

# The effects of methyl bromide alternatives on soil and seedling pathogen populations, weeds, and seedling morphology in Oregon and Washington forest nurseries

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**Abstract:** Five fumigation treatments (a conventional methyl bromide–chloropicrin application and four reduced-rate alternative fumigant treatments) and a nonfumigated treatment were evaluated at two forest nurseries in Oregon and one forest nursery in Washington for their effects on soil pathogen populations, weeds, and seedling morphology during a 2-year study. The effect of plastic tarp composition on fumigant efficacy was also evaluated (virtually impermeable film versus high-density polyethylene). All fumigant treatments reduced soil populations of *Fusarium* and *Pythium* for up to 7 months after fumigation and resulted in seedlings with significantly less pathogen colonization than those from the nonfumigated treatment. All fumigant treatments were more effective against pathogen inoculum buried at 15 cm rather than at 30 cm. *Fusarium commune* Skovgaard, O'Donnell et Nirenberg, *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen, isolates from the *Gibberella fujikuroi* Saw. complex, *Pythium irregulare* Buisman, *Pythium aff. spiculum* B. Paul 2006, *Pythium sylvaticum* Campbell & Hendrix, and *Pythium 'vipa'* Hermansen & Klemsdal were the most commonly isolated pathogens. Weed biomass and weeding times were significantly reduced by fumigation, but only at the Washington nursery with high weed pressure. No significant differences were observed in efficacy between the conventional methyl bromide–chloropicrin treatment and any of the reduced rate fumigants or between the two types of plastic tarp. Conifer seedling height, diameter, shoot volume, and root volume were significantly greater in all fumigated treatments compared with the nonfumigated treatment.

**Résumé :** Cinq traitements de fumigation (une application conventionnelle de bromure de méthyle et de chloropicrine et quatre autres traitements de fumigation à taux réduit) ainsi qu'un traitement sans fumigation ont été évalués dans deux pépinières forestières en Oregon et une autre pépinière forestière dans l'État de Washington. L'étude a duré deux ans et a porté sur l'effet des traitements sur les populations de pathogènes du sol, les mauvaises herbes et la morphologie des semis. L'effet de la composition de la bâche en plastique sur l'efficacité du fumigant a aussi été évalué (pellicule pratiquement imperméable versus polyéthylène haute densité). Tous les traitements de fumigation ont réduit les populations de *Fusarium* et de *Pythium* pendant une période dont la durée pouvait aller jusqu'à sept mois après la fumigation et les semis étaient significativement moins colonisés par les pathogènes que ceux qui n'avaient pas été fumigés. Tous les traitements de fumigation ont été plus efficaces contre l'inoculum des pathogènes enfoui à 15 cm plutôt qu'à 30 cm. *Fusarium commune* Skovgaard, O'Donnell et Nirenberg, *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen, des isolats appartenant au complexe *Gibberella fujikuroi* Saw., *Pythium irregulare* Buisman, *Pythium aff. spiculum* B. Paul 2006, *Pythium sylvaticum* Campbell & Hendrix et *Pythium « vipa »* Hermansen & Klemsdal sont les pathogènes qui ont été le plus souvent isolés. La fumigation a réduit de façon significative la biomasse des mauvaises herbes et la durée du désherbage mais seulement dans la pépinière de l'État de Washington où la pression des mauvaises herbes était forte. Aucune différence significative d'efficacité n'a été observée entre le traitement conventionnel, avec le bromure de méthyle et la chloropicrine, et n'importe quel des traitements de fumigation à taux réduit, ni entre les deux types de bâche en plastique. La hauteur, le diamètre, le volume des pousses et le volume des racines des semis de conifère avaient des valeurs significativement plus élevées dans tous les traitements de fumigation comparativement au traitement sans fumigation.

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## Introduction

Forest nurseries of the Pacific Northwest (PNW) of the United States (Oregon, Washington, and Idaho) produce field-grown bareroot tree seedlings to regenerate lands that have been harvested or destroyed by diseases, insects, or fire. These nurseries also provide seedlings for the Christmas tree and ornamental nursery industries. Many tree species are grown. However, most of the approximately 100 million conifer seedlings sold and subsequently planted are 2-year-old transplants of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (2010 industry sales data, unpublished).

Soilborne pathogens pose a significant challenge to tree seedling production in the PNW. Species within the genera *Cylindrocarpon*, *Fusarium*, *Phytophthora*, and *Pythium* cause damping off of seeds and young seedlings, as well as root rot in older plants when soil moisture is abundant (Dumroese and James 2005). Although pathogen soil populations have traditionally been assessed in a number of fumigation and (or) forest nursery studies, there are few reports that identify these genera to the species level. Knowledge of pathogen species identity is critical for determining which species cause the greatest economic damage and for the development of integrated pest management (IPM) practices that target the pathogens of interest. Targeted IPM practices are expected to become increasingly important to the forest nursery industry as the use of fumigants with broad activity against multiple pathogens becomes restricted due to increased state and federal regulations.

Weeds are also of concern in forest nurseries. In addition to competing with seedlings for nutrients, light, and water, some weed species such as yellow nutsedge (*Cyperus esculentus* L.) are considered quarantine pests in Oregon and Washington. By eliminating the weed seed bank in forest nursery soils via fumigation prior to planting, nursery managers can limit the number of subsequent herbicide applications and reduce costs associated with hand-weeding.

Historically, the fumigant methyl bromide (MB), in combination with chloropicrin (Pic), has been applied to forest nursery fields under high-density polyethylene (HDPE) plastic tarp to reduce soilborne pathogens and weed populations (Leon 2009). Many nurseries in the PNW crop nursery beds for two years and then leave the beds fallow for one season. In late summer of the fallow year, these beds are fumigated and continue in fallow until they are planted the following spring. From 2004 to 2006, approximately 95 ha of bareroot conifer nursery soil in the PNW were fumigated with MB each year under critical use exemption (CUE) permits (Environmental Protection Agency 2010). However, based on the terms of the United States Clean Air Act and the Montreal Protocol (Environmental Protection Agency 2009), the use of this fumigant under the CUE rule is expected to cease. As a consequence, forest nurseries have focused efforts on finding fumigants other than MB to meet their disease and weed management objectives without compromising seedling quality. Recently, new plastic chemistries such as virtually impermeable film (VIF) have become available. These low-permeability plastics increase fumigant retention in the soil, reduce emissions, and might allow reduced-rate fumigant formulations to be as efficacious in pathogen and weed control as full-rate applications under traditional HDPE plastic.

A study was initiated in 2008 to identify technically and economically viable, reduced-rate fumigant alternatives to MB. Objectives were to (i) compare the effectiveness of the standard MB/Pic fumigant in reducing pathogen and weed populations with those of reduced-rate alternative fumigants, (ii) contrast fumigant efficacy on eradication of inoculum buried at 15 and 30 cm depths, (iii) identify soilborne pathogen species in forest nurseries of Oregon and Washington, (iv) compare efficacy of VIF with HDPE when applied over the same reduced-rate formulation of methyl iodide (MI) on pathogen and weed populations, and (v) quantify seedling morphology, root infection, packable yields, and economic impact of alternative fumigants under reduced-rate management regimes.

## Materials and methods

### Nurseries

Field trials were established at one forest nursery in Washington (nursery A) and two forest nurseries in Oregon (nurseries B and C). Soil at nursery A is classified as a Cagey loamy sand, and soils at nurseries B and C are both classified as Canderly sandy loam. The last crop of Douglas-fir seedlings at each nursery was harvested by March 2008. Fields at nurseries A and B were then bare fallow until late July 2008. A sorghum-Sudan (*Sorghum bicolor* L.) cover crop was planted at nursery C in April 2008 and then chopped and tilled 4 weeks prior to fumigation. Soil organic matter content (OM), pH, cation exchange capacity (CEC), bulk density, and moisture content were determined at four locations in each nursery field. I-button temperature recorders (Maxim Integrated Products, Inc., Sunnyvale, California) recorded soil temperature under HDPE and VIF tarps at 15 and 30 cm depths and under bare soil (no plastic tarp) at 20 cm depth. Site weather data from July 2008 to December 2009 were gathered at each nursery.

### Experimental design

Six fumigation treatments (Table 1), including a conventional MB/Pic application and a nonfumigated treatment under HDPE as controls, were applied at each nursery in a randomized complete block design with four replicate blocks in early August 2008. Each treatment plot was approximately 12 × 46 m (nonfumigated plots 12 × 30 m). Fumigants were selected based on their efficacy in prior industry research trials and on the consensus of the nurseries involved. Reduced rates for each fumigant were selected to meet 2008 Environmental Protection Agency guidelines for a 30 m buffer zone. Twenty days after fumigation, approximately 40% of the VIF was fragmented due to the absence of a UV inhibitor in the material. Therefore, both HDPE and VIF plastic tarps were removed at that time. One-year-old Douglas-fir seedlings were transplanted into each nursery in April 2009. Soil and seedling samples were collected as described below.

### Pathology analyses

#### Sample collection

Nursery soils were sampled four times during the experiment: 1 week before fumigation (prefum); 1 month after fumigation (postfum); 1–2 weeks before planting (preplant);

**Table 1.** Fumigation treatments applied in August 2008 according to a randomized complete block design with four replicate blocks at three forest nurseries.

Treatment	Application rate	Plastic film type
NF HDPE	None	High-density polyethylene
MB/Pic HDPE	392 kg/ha (67:33)	High-density polyethylene
MI/Pic HDPE	273 kg/ha (50:50)	High-density polyethylene
MI/Pic VIF	273 kg/ha (50:50)	Virtually impermeable film
MS/Pic VIF	468 L/ha + 137 kg/ha	Virtually impermeable film
DMDS/Pic VIF	561 L/ha (205 kg + 54 kg)	Virtually impermeable film

**Note:** NF, nonfumigated; HDPE, high-density polyethylene; MB, methyl bromide; Pic, chloropicrin; MI, methyl iodide; VIF, virtually impermeable film; MS, metam sodium; DMDS, dimethyl disulfide.

and at the end of the growing season in late October – early November 2009 (postplant). Samples were collected by taking twenty 2 cm diameter soil cores in a randomized pattern to a depth of 30 cm from each treatment plot. Soil samples were bulked by plot and mixed thoroughly to generate 24 composite samples from each nursery. Each composite sample was then divided to provide separate samples for *Fusarium* and *Pythium* analyses. Soil samples were stored at 4 °C until assays were completed.

Douglas-fir seedlings were sampled prior to planting (preplant) and at the end of the growing season (postplant). Two sets of 10 preplant and 25 postplant seedlings per treatment plot were collected according to a randomized design for assays of *Fusarium* and *Pythium* root colonization, respectively. Seedlings were stored at 4 °C until assays were completed.

### Soil and seedling pathogen populations

#### *Fusarium*

**Soil** *Fusarium* colonies from soil samples were enumerated on Komada's medium (Komada 1975), and colony-forming units (CFU/g) were determined on a dry mass soil basis. One gram of soil from each composite sample was diluted in 80 mL of 0.1% water agar, and a 0.40 mL aliquot of the soil-water agar slurry was placed in three replicate Petri plates. Prepared Komada's medium was cooled to 38 °C, poured into plates containing the slurry, and then mixed by gently stirring the plates. Plates were then placed in an incubator at 25 °C with 16 h-day<sup>-1</sup> of fluorescent light for 1 week. Isolates were identified as described below.

**Seedlings** Roots of each seedling were washed free of soil, cut into ten 1 cm long segments, sanitized in 10% Clorox for 10 min, and rinsed in distilled water. Root segments were then plated on Komada's medium and incubated as described above, and the percentages of *Fusarium*-positive root segments for each seedling were calculated.

**Species identification** Single-spore *Fusarium* isolates (47–92 isolates from each nursery) were initially grown on potato dextrose agar (PDA) for 1 week. Approximately 100 mg of mycelia per isolate was homogenized in a 2.0 mL Lysing Matrix A tube (MP Biomedical, Irvine, California) containing one 6.35 mm ceramic sphere, 400 µL stock solution Buffer AP1, and 4 µL RNase A from a Qiagen DNeasy Plant Mini Kit (Valencia, California) using a Bio101 (LaJolla, California) FastPrep #FP120 machine at speed setting 4.0 for 20 s. Steps 8–18 of the kit protocol were then

followed with these modifications: samples were not incubated on ice after step 9; 600 µL of Buffer AP3 were added to the lysate in step 13; 50 µL Buffer AE were added to the DNeasy Mini Spin Column in step 18; and the second aliquot of Buffer AE in step 19 was omitted to increase DNA yield.

One microlitre of DNA extract was added to a 29.1 µL PCR reaction mixture containing 1× PCR buffer (USB Corporation, Cleveland, Ohio), 0.2 mmol/L dNTPs, 1.5 mmol/L MgCl<sub>2</sub>, 0.1 U *Taq* polymerase, and 3 µmol/L each of translation elongation factor 1α (EF-1α) primers EF1 and EF2 (O'Donnell et al. 1998) or mitochondrial small subunit (mtSSU) primers MS1 and MS2 (White et al. 1990). Amplification was performed in a Bio-Rad MyCycler Personal Thermal Cycler (Hercules, California) following the temperature profiles of Stewart et al. (2006). PCR products were electrophoresed in 0.83% TBE agarose gels containing 2 µL of 1:10 SYBR Green I (Sigma-Aldrich, St. Louis, Missouri), purified using ExoSAP-IT (USB Corporation, Cleveland, Ohio), and then sequenced at the University of Washington Department of Biochemistry DNA Sequencing Facility (Seattle, Washington). *Fusarium* sequences were compared with those available in GenBank using BLAST to identify each isolate to species. Isolate identity was confirmed on the basis of spore characteristics (Leslie and Summerell 2006) from cultures grown on Spezieller Nährstoffarmer Agar (SNA) (Singleton et al. 1992).

#### *Pythium*

**Soil** Ten grams of soil from each composite sample were added to 90 mL of 0.2% water agar and shaken for 45 min at 150 rpm. Aliquots of the suspension (0.5 mL) were then spread on 10 Petri plates containing PARP, a semiselective medium for Pythiaceae species (Kannwischer and Mitchell 1978). Plates were incubated at room temperature for 2 days, and the number of *Pythium* isolates per plate were counted and expressed as CFU/g on a dry mass soil basis.

**Seedlings** Roots of each seedling were rinsed under running tap water for 10 min, and ten 1 cm long segments from each seedling were plated on PARP. Plates were incubated at room temperature for 2 days, and the percentages of *Pythium*-positive root segments for each seedling were calculated.

**Species identification** *Pythium* isolates obtained from preplant and postplant seedling roots (50–80 randomly selected isolates from each nursery) were identified on the

basis of the internal transcribed spacer (ITS) region and morphology according to methods described previously (Weiland 2011). *Pythium* isolates obtained from prefumigation soil samples (100 randomly selected isolates from each nursery) were identified and reported previously (Weiland 2011).

### **Buried inoculum experiment**

#### *Fusarium*

Three *Fusarium* isolates obtained from cull pile seedlings and three isolates from prefumigation soil samples were collected from each nursery for the production of buried inoculum bags. Each isolate was cultured on SNA medium prior to inoculating 500 mL jars containing sterilized rye seed (15 g rye seed suspended in 70 mL Komada's medium without agar and autoclaved for 60 min at 15 psi). Inoculated rye seed was incubated for 2 weeks at 25 °C before transferring each isolate into individual 2.54 cm<sup>2</sup> nylon bags. Inoculum was then incubated in the nylon bags for another week at 25 °C. Isolate viability was assessed prior to burial by plating rye grains from each jar onto Komada's medium. Six inoculum bags for each nursery were attached to a plastic strip and stored at 3 °C for 1–4 days before burial. Isolates were buried in the same nursery from which they were originally collected to prevent pathogen movement between nurseries. Inoculum bag strips were buried within each treatment plot at depths of 15 and 30 cm 1–3 days before fumigation. Soil was replaced and tamped to approximate the undisturbed soil bulk density.

Inoculum bags were recovered from the soil 1 month after fumigation. Twenty rye seeds per bag were plated onto Komada's medium and the percentage of seeds yielding *Fusarium* was recorded. Isolate identity was confirmed with DNA analysis.

#### *Pythium*

One *Pythium* isolate obtained from cull pile seedlings was collected from each nursery for the production of buried inoculum bags (nursery A, *P. irregulare* Buisman; nursery B, *P. macrosporum* Vaartaja & van der Plaats-Niterink; and nursery C, *P. dissotocum* Drechsler). Inoculum was produced by growing a single-spore culture of each isolate on 20 mL plates containing V8 juice agar (200 mL clarified V8 juice in 800 mL distilled water and 17 g agar) for 7 days at room temperature. Colonized agar from each plate was then cut into approximately 20 equal-sized pieces and placed into a 52.5 × 20 × 11.9 cm autoclavable polyethylene spawn bag with a contaminant barrier filter (Fungi Perfecti, Olympia, Washington) filled with 3 L of dilute V8 juice (75 mL V8 juice per 1 L DI) and vermiculite (700 mL dilute V8 juice plus 1000 mL vermiculite) that had been autoclaved three times at 48 h intervals. Spawn bags were then incubated in the dark for 60 days at 20 °C with periodic mixing of contents by hand. *Pythium* inoculum was then removed from the bags and placed on individual trays to air dry for 3 days, followed by storage in resealable polyethylene bags at 20 °C for 3 days until used in buried inoculum preparation. Isolate viability was assessed by plating approximately 2 cm<sup>3</sup> of colonized vermiculite onto PDA. Soil collected from each nursery for inoculum production was autoclaved twice at 48 h intervals, and colonized vermiculite was mixed with the soil at a

1% rate (v/v) using twin-shell stainless steel dry blenders. Inoculum bags for burial were prepared by filling fine nylon mesh bags (Kayser-Roth Corp., Greensboro, North Carolina) with 50 cm<sup>3</sup> of the inoculum mixture. The bags were twisted and inverted twice to create a tripled outer layer, knotted at the end, and then stored at 4 °C for 4–6 days before burial in their respective field plots. Isolates were buried in the same nursery from which they were originally collected to prevent pathogen movement between nurseries. Four bags were buried within each treatment plot at depths of 15 and 30 cm 1–3 days before fumigation. Soil was replaced and tamped to approximate the undisturbed soil bulk density.

Inoculum bags were recovered from the soil 1 month after fumigation. Infested soil from each inoculum bag was assayed for each *Pythium* isolate by plating 0.5 mL of a 10% soil solution (w/v) in 0.2% water agar onto each of five plates containing PARP. Plates were incubated at room temperature for 2 days, and the number of *Pythium* isolates per plate was counted.

### **Weed analyses**

Weed sampling was conducted in November 2008 prior to any glyphosate application and in June, August, and October 2009 after seedling planting and the application of pre-emergent herbicides had occurred. Two 50.8 × 50.8-cm (0.26 m<sup>2</sup>) quadrats were placed approximately 4.5 or 6.1 m from the midpoint of each nonfumigated or fumigated plot, respectively, on either side of the midpoint. Weeds were identified to species at all sampling dates, and aboveground biomass was taken during the June, August, and October sampling. Weeds within each quadrat were clipped at the soil surface and dried at 75 °C for at least 3 days, and the resulting dry mass was recorded. Two separate beds within each plot were sampled at each time, resulting in a total of four quadrats per plot for each sampling date. Hand-weeding of plots was conducted separately at each site by nursery maintenance crews during the 2009 growing season to achieve a weed-free condition: two weedings at nursery C (20 May and 17 July) and once each for nurseries A and B (25–26 June and 19 June, respectively). Weeding time for each plot was recorded, and total weeding time per treatment per 100 lineal bed feet (LBF) was calculated (minutes per person per plot). To minimize impact on weed measurements, weed counts and biomass sampling occurred prior to hand-weeding at nurseries A and B and 1 month after the first hand-weeding at nursery C.

### **Seedling morphology analyses**

Douglas-fir seedlings were collected prior to planting in April 2009 (preplant) and at the end of the growing season in late October – early November 2009 (postplant). At each time period, 10 preplant or 25 postplant seedlings per treatment plot were collected according to a randomized design. Seedling height (cm), stem diameter (mm), shoot volume (g), and root volume (g) were measured, and seedling height–stem diameter ratio and root–shoot volume ratio were calculated.

### **Grading and cost analyses**

Twenty-five seedlings from each treatment plot and nursery were lifted in October–November 2009 and measured for

stem diameter, height, root–shoot ratio, and root infection by *Fusarium*. A standard set of cull parameters, below which a seedling would be expected to be discarded, was used to estimate packable yields. These parameters were set at 30.5 cm height, 6 mm stem diameter, and a root–shoot ratio of 0.3 (morphology grade). The percentage of seedlings with  $\geq 20\%$  *Fusarium* root infection (pathology grade) was calculated as described above. This estimate was based on our experience at these nurseries in which seedlings with  $\geq 20\%$  *Fusarium* colonization exhibited increased seedling mortality in storage and reduced establishment performance.

Seedling quality was also operationally graded by each nursery using their independent commercial standards. In February–March 2010, crews lifted seedlings from a 15 m long section from the center bed of each plot. Seedlings  $< 30.5$  cm height,  $< 6$  mm stem diameter, or with obvious defects were counted as operational culls at nurseries B and C, whereas nursery A graded seedlings  $< 25.4$  cm height and  $< 5$  mm stem diameter as culls. Operational culls tracked by this method were considered as an independent stakeholder-based assessment of actual seedling loss for each treatment in comparison with the morphology and pathology cull estimates described above.

The average cost of each treatment, including plastic tarp application, was determined for every 1000 seedlings produced. Cull percentages from each of the three grades were used to calculate the expected number of culls for each treatment at each nursery. These numbers were then subtracted from the initial number of planted seedlings per hectare to estimate the total number of packable seedlings per hectare. Fumigant application costs from 2010 (Table 5) were then applied to these totals to arrive at a final fumigation cost.

### Statistical analyses

*Fusarium* and *Pythium* field soil populations and counts from the buried inoculum experiment were analyzed using the Mann–Whitney, Kruskal–Wallis, and Scheirer–Ray–Hare tests for effects of nursery, time, treatment, and factor interactions (Sokal and Rohlf 1995). These methods are nonparametric approaches for comparing independent groups of sampled data and are used for data that do not meet normal distribution and equal variance assumptions. *Fusarium* and *Pythium* seedling root colonization were calculated as the proportion of root segments from which each pathogen was cultured. Proportions were transformed by the arcsine of the square root to homogenize variances before being subjected to mixed-model ANOVA, with treatment and time as fixed effects and nursery and block as random effects. Because of differences in weeding regimes at each nursery, weed data were only analyzed for effects of treatment using ANOVA for each nursery and time period separately. Seedling morphology data were analyzed using a mixed-model ANOVA, with treatment as a fixed effect and nursery and block as random effects. All ANOVA computations were separated using Fisher's protected least significant difference statistic and Tukey's test for multiple comparisons at  $p \leq 0.05$ . Analyses were performed using Minitab Statistical Software (release 15; Minitab Inc., State College, Pennsylvania), SAS 9.2 (SAS Institute Inc., Cary, North Carolina), or SPSS Statistics (version 17.0; SPSS Inc., Chicago, Illinois).

## Results

### Soil parameters

Soil physical and chemical properties before fumigation were generally similar among the three nurseries: OM =  $5.0\% \pm 0.8\%$  (mean  $\pm$  standard error); pH =  $5.6 \pm 0.2$ ; CEC =  $5.9 \pm 1.3$  meq/100 g; bulk density at 15 cm =  $1.03 \pm 0.04$  g/cm<sup>3</sup>; bulk density at 30 cm =  $1.13 \pm 0.09$  g/cm<sup>3</sup>; and moisture content =  $15.7\% \pm 1.5\%$ . Representative soil temperatures from nursery B ranged from 17 to 38 °C and 17 to 41 °C at 15 cm depth under HDPE and VIF, respectively. At 30 cm depth, soil temperatures ranged from 18 to 31 °C and 19 to 32 °C under HDPE and VIF, respectively. Temperatures under bare soil (no plastic tarp) ranged from 17 to 26 °C (20 cm depth). Data were similar for the other nurseries (data not shown). After the VIF tarp disintegrated at the three nurseries, soil temperature profiles in the affected plots were similar to nontarped soil.

### Pathology analyses

#### Soil and seedling pathogen populations

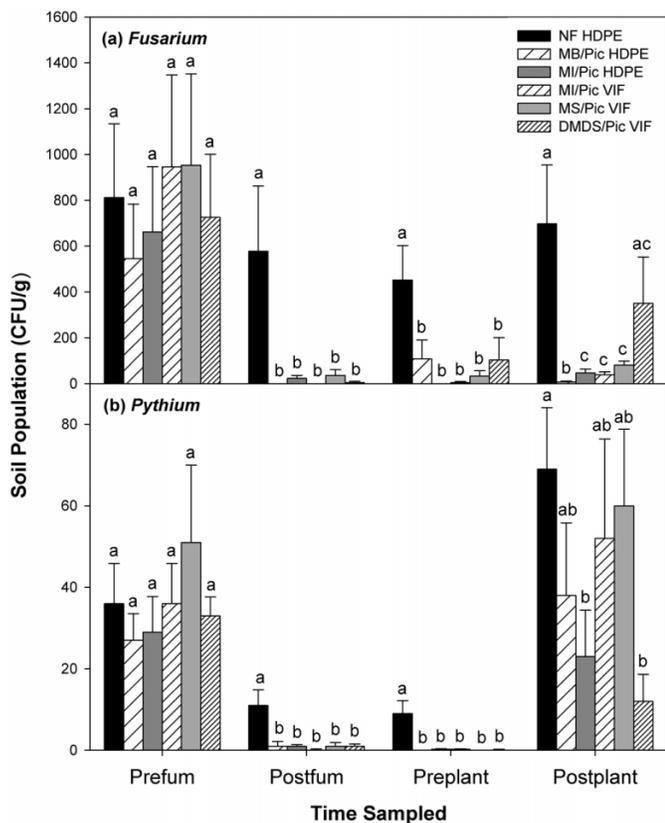
##### *Fusarium*

**Soil** Before fumigation (prefum), *Fusarium* populations (mean  $\pm$  standard error) were greater at nursery C ( $1958 \pm 241$  CFU/g) than at nurseries A ( $93 \pm 29$  CFU/g) or B ( $272 \pm 67$  CFU/g). Differences between each nursery were significant ( $p < 0.001$ ). Population means (pooled nursery data) were similar among treatments ( $p = 0.848$ ) (Fig. 1a), and no nursery  $\times$  treatment interaction ( $p = 0.994$ ) was observed.

One month after fumigation (postfum), *Fusarium* populations were reduced by at least 87% in all fumigant treatments ( $p < 0.001$ ). These populations remained low until 1–2 weeks before planting (preplant,  $p \geq 0.167$ ) (Fig. 1a). In contrast, populations in nonfumigated plots were similar to those observed prior to fumigation and remained relatively constant ( $88$ – $1509$  CFU/g) until 1–2 weeks before planting ( $p \geq 0.323$ ). Nonfumigant treatment populations were greater than those from any of the fumigant treatments at either sampling date ( $p \leq 0.015$ ). No difference in efficacy was observed between each reduced-rate fumigant and the conventional application of MB/Pic HDPE ( $p \geq 0.071$ ) or between HDPE and VIF of the MI/Pic treatments ( $p \geq 0.071$ ). There were no effects of nursery ( $p \geq 0.092$ ), and no factor interactions were observed ( $p \geq 0.834$ ).

At the end of the growing season (postplant), *Fusarium* populations varied significantly by fumigant treatment ( $p = 0.001$ ) (Fig. 1a). The MB/Pic HDPE treatment provided the best control for *Fusarium*. Soil populations for this treatment were similar to those present before planting ( $p \geq 0.149$ ) and were always less than those of the nonfumigant and reduced-rate fumigant treatments ( $p \leq 0.046$ ). Treatments MI/Pic HDPE, MI/Pic VIF, and MS/Pic VIF provided the second-best control for *Fusarium*. Soil populations in each of these treatments were similar ( $p = 0.133$ ) and had increased 40 CFU/g, on average, over the populations present before planting ( $p \leq 0.021$ ). Soil populations in the DMDS/Pic VIF treatment were the greatest of any of the fumigant treatments and had increased 250 CFU/g, on average, over those present be-

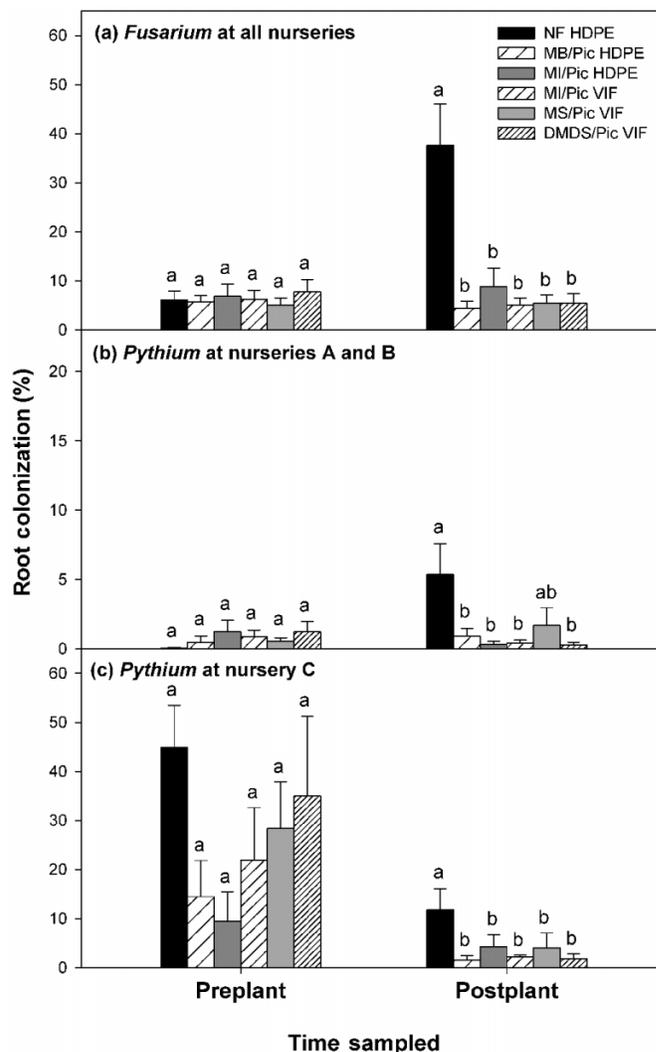
**Fig. 1.** Means (+ standard error) of (a) *Fusarium* and (b) *Pythium* soil populations before soil fumigation in early August 2008 (Prefum), 1 month after fumigation (Postfum), 1–2 weeks before planting seedlings in April 2009 (Preplant), and at the end of the growing season in October–November 2009 (Postplant) among six fumigation treatments at three forest nurseries ( $n = 12$ ). Treatments labeled with the same letter within each time period are not significantly different ( $p < 0.05$ ) according to Mann–Whitney and Kruskal–Wallis nonparametric statistical tests. Note differences in scales between Figs. 1a and 1b.



fore planting ( $p = 0.008$ ). However, most of the population increase occurred at nurseries B and C, not nursery A (data not shown). Although *Fusarium* populations in DMDS/Pic VIF were not significantly different from those of the other reduced-rate fumigant treatments ( $p = 0.094$ ), they were also not significantly different from those in the nonfumigated treatment ( $p = 0.174$ ). Despite the population increase in each reduced-rate fumigant treatment since planting, these populations were always less than those observed before fumigation ( $p \leq 0.018$ ). *Fusarium* populations in the nonfumigated treatment were always greater than those in any fumigant treatment ( $p < 0.001$ ), except for DMDS/Pic VIF as noted, and had remained relatively constant throughout the study ( $p \geq 0.563$ ). No differences were observed between HDPE and VIF of the MI/Pic treatments ( $p \geq 0.953$ ). An effect of nursery ( $p = 0.008$ ) was observed due to the larger population increase in DMDS/Pic VIF treatment at nurseries B and C as noted, which led to a nursery  $\times$  treatment interaction ( $p = 0.001$ ).

**Seedlings** Before planting (preplant), seedling roots at all three nurseries were infrequently colonized by *Fusarium*

**Fig. 2.** Means (+ standard error) of (a) *Fusarium* and (b and c) *Pythium* Douglas-fir root colonization at planting in April 2009 (Preplant) and at the end of the growing season in October–November 2009 (Postplant) among six fumigation treatments at three forest nurseries ( $n = 12$ ). Treatments labeled with the same letter within each time period are not significantly different ( $p < 0.05$ ) according to Tukey's test for multiple comparisons. Note differences in scales between Figs. 2a, 2b, and 2c.



(1%–14%) (Fig. 2a), and no effects of nursery, treatment, or interaction were observed ( $p \geq 0.103$ ). At the end of the growing season (postplant), colonization in all fumigant treatments was the same as that observed before planting ( $p = 0.918$ ). Colonization in nonfumigated plots, however, had at least quadrupled since planting ( $p = 0.004$ ), and seedling roots lifted from these plots were more heavily colonized than those lifted from any fumigant treatment ( $p < 0.001$ ). The increase in colonization was much greater at nursery C (11% to 73%, respectively) than at nurseries A (3% to 20%, respectively) or B (5% to 20%, respectively), which led to an effect of nursery ( $p = 0.010$ ) and a time  $\times$  treatment interaction ( $p = 0.015$ ). However, no nursery  $\times$  treatment interaction ( $p = 0.521$ ) was observed. There was no difference in efficacy between each reduced-rate fumigant and the conventional application of MB/Pic HDPE ( $p =$

0.814) or between HDPE and VIF of the MI/Pic treatments ( $p = 0.286$ ).

**Species identification** *Fusarium commune* Skovgaard, O'Donnell et Nirenberg, *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen, and isolates belonging to the *Gibberella fujikuroi* Saw. complex (teleomorph of *Fusarium* spp.) were isolated from soil and seedlings at all three nurseries. At nursery A, *F. oxysporum* was the most prevalent species (66%), followed by *F. commune* (23%) and *G. fujikuroi* (11%). Nursery B had similar frequencies of each (56%, 30%, and 14%, respectively). In contrast, most isolates at nursery C were *G. fujikuroi* (50%), with intermediate levels of *F. oxysporum* (32%), and low levels of *F. commune* (18%).

### *Pythium*

**Soil** Before fumigation (prefum), soil *Pythium* populations (mean  $\pm$  standard error) were greatest at nurseries A ( $40 \pm 5$  CFU/g) and B ( $45 \pm 12$  CFU/g) and least at nursery C ( $19 \pm 3$  CFU/g). Differences were only significant between either nurseries A or B and nursery C ( $p = 0.007$ ). Population means (pooled nursery data) were similar among treatments ( $p = 0.636$ ) (Fig. 1b), and no nursery  $\times$  treatment interaction was observed ( $p = 0.638$ ).

One month after fumigation (postfum), *Pythium* populations were reduced by at least 92% in all fumigant treatments ( $p \leq 0.047$ ). These populations remained low until 1–2 weeks before planting (preplant,  $p \geq 0.083$ ) (Fig. 1b). Populations in nonfumigated plots also decreased and were reduced 56%–100% from prefumigation levels by 1–2 weeks before planting. Despite this reduction, populations in nonfumigated plots were greater than those in any of the fumigant treatments at nurseries A and B for either sampling date ( $p \leq 0.001$ ). However, *Pythium* was not detected in any of the nonfumigated plots sampled 1–2 weeks before planting at nursery C. No difference in efficacy was observed between each reduced-rate fumigant and the conventional application of MB/Pic HDPE ( $p \geq 0.148$ ) or between HDPE and VIF of the MI/Pic treatments ( $p \geq 0.342$ ) at either sampling date. Although there was an effect of nursery 1 month after fumigation ( $p = 0.003$ ), this effect was not detected 1–2 weeks before planting ( $p = 0.070$ ), and no factor interactions were observed ( $p \geq 0.396$ ).

At the end of the growing season (postplant), few differences in *Pythium* populations were observed among the different treatments ( $p = 0.108$ ) (Fig. 1b). Populations within most treatments had increased 40 CFU/g, on average, over those observed before planting ( $p \leq 0.018$ ) and were similar to those found before fumigation ( $p \geq 0.053$ ). The only exception was DMDS/Pic VIF, which had significantly lower populations than found prior to fumigation ( $p = 0.020$ ). No difference in efficacy was observed between each reduced-rate fumigant and the conventional application of MB/Pic HDPE ( $p \geq 0.485$ ) or between HDPE and VIF of the MI/Pic treatments ( $p \geq 0.833$ ). No effects of nursery ( $p = 0.070$ ) or factor interactions ( $p = 0.286$ ) were detected.

**Seedlings** Before planting (preplant), seedling roots at nurseries A and B were infrequently colonized by *Pythium* (0%–2%) (Fig. 2b) in comparison with those at nursery C

(10%–45%) (Fig. 2c). This difference between nurseries was significant ( $p < 0.001$ ), but no effects of treatment or interaction were observed ( $p \geq 0.221$ ). At the end of the growing season (postplant), colonization in all fumigant treatments at nurseries A and B was the same as that observed before planting ( $p = 0.607$ ). Colonization in the nonfumigated plots, however, was greater than that found at planting ( $p = 0.003$ ) and was also greater than the colonization observed in any fumigant treatment ( $p \leq 0.039$ ) except MS/Pic VIF ( $p = 0.098$ ). In contrast, colonization at the end of the growing season in nursery C was always less than that observed at planting for each respective treatment, including the nonfumigated treatment ( $p < 0.001$ ). Roots from the nonfumigated treatment, however, were more heavily colonized than those from any of the fumigant treatments ( $p = 0.013$ ). No factor interactions were observed ( $p \geq 0.293$ ) except for an effect of nursery  $\times$  time ( $p < 0.001$ ). Regardless of the nursery tested, no differences were observed between the reduced-rate fumigants and the conventional application of MB/Pic HDPE ( $p \geq 0.863$ ) or between HDPE and VIF of the MS/Pic treatments ( $p \geq 0.918$ ).

**Species identification** Five *Pythium* species were commonly isolated from seedlings at the three nurseries (>10% each), though the frequency of each species was dependent on the nursery from which it was recovered. *Pythium irregulare* and *P. aff. spiculum* B. Paul 2006 were isolated from seedlings at all three nurseries. At nursery A, *P. irregulare* was the most common species isolated (54%), followed by *P. aff. spiculum* (26%) and *P. mamillatum* Meurs (13%). *Pythium irregulare* was also the most common species at nursery B (34%), although *P. aff. spiculum* (23%), *P. sylvaticum* Campbell & Hendrix (19%), and *P. 'vipa'* (16%) were also frequently isolated. In contrast, *P. sylvaticum* was the most common species (73%) at nursery C, with very little *P. irregulare* or *P. aff. spiculum* present (6% each). Other *Pythium* species (data not shown) were only rarely detected ( $\leq 10\%$  of the population).

### **Buried inoculum experiment**

#### *Fusarium* and *Pythium*

Fumigation, regardless of the fumigant used, was most effective on *Fusarium* and *Pythium* inoculum buried at 15 cm rather than at 30 cm ( $p \leq 0.001$ ) (Table 2). *Pythium* inoculum in nonfumigated plots also exhibited greater survival at 30 cm ( $p \leq 0.001$ ), but *Fusarium* inoculum survived better at 15 cm ( $p = 0.007$ ). All inoculum regardless of species or depth of burial survived better in nonfumigated plots than in any of the fumigant treatment plots ( $p \leq 0.001$ ). Most reduced-rate fumigant treatments were similar in efficacy to the conventional MB application (Table 2), and no differences in efficacy were observed between HDPE and VIF of the MI/Pic treatments ( $p \geq 0.227$ ). Although an effect of nursery was observed for both pathogen genera ( $p > 0.001$ ), there was no evidence of interaction ( $p \geq 0.324$ ).

### **Weed analyses**

Initial weed counts were much greater at nursery A (17 weeds/quadrat) than at either nursery B (<1 weed/quadrat) or nursery C (1 weed/quadrat). Weed pressure at the latter

**Table 2.** Mean survival ( $\pm$  standard error) of *Fusarium* in inoculated rye seeds (%) and *Pythium* in infested soil (CFU/g) at 15 and 30 cm depths 1 month after fumigation at three forest nurseries ( $n = 144$  for *Fusarium*,  $n = 72$  for *Pythium*).

Treatment	Mean survival of <i>Fusarium</i> (%)		Mean survival of <i>Pythium</i> (CFU/g)	
	15 cm	30 cm	15 cm	30 cm
NF HDPE	97 (1.0)a	91 (9.8)a	19 (1.3)a	31 (1.4)a
MB/Pic HDPE	3 (0.7)b	33 (3.5)b	0 (0.1)b	1 (0.3)b
MI/Pic HDPE	9 (2.1)bc	30 (3.6)b	0 (0.0)b	1 (0.2)b
MI/Pic VIF	12 (2.3)c	34 (3.7)b	1 (0.3)b	1 (0.3)b
MS/Pic VIF	12 (2.5)c	25 (3.2)b	0 (0.0)b	5 (0.7)c
DMDS/Pic VIF	21 (2.9)d	30 (3.5)b	0 (0.1)b	2 (0.6)bc

**Note:** NF, nonfumigated; HDPE, high-density polyethylene; MB, methyl bromide; Pic, chloropicrin; MI, methyl iodide; VIF, virtually impermeable film; MS, metam sodium; DMDS, dimethyl disulfide. Treatments followed by the same letter in each column are not significantly different ( $p < 0.05$ ) according to Mann-Whitney and Kruskal-Wallis nonparametric statistical tests.

two nurseries remained low throughout the study, therefore treatment effects were rarely observed (data not shown). Weed pressure at nursery A, however, remained high throughout the study, and all fumigation treatments reduced weed parameters by  $\geq 70\%$  for all sample dates compared with the nonfumigated treatment (Table 3). As a consequence of this high weed pressure, hand-weeding in all fumigant plots took almost twice as long at nursery A (5.2 min/LBF) than at nursery C (3.3 min/LBF), and 17 times as long as at nursery B (0.3 min/LBF). Furthermore, weeding times at nursery A were reduced in all fumigant treatments by 95% compared with the nonfumigated treatment. There was no difference in efficacy among reduced-rate fumigants and MB/Pic HDPE or between HDPE and VIF of the MI/Pic treatment ( $p > 0.05$ ) at nursery A, except in October 2009 when weed biomass measurements in DMDS/Pic VIF, MI/Pic HDPE, and MS/Pic VIF plots were similar to those in nonfumigated plots ( $p > 0.05$ ). The predominant weed species found in nonfumigated plots at all sampling dates were common pearlwort (*Sagina apetala* Ard.), common chickweed (*Stellaria media* (L.) Vill.), shepherd's-purse (*Capsella bursa-pastoris* (L.) Medic.), and annual bluegrass (*Poa annua* L.).

### Seedling morphology analyses

At planting, seedlings were uniform in morphology within each nursery (data not shown). Seedlings were taller (mean  $\pm$  standard error) at nurseries B (17 cm  $\pm$  0.2) and C (16 cm  $\pm$  0.2) than at nursery A (11 cm  $\pm$  0.2). The other morphological traits followed similar distributions with the greatest values occurring at nursery B (data not shown).

Morphological traits at lift in November varied by nursery and treatment ( $p \leq 0.021$ ). Seedling height was greatest in all fumigant treatments at nursery A (53 cm  $\pm$  0.9) and least at nursery C (44 cm  $\pm$  0.9). Other morphological traits from the fumigant treatments followed similar distributions, with the greatest values occurring at nursery A (data not shown) and the least at nursery C. Reduced-rate fumigant treatments usually yielded results similar to ( $p \geq 0.133$ ) or greater than ( $p \leq 0.001$ ) those from the conventional MB/Pic HDPE application (Table 4). The one exception was for the height-diameter ratio, in which DMDS/Pic VIF had a lower ratio than MB/Pic HDPE ( $p \geq 0.001$ ). Morphological traits from

the nonfumigated treatment were usually less than from the fumigant treatments, though this was not always significant (Table 4). No difference in efficacy was observed between HDPE and VIF of the MI/Pic treatments ( $p \geq 0.551$ ).

### Grading and cost analyses

The actual percentage of culls removed within each treatment by line workers during operational grading was different than the percentages estimated for each treatment by morphology and pathology grading (Table 5). In the operational grade, more culls were removed from the nonfumigated treatment (12%) than from any of the fumigant treatments (7%–8%). Cull estimates based on the morphology grade, in contrast, were 13%–26% age points greater than the operational cull percentages for each treatment, and there was no detectable difference between the percentages of culls from the nonfumigated treatment and the five fumigant treatments. Cull estimates based on the pathology grade, however, exhibited a large difference in the amount of culls from the nonfumigated treatment (almost 60%) in comparison with those from the five fumigant treatments (1%–14%).

Because of their initial price, both MI/Pic treatments were the most expensive fumigant option, regardless of the grading method used (Table 5). The lowest cost/1000 seedlings was always represented by the nonfumigated treatment. However, when potential losses due to *Fusarium* species infection were taken into account by the pathology grade method, costs for the nonfumigated treatment were similar to those of MB/Pic HDPE and MS/Pic VIF.

### Discussion

This study represents the first replicated operational scale trial of MI/Pic, MS/Pic, and DMDS/Pic using HDPE and VIF in the PNW. Participating nurseries transplanted and applied best cultural practices to their Douglas-fir crop throughout the study. Therefore, the results of this study represent the readiness of bare-root conifer growers in the region to adopt projected EPA fumigant guidelines. However, given the results of the buried inoculum portion of this study, efficacy of reduced-rate fumigants would likely be improved by management practices that decrease soil bulk density at 30 cm, thus allowing better fumigant infiltration. In addition, sanitation measures to reduce the amount of seedling residues

**Table 3.** Means of weed counts, weed biomass, and weeding times ( $\pm$  standard error) among six fumigation treatments at forest nursery A ( $n = 12$ ).

Treatment	Weed count						Weed biomass (kg)						Weeding time (min)	
	November 2008	June 2009	August 2009	October 2009	June 2009	October 2009	June 2009	August 2009	October 2009	June 2009	October 2009	June 2009	October 2009	
NF HDPE	16.88 (3.37)a	0.84 (0.34)a	1.09 (0.33)a	6.97 (1.93)a	0.20 (0.11)a	8.01 (2.72)a	0.20 (0.11)a	8.01 (2.72)a	7.25 (3.23)ab	0.20 (0.11)a	8.01 (2.72)a	107.48 (33.71)a	7.25 (3.23)ab	
MB/Pic HDPE	0.56 (0.27)b	0.00 (0.00)b	0.31 (0.12)b	0.16 (0.07)b	0.00 (0.00)b	0.52 (0.24)b	0.00 (0.00)b	0.52 (0.24)b	0.87 (0.61)c	0.00 (0.00)b	0.52 (0.24)b	5.00 (0.20)b	0.87 (0.61)c	
MI/Pic HDPE	0.06 (0.11)b	0.09 (0.04)b	0.34 (0.08)b	0.34 (0.10)b	0.00 (0.00)b	1.76 (0.48)b	0.00 (0.00)b	1.76 (0.48)b	2.24 (0.65)bc	0.00 (0.00)b	1.76 (0.48)b	4.95 (0.51)b	2.24 (0.65)bc	
MI/Pic VIF	0.25 (0.06)b	0.06 (0.09)b	0.28 (0.12)b	0.25 (0.10)b	0.01 (0.00)b	1.17 (1.16)b	0.01 (0.00)b	1.17 (1.16)b	0.91 (1.26)c	0.01 (0.00)b	1.17 (1.16)b	5.65 (0.16)b	0.91 (1.26)c	
MS/Pic VIF	0.19 (0.10)b	0.03 (0.03)b	0.28 (0.10)b	0.22 (0.07)b	0.01 (0.01)b	0.74 (0.40)b	0.01 (0.01)b	0.74 (0.40)b	1.31b (0.55)c	0.01 (0.01)b	0.74 (0.40)b	5.17 (0.16)b	1.31b (0.55)c	
DMDS/Pic VIF	0.31 (0.22)b	0.09 (0.05)b	0.34 (0.12)b	0.75 (0.14)b	0.02 (0.01)b	0.65 (0.39)b	0.02 (0.01)b	0.65 (0.39)b	10.87 (3.83)a	0.02 (0.01)b	0.65 (0.39)b	5.02 (0.27)b	10.87 (3.83)a	

**Note:** NF, nonfumigated; HDPE, high-density polyethylene; MB, methyl bromide; Pic, chloropicrin; MI, methyl iodide; VIF, virtually impermeable film; MS, metam sodium; DMDS, dimethyl disulfide. Weed count: number of weeds within a 0.26 m<sup>2</sup> quadrat; weed biomass, dry mass of weeds within a 0.26 m<sup>2</sup> quadrat; weeding time, time required to weed Douglas-fir nursery beds (minutes per person per 100 lineal bed feet) weeded. Treatments followed by the same letter in the same column are not statistically different ( $p < 0.05$ ) according to Fisher's protected LSD.

following harvest may also improve efficacy by removing inoculum sources that may be more resistant to fumigant penetration.

Although the reduced-rate MB-alternative fumigants reported here usually achieved similar results as the conventional application of MB/Pic HDPE for pathogen and weed control and for seedling size and yields, each alternative fumigant exhibited at least one disadvantage that may limit its adoption by the forest nursery industry. Commercial application of MI is currently limited by cost, which is approximately twice that of MB. Furthermore, MI is not registered for use in Washington State. Both DMDS and MS applications, in contrast, are priced similar to MB but are associated with strong odors that limit their use near residential and commercial areas. Long-term benefits of MS application may be further restricted because its efficacy has been reported to decrease with repeated use (Triky-Dotan et al. 2010). Additionally, the DMDS/Pic VIF application in this study was associated with significantly greater *Fusarium* soil populations at the end of the growing season in two of the three nurseries. In any case, the chief limitation in the use of all fumigants is expected to be the size of the buffer zone required by the Environmental Protection Agency. Many nurseries must farm fenceline to fenceline to stay profitable, and the encroachment of residential and commercial areas has the potential to drastically reduce field fumigation. The use of low-permeability plastic films such as VIF or totally impermeable film (TIF) during fumigation might significantly decrease fumigant emissions and reduce the buffer zones required by regulatory agencies if the integrity of the material can be maintained and if the material is applied correctly. As noted in the present study, no differences were observed in efficacy between HDPE and VIF of the MI treatment, but research with these films in combination with even lower fumigant rates should be investigated for their suitability in forest seedling production.

Relatively little information has been available regarding the species identity of *Fusarium* and *Pythium* isolates obtained from forest nursery soils and seedlings. Of those identified in the PNW and western Canada, *F. commune*, *F. oxysporum*, *P. irregulare*, *P. mamillatum*, *P. sylvaticum*, and *P. ultimum* Trow were the most common species noted (Axelrood et al. 1998; Hansen et al. 1990; James 2000; Skovgaard et al. 2003; Stewart et al. 2006; Vaartaja 1975). To our knowledge, this study is the first to report *F. commune* from forest nursery soils and Douglas-fir seedlings in Oregon and Washington and *Pythium aff. spiculum*, *P. sylvaticum*, and *P. 'vipa'* from Douglas-fir seedling roots. Knowledge regarding species identity is critical for the evaluation of species pathogenicity to conifer seedlings and for the development of nonfumigant-based pathogen control measures.

The frequency of *Pythium* species identified from preplant Douglas-fir seedlings in this study differed greatly from the frequency of *Pythium* species identified from the prefumigation soil (Weiland 2011). For example, at nursery A, 78% of the 100 soil isolates isolated by the dilution plate method were *P. irregulare* compared with only 54% of the seedling isolates. Conversely, *P. aff. spiculum* represented only 5% of the soil isolates but composed 26% of the seedling isolates. Furthermore, several species that were frequently detected from nursery soils were rarely or never detected from seed-

**Table 4.** Means ( $\pm$  standard error) of Douglas-fir transplant morphological traits at the end of the growing season in October–November 2009 among six fumigation treatments at three forest nurseries ( $n = 300$ ).

Treatment	Height (cm)	Diameter (mm)	Height–diameter ratio	Shoot volume (g)	Root volume (g)	Root–shoot ratio
NF HDPE	42 (0.5)a	6.9 (0.08)a	6.2 (0.07)a	37 (0.9)a	20.1 (0.45)a	0.58 (0.011)a
MB/Pic HDPE	49 (0.6)b	7.4 (0.09)bc	6.8 (0.07)cd	50 (1.3)b	21.7 (0.48)ab	0.48 (0.011)b
MI/Pic HDPE	48 (0.5)b	7.5 (0.09)bc	6.6 (0.07)bc	51 (1.3)b	22.7 (0.56)b	0.48 (0.010)b
MI/Pic VIF	48 (0.5)b	7.5 (0.10)bc	6.6 (0.06)bc	50 (1.4)b	22.3 (0.51)b	0.48 (0.010)b
MS/Pic VIF	49 (0.6)b	7.2 (0.10)ab	6.9 (0.07)d	52 (1.4)b	20.9 (0.51)ab	0.43 (0.007)c
DMDS/Pic VIF	49 (0.6)b	7.8 (0.09)c	6.4 (0.06)ab	52 (1.4)b	22.2 (0.52)b	0.46 (0.007)bc

**Note:** NF, nonfumigated; HDPE, high-density polyethylene; MB, methyl bromide; Pic, chloropicrin; MI, methyl iodide; VIF, virtually impermeable film; MS, metam sodium; DMDS, dimethyl disulfide. Means followed by the same letter in the same column are not significantly different ( $p < 0.05$ ) according to Tukey's test for multiple comparisons.

**Table 5.** Fumigation application costs per hectare,<sup>a</sup> mean percentage of culls, and mean cost per 1000 seedlings<sup>a</sup> of six fumigation treatments according to three grading methods at three forest tree nurseries.

Treatment	Cost/ha	Operational grade <sup>b</sup>		Morphology grade <sup>c</sup>		Pathology grade <sup>d</sup>	
		Cull (%)	Cost/1000	Cull (%)	Cost/1000	Cull (%)	Cost/1000
NF HDPE	1 940	12	5.38	29	6.67	59	11.56
MB/Pic HDPE	4 967	7	12.94	31	17.59	1	12.18
MI/Pic HDPE	10 984	7	28.68	24	35.14	8	28.87
MI/Pic VIF	12 133	8	32.03	22	37.82	3	30.51
MS/Pic VIF	5 115	8	13.47	34	18.94	5	13.09
DMDS/Pic VIF	6 215	8	16.50	21	19.05	14	17.60

**Note:** NF, nonfumigated; HDPE, high-density polyethylene; MB, methyl bromide; Pic, chloropicrin; MI, methyl iodide; VIF, virtually impermeable film; MS, metam sodium; DMDS, dimethyl disulfide.

<sup>a</sup>Costs include HDPE or VIF tarp application and are based on 2010 prices, US dollars.

<sup>b</sup>Actual percentage of seedlings culled from each treatment operationally.

<sup>c</sup>Estimate of the percentage of seedlings culled from each treatment based on morphological measurements with a height < 30.5 cm, stem diameter < 6 mm, and a root–shoot ratio < 0.3.

<sup>d</sup>Estimate of the percentage of seedlings culled from each treatment based on seedlings  $\geq 20\%$  *Fusarium* root colonization.

lings (e.g., *P. dissotocum* and *P. ultimum* var. *ultimum*). These differences might be explained by the fact that the one-year-old seedlings were initially grown in different fields, sometimes at different nurseries, before being dug and placed into storage over winter and then planted into this study. If the initial seedling beds contained a different assemblage of *Pythium* species, those differences might carry over into the present study. Seedling storage might further modify root communities by selecting for *Pythium* species that are more likely to survive or reproduce in the storage environment than in the soil. This might have been the case at nursery C, where the root colonization observed at preplant was always greater than that found after planting in the field. Finally, some *Pythium* species found in nursery soils may be only weakly virulent or nonpathogenic to Douglas-fir seedlings. These species would therefore be less frequently isolated from Douglas-fir roots. This disparity between soil and seedling populations calls attention to the importance of pathogen transport, as planting infected seedlings might reinfest newly fumigated fields or introduce new *Pythium* species into locations where they had not previously occurred. It is also important to note that the nursery with the smallest seedlings at the end of the growing season (nursery C) was the same nursery with significantly greater preplant root colonization by *Pythium* than either nursery A or nursery B. This is in direct contrast to nursery A, which had the tallest seedlings at the end of the growing season and the least amount of pre-

plant root colonization. Additional research is needed to identify which *Pythium* species are favored by storage and then determine how these species interact with those in the soil, affect subsequent seedling growth, and contribute to pathogen recolonization of field soils.

In addition to providing management of soilborne pathogen populations, forest nursery managers expect fumigant treatments to provide weed control. Each of the reduced-rate fumigant treatments in this study controlled resident weed species for  $\geq 12$  months. These species (common pearlwort, common chickweed, and shepherd's-purse) are not wind-dispersed, so they were most likely present in the field plots prior to fumigation. However, other weed species including common groundsel (*Senecio vulgaris* L.), common catsear (*Hypochaeris radicata* L.), annual sowthistle (*Sonchus oleraceus* L.), willow-weed (*Epilobium* species), black cottonwood (*Populus trichocarpa* L.), and bull thistle (*Cirsium vulgare* (Savi) Ten.) were also found in the same plots. These weeds are wind-dispersed species that may have germinated from seeds blown into the fields after fumigation. Consequently, reduced-rate fumigant efficacy against weeds could prove greater than that observed in the current study if future experiments take these wind-dispersed weed species into account.

Estimates of cull percentages for each treatment through morphology and pathology grading methods were quite different than the operational culls reported by each nursery.

Cull estimates based on morphology were always greater than the operational cull percentages, which might be due to the greater precision and amount of time spent in measuring seedling height and stem diameter during the morphological grading process. Although this process was illustrative for research purposes, adoption of the morphological grading method by the nursery industry is impractical during spring packing operations when time is at a premium. Large volumes of nursery stock must be processed, packed, and shipped within a relatively short period of time to ensure that the material arrives at its destination in good condition and at the proper time for planting. In contrast, cull estimates based on pathology were similar to operational cull percentages for all fumigant treatments. However, almost 60% of seedlings from nonfumigated treatments had  $\geq 20\%$  root colonization. Currently, pathology grading is not practiced within the nursery industry due to time and resource constraints, and root infection in the nursery does not necessarily equate to greater mortality once the seedlings are planted into forest sites. Several reports, summarized in Dumroese and James (2005), for example, find that root infection in the nursery by *F. oxysporum* and *F. proliferatum* (Matsus.) Nirenberg seems to have little impact on seedling survival and growth once seedlings are outplanted. However, in our experience at the nurseries in this study, seedlings with  $\geq 20\%$  root colonization exhibited mortality in storage and poor establishment once outplanted. *Fusarium* species or isolates at these locations might be more virulent than those found in previous studies. Future trials should investigate the effects of seedling infection by other pathogens such as *F. commune* and *Pythium* species on outplanting success.

Reduced rates of MI/Pic, MS/Pic, and DMDS/Pic, in combination with VIF, show potential as alternatives to conventional MB/Pic HDPE application in forest nurseries. Rates used in this study were effective for pathogen and weed management and did not negatively impact the size or yield of forest seedlings. As fumigant use becomes increasingly regulated at both the federal and state levels, forest nurseries are beginning to explore nonfumigant-based integrated pest management practices (IPM). Because fumigants act as nonselective agents against a broad array of pathogens and weed species, relatively little was known about the species of *Fusarium* or *Pythium* present in forest nursery soils. IPM approaches, on the other hand, require increased knowledge about the identity and biology of key soilborne pathogens and weeds to develop and establish effective management practices. Information regarding pathogen identity and biology can guide future research to determine disease risk and economic threshold values for important pathogen species. The establishment of long-term, nonfumigated field plots would aid the transition away from fumigant-intensive management and allow researchers to evaluate the effects of nursery management on pathogen and weed populations and seedling productivity in the absence of fumigation.

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