PYTHIUM AND *FUSARIUM* SPECIES ASSOCIATED WITH PRODUCTION OF DOUGLAS-FIR SEEDLINGS

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Introduction. Forest tree nurseries grow tree seedlings that are typically used to regenerate forest lands that have been harvested or destroyed by disease or fire. Seedlings are also grown for the Christmas tree and ornamental nursery industries. Douglas-fir (*Pseudotsuga menziesii*) is the most commonly grown species in the Pacific Northwest. Seeds are generally sown in field plots in April and seedlings harvested from January through March of the following year. Harvested seedlings, most often sold as bareroot, may then be sold directly to the customer or held in cold storage to be transplanted back into the nursery in spring for additional growth.

A typical nursery acre is in production for two years and then left fallow for one season. In late summer of the fallow year, the fields are fumigated and continue in fallow until they are planted the following spring. Traditionally, methyl bromide (MB) + chloropicrin (PIC) at 67:33 has been flat-fumed, shanking 350 lbs/acre and tarped with high density polyethylene (HDPE). Fumigation is primarily targeted towards soilborne pathogens and weeds. Most damage by soilborne pathogens occurs within the first few months following germination as damping off of newly emerged seedlings. Typical pathogens that cause damage include *Cylindrocarpon*, *Fusarium*, *Phytophthora*, and *Pythium* species. Weed species that are often targeted for control include yellow nutsedge (*Cyperus esculentus*), quackgrass (*Elytrigia repens*), and pearlwort (*Sagina* species).

The development of virtually impermeable film (VIF) may allow the application of lower doses of MB alternatives with similar efficacy to higher doses applied under HDPE. The specific objectives of this project are:

1. To compare the efficacy of lower doses of chemical fumigants under VIF to the traditional Methyl Bromide + Chloropicrin treatment under HDPE on soil populations of *Fusarium*, *Pythium*, and existing weed species.
2. To assess the economic viability of each treatment.
3. To conduct educational outreach to project stakeholders.

Approach. Six treatments (Table 1) were applied according to a randomized complete block design with four replicate blocks at three conifer nurseries in western Oregon and Washington. Soil samples were collected just prior to fumigation in early August 2008 and at 1 and 7 months after fumigation. In addition, inoculum packets containing rye inoculated with six *Fusarium* isolates or soil infested with two *Pythium* isolates were buried at each nursery in fumigation plots 1-3 days before fumigation and then removed one month after fumigation. Inoculum packets were buried at 6 and 12 inches below the soil surface. Two-year-old transplants of Douglas-fir (*Pseudotsuga menziesii*) were transplanted into all plots in May 2009. Prior to planting, a subset of transplants were sampled for colonization by *Fusarium* and *Pythium* species. *Fusarium* and *Pythium* populations were assessed from soil, inoculum packets, and root samples by plating on Komada's medium and PARP, which are semiselective media for *Fusarium* and *Pythium* species, respectively. *Fusarium* isolates were sequenced using mitochondrial rDNA (mtSSU) and elongation factor 1-alpha (EF-1α) to characterize *Fusarium* species, with specific emphasis placed on determining the efficacy of fumigation against *Fusarium oxysporum* and *Fusarium commune*, species that are non-pathogenic and pathogenic respectively. *Pythium* isolates were identified on the basis of the internal transcribed spacer (ITS) region.

Results. Average prefumigation counts of *Pythium* for each nursery were greatest at nursery B (45 cfu/g dry soil) and A (40 cfu/ g dry soil), and least at nursery C (19 cfu/g dry soil). Counts of *Pythium* in all fumigant treated plots were reduced by at least 86% one month after fumigation or by at least 95% seven months after fumigation (Fig. 1). Nonfumigated plots also experienced a reduction in *Pythium* counts by 60-92% one month after fumigation or by 68-98% seven months after inoculation. Analyses of variance on the counts of *Pythium* indicated an effect of treatment one month after fumigation ($P = 0.036$), but not after seven months ($P = 0.313$). Only nonfumigated control treatments had significantly less reduction in *Pythium* counts than those measured from plots treated with any of the five fumigant treatments. No fumigant treatment was significantly different from the conventional application of methyl bromide/chloropicrin. No difference in efficacy was observed between HDPE and VIF. *Pythium irregulare*, *P. dissotocum*, and *P. macrosporum* are predominate at all three nurseries.

At Nursery A, *Fusaria* present in plots fumigated with alternative fumigants were not significantly different than plots fumigated with methyl bromide (Figure 2). Nusery B showed no significant difference in percent *Fusaria* present between alternative treatments and methyl bromide with the exception of methyl iodide (VIF) which had higher percent *Fusaria* present. At Nursery C, only DMDS had a significantly higher percent *Fusaria* present than methyl bromide and all other treatments showed no significant difference . Only nursery A showed differences in percent *Fusaria* present as a function of depth (Figure 2). The soil data were inconclusive, though Nursery A and Nursery B showed a trend of alternative chemical treatments working as well as methyl bromide as controls for *Fusarium spp* (Figure 3). Higher levels of *F. oxysporum* root colonization were found at Nursery C on some seedlots (0 - 23%) compared to Nursery A (0 – 15%) and B (2 – 13%). A preliminary pathogenicity test was run against these root isolates using stratified Douglas-fir seed. Most isolates caused extensive pre- and post-emergent disease relative to non-inoculated controls (Table 2).

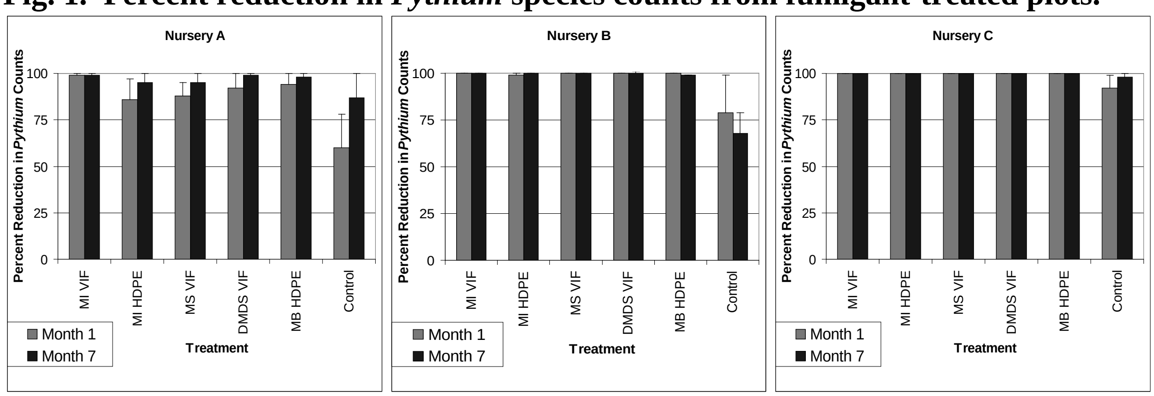
Table 1. Fumigant treatments.

Treatment	Rate of Application	Film Type
Untreated Control		
Methyl Bromide + Chloropicrin	350 lbs/A (67:33)	HDPE
Methyl Iodide + Chloropicrin	244 lbs/A (50/50)	VIF
Methyl Iodide + Chloropicrin	244 lbs/A (50/50)	HDPE
Metam Sodium + Chloropicrin	50 gal/A + 122 lb/A	VIF
DMDS + Chloropicrin (Paladin)	60 gal/A (453 lb + 120 lb)	VIF

Table 2. Various *Fusarium* species isolated from pre-transplant DF seedlings at various nurseries (A, B, C) and their impact on DF seed germination in vitro.

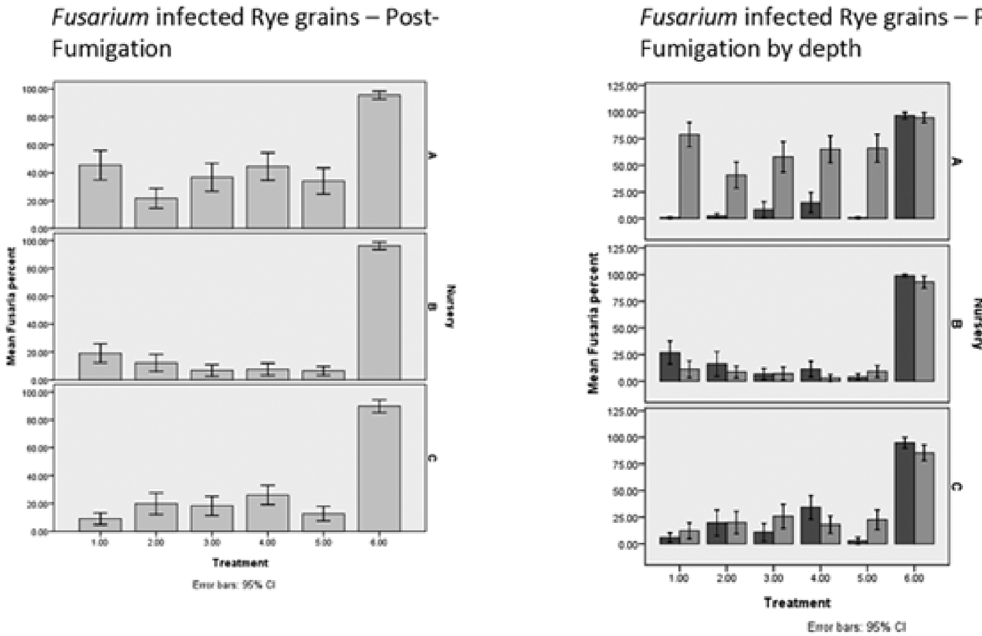
Identity	Origin	% healthy germ
Fusarium roseum	A	33
Fusarium oxysporum	A	28
Fusarium roseum	A	17
Fusarium oxysporum	A	6
Fusarium oxysporum	A	6
Fusarium oxysporum	A	6
Fusarium oxysporum	A	6
Fusarium roseum	A	11
Fusarium	A	6
Fusarium solani	B	0
Fusarium oxysporum	B	6
Fusarium oxysporum	B	28
Fusarium oxysporum	B	6
Fusarium oxysporum	B	17
Fusarium oxysporum	B	0
Fusarium oxysporum	B	11
Fusarium oxysporum	B	0
Fusarium oxysporum	B	28
Fusarium oxysporum	B	11
Fusarium proliferatum	B	0
Fusarium oxysporum	C	0
Fusarium oxysporum	C	44
Fusarium oxysporum	C	11
Fusarium roseum	C	50
Fusarium roseum	C	0
Control	none	100

Fig. 1. Percent reduction in *Pythium* species counts from fumigant-treated plots.



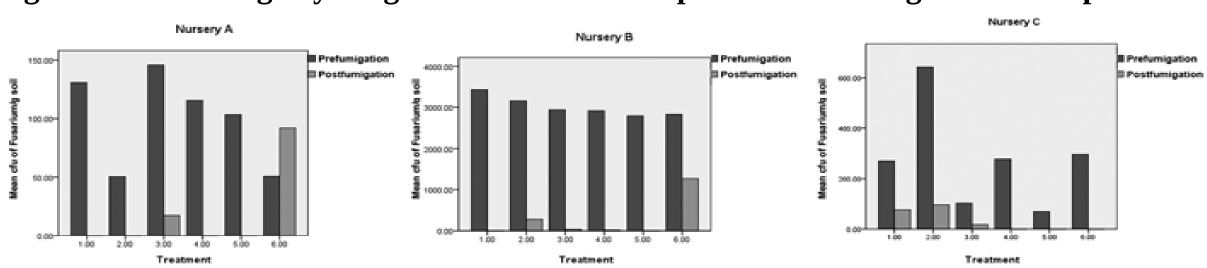
MI = methyl iodide, MS = metam sodium, DMDS = dimethyl disulfide, MB = methyl bromide, VIF = virtually impermeable film, HDPE = high density polyethylene.

Fig. 2. Percent *Fusaria* present in inoculum packets buried in fumigant-treated plots.



Treatment 1 = Methyl Iodide + Chloropicrin (VIF), 2 = Metam Sodium + Chloropicrin (VIF), 3 = Methyl Iodide + Chloropicrin (HDPE), 4 = DMDS + Chloropicrin (VIF), 5 = Methyl Bromide + Chloropicrin (HDPE), 6 = Untreated Control (none).

Fig. 3. Mean CFU/g dry weight soil of *Fusarium* species from fumigant-treated plots.



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