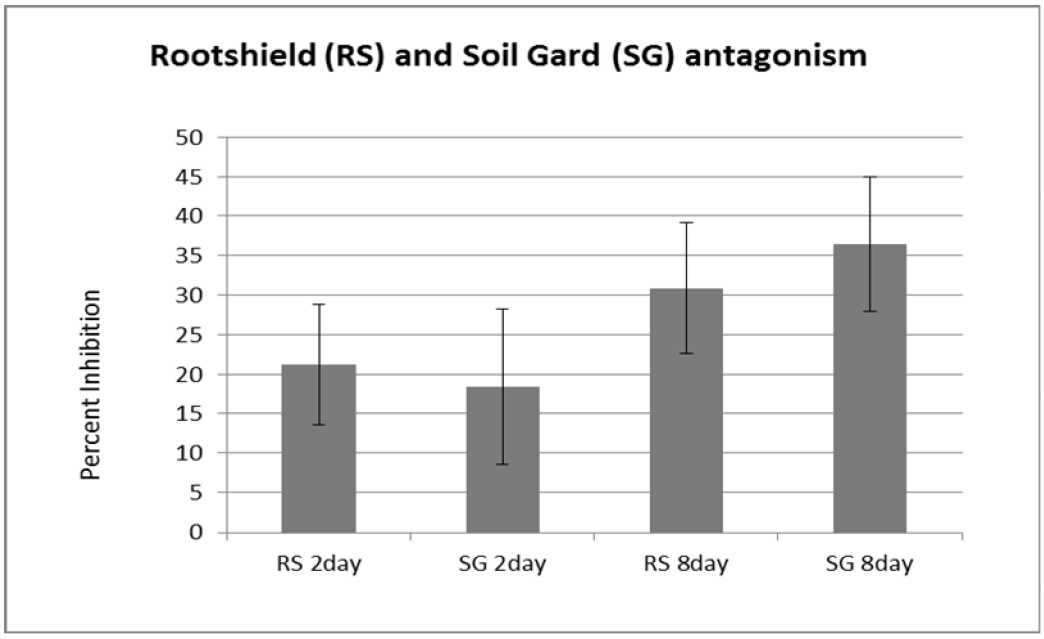

Figure 3. Comparison of biocontrol efficacy of RootShield and SoilGard against Fusarium.