Efficacy of reduced rate fumigant alternatives and methyl bromide against soilborne pathogens and weeds in western forest nurseries.

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ABSTRACT

Preplant soil fumigation is commonly used to control soilborne pathogens and weeds in forest seedling nurseries of Oregon and Washington. However, lower chemical inputs are desired to meet state and federal application regulations, to minimize buffer zone size requirements, and to help protect the environment. Therefore, the objectives of this research were to evaluate the efficacy of three reduced rate soil fumigants under totally impermeable film (TIF) in managing soilborne diseases and weeds, and to determine if combined applications of up to four biocontrol agents improved soilborne disease control. Reduced rates of methyl bromide, metam sodium, and 1,3-dichloropropene, all applied in combination with chloropicrin, were effective in

decreasing soil populations of *Pythium* and *Fusarium* as well as the presence of *Pythium* in root debris from the previous crop. The roots of Douglas-fir (*Pseudotsuga menziesii*) seedlings transplanted into each fumigant treatment were also colonized less by *Pythium* and *Fusarium* than those transplanted into nonfumigated control plots. However, biocontrol treatments were not effective against either pathogen. Weed biomass and weeding times were also significantly reduced by fumigation. Application costs were similar for all three fumigant treatments, but seedling size was largest from the methyl bromide and metam sodium treatments followed by the 1,3-dichloropropene treatment. Based on the results of this study, reduced rates of methyl bromide, metam sodium, and 1,3-dichloropropene show promise in managing soilborne diseases and weeds in forest nurseries.

Keywords: soil fumigation; methyl bromide alternatives; *Pythium*; *Fusarium*; weed control; forest nursery; chloropicrin; metam sodium; 1,3-dichloropropene.

1. Introduction

Forest nurseries in the western states of California, Idaho, Montana, Oregon, and Washington produce approximately 200 million tree seedlings each year, mainly for reforestation purposes (Weiland et al., 2013b). Most seedlings sold in the region are Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). Soilborne disease and weed control in forest nurseries has been primarily achieved through preplant soil fumigation with methyl bromide combined with chloropicrin (MBC, 67:33) at 392 kg/ha under a Quarantine Preshipment Exemption to the Montreal Protocol (Weiland et al., 2013b). The most common soilborne pathogens are those causing damping-off and root rot, such as those in the genera *Pythium*, *Fusarium*, and

Cylindrocarpon (Dumroese and James, 2005; Stewart et al., 2011; Weiland et al., 2013a; Weiland et al., 2011; Weiland et al., 2013b). Typical symptoms of infection by these pathogens include chlorosis, stunting, fine root mortality, and in severe cases, seedling death. Left untreated, damping-off and root rot can significantly reduce seedling yield and quality (Stewart et al., 2011; Weiland et al., 2013a; Weiland et al., 2013b; Weiland et al., 2014). Eventually, forest nurseries will lose methyl bromide for disease and weed control, but in the meantime most will continue to use the product due to the lack of suitable alternatives.

Previous research in Oregon and Washington has shown that reduced rates (defined as less than label rate) of the alternative fumigants metam sodium, dimethyl disulfide, and methyl iodide, when combined with chloropicrin, are as effective at controlling *Pythium*, *Fusarium*, *Cylindrocarpon*, and weeds as the standard, full rate of MBC at 392 kg/ha (Weiland et al., 2011; Weiland et al., 2013b). Reduced rate applications are desirable because they decrease the size of the required buffer zone (nonfumigated areas separating fumigated fields and neighboring property) and reduce costs because less product is applied. Unfortunately, one of the reduced rate alternative fumigants previously identified as effective, methyl iodide (iodomethane), was removed from the U.S. market in 2012, and another fumigant, dimethyl disulfide, had an objectionable, strong odor that resulted in neighbor complaints when used (Weiland et al., 2013b). As a result, there are few alternatives for replacing methyl bromide that have been tested at reduced rates in the western region of the U.S. There is also industry interest in testing the efficacy of reduced rate applications of methyl bromide because this product is still in use at approximately 70% of the region's nurseries (Weiland et al., 2013b).

Biocontrol agents have also been tested at western forest nurseries for their ability to control soilborne pathogens. For the most part, results from greenhouse and field studies have

been mixed, with some treatments succeeding (Dumroese et al., 2012; Mousseaux et al., 1998), while others fail (Dumroese et al., 1996; Hildebrand et al., 2004; James et al., 2004; Linderman et al., 2008). This lack of consistency and limited success is one reason that biocontrol agents are not used more widely in forest nursery seedling production (Weiland et al., 2013b). However, previous studies mostly relied on single applications of a single biocontrol agent and there is evidence that multiple applications with several biocontrol agents might prove more effective in controlling soilborne pathogens (Guetsky et al., 2002; Whipps, 2001).

A field experiment was initiated in 2010 at two forest nurseries to evaluate reduced rate soil fumigants and biocontrol agents for efficacy against soilborne diseases and weeds. The objectives were to: 1) evaluate the efficacy of reduced rate formulations of methyl bromide and alternative fumigants in order to meet 2010 Environmental Protection Agency guidelines for a 7.6 m (25 ft) buffer zone; and, 2) determine whether applying combined mixtures of biocontrol agents can provide supplemental soilborne disease control in fumigated soils.

2. Materials and methods

2.1. Nurseries

Field trials were established at two forest nurseries (located 12 km apart) in western Oregon that had been used in previous methyl bromide alternatives research and designated nursery B and nursery C (Weiland et al., 2011). Soil at both of these nurseries is classified as Canderly sandy loam and the last crop of Douglas-fir seedlings were harvested at each nursery by March 2010. Fields at both nurseries were then bare fallow until fumigation occurred in mid-August 2010.

2.2 Experimental design

Four fumigation treatments, consisting of three fumigant treatments and a nonfumigated negative control treatment (NF), were applied under totally impermeable film (TIF) at each nursery in a randomized complete block design (RCBD) with four replicate blocks in mid-August 2010. The three fumigant treatments were: 1) 50% methyl bromide + 50% chloropicrin applied at 280 kg/ha (MBC); 2) metam sodium + chloropicrin applied at 253 l/ha and 168 kg/ha, respectively (MSC); and, 3) 40% 1,3-dichloropropene + 60% chloropicrin (Pic-Clor 60) applied at 319 kg/ha (DPC). All fumigants were selected based on their efficacy in prior industry research trials (unpublished data) and on the consensus of the two nurseries involved. Reduced rates (less than label rate) for each fumigant were selected to meet 2010 Environmental Protection Agency guidelines for a 7.6 m (25 ft) buffer zone.

2.3 Application of fumigation treatments

Fumigants were applied by a commercial applicator (Trident Agricultural Products, Toppenish, WA). Both the MBC and DPC treatments were chisel injected into the soil 23 cm deep and 30 cm apart with a D4D crawler (Caterpillar Inc., Peoria, IL) equipped with a noble plow rig. For the MSC treatment, metam sodium was injected at a 10 cm and 20 cm soil depth with a John Deere 4960 tractor (Deere & Co., Moline, IL) equipped with backswept shanks spaced 30 cm apart. Chloropicrin was then injected into the soil 23 cm deep and 30 cm apart with a D4D crawler equipped with a noble plow rig. TIF was applied immediately after fumigation to all fumigation treatment plots with the D4D crawler, including the NF plots. Fumigated plots were 46 × 13 m and NF plots were slightly shorter at 30 × 13 m to minimize economic losses due to the absence of disease and weed control provided by fumigation. This difference in size between fumigated and nonfumigated plots did not affect results as indicated by the lack of significant differences among treatment plots in both prefumigation soil pathogen

populations and root debris colonization by *Pythium* and *Fusarium* (see Results). TIF was removed one month after fumigation and plots remained fallow until planting the following spring.

2.4 Planting and application of biocontrol treatments

One-year-old, bareroot 1+0 Douglas-fir seedlings were transplanted into each nursery in early to mid-May 2011. All plots, including the NF plots, consisted of six nursery beds with an average of 29 seedlings planted per linear 30 cm of bed. Three biocontrol treatments and a nontreated negative control treatment (no biocontrol agent) were then applied to four 7.6×1.2 m subplots placed according to a split-plot design in the center bed of all MSC, DPC, and NF main plots for a total of 48 biocontrol subplots at each nursery. No biocontrol treatments were applied within the MBC treated plots because methyl bromide is expected to be phased out in the future. The three biocontrol treatments were applied using hand sprinklers to their respective subplots in a 7.6 l volume of water. The biocontrol treatments were a combination of biocontrol products at soil drench label rates (Table 1) and consisted of: 1) Actinovate + Cease; 2) SoilGard + RootShield; and, 3) Actinovate + Cease + SoilGard + RootShield. The control consisted of nontreated water and was applied at the same volume/subplot. All biocontrol treatments and the control treatment were applied three times throughout the 2011 growing season: early June, late July to early August, and early October.

2.5 Soilborne and seedling pathogens

Soil in each of the 16 fumigation plots (4 fumigation treatments $\times$ 4 blocks) at each nursery was assessed for soilborne pathogen populations (*Pythium*, *Fusarium*, and *Cylindrocarpon* species) two weeks before fumigation (prefumigation, late July 2010), six weeks after fumigation (postfumigation, late September 2010), just before planting (preplant, mid-May

2011), and at the end of the growing season (harvest, early- to mid-December 2011). Soil samples were collected by taking twenty 2-cm-diameter $\times$ 30-cm-depth soil cores in a randomized pattern from each plot. Soil samples from the biocontrol subplots were obtained using the same sampling strategy, but were only collected at harvest. Soil samples were bulked by plot or subplot and mixed thoroughly to generate composite samples for each fumigation plot and biocontrol subplot at each nursery. Half of each composite soil sample was then plated on PARP (Kannwischer and Mitchell, 1978) to assess *Pythium* soil populations and the remaining half was plated on Komada's medium (Komada, 1975) to assess *Fusarium* and *Cylindrocarpon* soil populations according to previous methods (Weiland et al., 2011). Populations of each pathogen were expressed as propagules per gram (ppg) of dry soil. Based on previous data (Weiland et al., 2011), *Fusarium* soil populations remain relatively low and constant after fumigation until seedlings are transplanted into the field. Therefore, postfumigation levels of this pathogen were not evaluated until the preplant sampling period. Although *Cylindrocarpon* species were found in the soil during this study, populations were never distributed evenly enough across field plots, nor were populations consistently large enough to adequately quantify and show treatment effects. Therefore, *Cylindrocarpon* soil populations were not assessed further.

In order to determine whether residual root debris (dead seedling roots remaining in field soil from the previous 2010 seedling crop) allows soilborne pathogens to avoid lethal doses of fumigant at reduced application rates, root debris was assessed for percent colonization by *Pythium*, *Fusarium*, and *Cylindrocarpon* species. Living roots of the Douglas-fir seedlings planted into each nursery were also assessed to evaluate for effects of fumigation and biocontrol treatments. Residual root debris was sieved from soil collected from 0.6 $\times$ 0.6 m sampling

quadrats within each of the 16 fumigation plots at each nursery before and after fumigation. Root debris was no longer recoverable at preplant due to decomposition, therefore it was not evaluated at either preplant or harvest times. Living Douglas-fir seedling roots were sampled just before planting (preplant) and at the end of the growing season (harvest) by randomly selecting 25 and 16 seedlings per plot for *Pythium* and *Fusarium/Cylindrocarpon*, respectively. Living Douglas-fir seedling roots from biocontrol subplots were obtained using the same sampling strategy, but were only collected at harvest. Percent colonization of both residual root debris and living roots were evaluated by plating twenty 1-cm-long debris roots or ten 1-cm-long root segments from each seedling onto PARP (*Pythium*) or Komada's (*Fusarium* and *Cylindrocarpon*) media, respectively, according to previous methods (Weiland et al., 2011). Although *Cylindrocarpon* species were occasionally found in root debris and on seedling roots, root colonization was never consistent or frequent enough to adequately quantify and show treatment effects. Therefore, *Cylindrocarpon* root debris and seedling root colonization were not assessed further.

2.6 Weeds

Weed cover was visually estimated at three times following fumigation in August 2010: July 20 and September 7, 2011, and January 11, 2012. Two 50.8×50.8 cm (0.26 m^2) quadrats were placed approximately 4.5 or 6.1 m from the midpoint of each control or fumigated plot, respectively, one on either side of the midpoint. Two separate beds within each plot were sampled at each time, resulting in a total of four quadrats per plot for each sampling date. Weed cover was not analyzed within biocontrol subplots. Percent weed cover within each quadrat was recorded by species for all sampling dates. As another measure of weed pressure (and not as a standard nursery practice for controlling weeds), hand-weeding of all plots was conducted at nursery B on July 28, 2011 and total weeding time per treatment was calculated (m per person

per min per plot). Weeding time was not evaluated at nursery C because the weed pressure was low in July 2011 ($\leq 9\%$ weed cover).

2.7 Seedling morphology, cull parameters, and cost analyses

Twenty five seedlings, selected at random from each fumigation treatment plot at each nursery, were lifted at harvest and measured for stem height (cm), stem diameter (mm), shoot weight (g), and root weight (g). The root:shoot ratio was also calculated. The seedlings were then evaluated against standard sets of cull parameters, below which a seedling would be discarded, to estimate packable yields. Cull parameters vary depending on customer needs for seedling size and quality. These are standard operational parameters used by the forest nursery industry and are established at heights of 25.4, 27.9, 30.5, 33.0, 35.6, and 38.1 cm, stem diameters of 4, 5, 6, 7, 8 and 9 mm, and/or root:shoot ratios of 0.33 and 0.50. The number and percentage of culls (out of the number of seedlings produced, calculated at 421,994 seedlings/ha) was estimated for each treatment based on each of these parameters separately, but focused on a combined cull parameter set to remove seedlings that were < 30.5 cm tall, < 6 mm in stem diameter, and with a root:shoot ratio < 0.33 to compare application costs as agreed upon by both nurseries. Cull estimates based on this combined cull parameter set were then used to determine the number of packable seedlings sold/ha for each fumigation treatment (number of seedlings produced - number of seedlings culled). Costs of application for each of the fumigation treatments were obtained from the commercial applicator. Finally, the average application cost of each treatment, including TIF application, was determined for every 1000 seedlings produced at 2015 prices ($1000 \times \text{application cost}/\text{number of seedlings sold}$) and the relative cost to MBC was calculated ($\text{cost of fumigation treatment}/\text{cost of MBC}$).

2.8 Statistical analyses

All pathology, weed, and seedling morphology data from the fumigation treatments were collected on a RCBD and were analyzed accordingly. Because data from *Pythium* and *Fusarium* soil populations did not meet assumptions of normal distribution and equal variance, these data were analyzed using Mann-Whitney, Kruskal-Wallis, and Scheirer-Ray-Hare nonparametric tests for effects of nursery, block, fumigation treatment, and interactions for each time period (prefumigation, postfumigation, preplant, and harvest) separately (Sokal and Rohlf, 2012). *Pythium* and *Fusarium* colonization of root debris and seedling roots were calculated as the proportion of root segments from which each pathogen was isolated. Proportion data were transformed using the arcsine of the square root to homogenize variances and then analyzed for effects of nursery, block, fumigation treatment, and interactions for each time period separately using a mixed-model ANOVA with fumigation treatment as a fixed effect and nursery and block as random effects. Because of differences in weeding regimes at each nursery, weed data were only analyzed for effects of fumigation treatment using ANOVA for each nursery and time period separately. Seedling morphology data were analyzed using a mixed-model ANOVA, with fumigation treatment as a fixed effect and nursery and block as random effects. No interactions were observed for any of the pathology (above) or seedling morphology analyses ($P \geq 0.294$). Therefore, treatment means are averaged from both nurseries. All ANOVA computations were separated using Fisher's protected least significant difference statistic and Tukey's test for multiple comparisons at $P \leq 0.05$.

For the biocontrol treatments, both soil population and seedling root colonization data were analyzed according to a split-plot design with fumigation treatment as the main plot and biocontrol treatment as the subplot. Both sets of data met assumptions of normality and equal variance, and root colonization data were transformed as described above by the arcsine of the

square root of the proportion before analyzing for effects of block, fumigation, biocontrol treatment, and interactions. Analyses were performed using Minitab Statistical Software (release 16; Minitab Inc., State College, Pennsylvania), SAS 9.3 (SAS Institute Inc., Cary, North Carolina), or SPSS Statistics (version 17.0; SPSS Inc., Chicago, Illinois).

3. Results

3.1 Soilborne and seedling pathogens

Initial prefumigation *Pythium* soil populations (Fig. 1A) were greater at nursery B (174 ppg) than nursery C (21 ppg) ($P < 0.001$), and no differences were observed among treatment plots within each nursery at that time ($P = 0.762$). After fumigation, *Pythium* populations were reduced by at least 99% to at or near undetectable levels (0-1 ppg) by each fumigant treatment compared to the NF plots ($P < 0.001$). There were no differences in efficacy between the three fumigant treatments ($P = 0.363$). By preplant, *Pythium* populations had increased in all fumigant treatments, but never reached levels observed in NF plots ($P < 0.001$). At harvest, *Pythium* populations were at or above prefumigation levels in all treatments. Populations were smallest in the DPC plots, followed by MBC and then MSC plots, with the largest populations occurring in NF plots ($P < 0.001$).

As observed for *Pythium*, initial prefumigation *Fusarium* soil populations (Fig. 1B) were greater at nursery B (2352 ppg) than nursery C (188 ppg) ($P < 0.001$), and were similar across all treatment plots within each nursery ($P = 0.570$). Each of the three fumigant treatments, as quantified at preplant, reduced *Fusarium* populations by at least 85% to undetectable or low levels (0-31 ppg) compared to the NF plots ($P < 0.001$), and by harvest, *Fusarium* populations in the fumigant treatments had increased only slightly to 31-175 ppg. There were no differences in

efficacy among the three fumigant treatments ($P = 0.769$). In contrast, NF plots had the largest soil populations of *Fusarium* ($P = 0.049$) at both preplant and harvest.

Initial prefumigation root debris colonization by *Pythium* was greater at nursery B (51%) than at nursery C (10%), and was similar across all treatment plots within each nursery ($P = 0.737$) (Table 2). After fumigation, *Pythium* was not detected on root debris from any of the three fumigant treatments. However, *Pythium* was still evident on root debris in NF plots at low, but significant levels (5% in both nurseries, $P = 0.002$). In contrast, prefumigation root debris colonization by *Fusarium* was either too inconsistent or low ($< 6\%$) to be meaningful. Most root debris at nursery B was colonized less than 6% of the time, while *Fusarium* was undetectable on root debris at nursery C. Results were similar at postfumigation.

Living roots of seedlings transplanted into each nursery had similar initial levels of colonization by *Pythium* (nursery B = 15%, nursery C = 29% ($P = 0.302$)) (Table 2). By harvest, however, root colonization had increased in NF plots at both nurseries to 71% while remaining lower in all three fumigant treatments (26-36%) ($P = 0.039$). There were no differences in seedling root colonization by *Pythium* among the three fumigant treatments at either nursery ($P = 0.658$). For *Fusarium*, roots of seedlings transplanted into nursery B had lower initial levels of colonization (6%) than at nursery C (19%) ($P = 0.005$). By harvest, root colonization had increased in NF plots at nursery B (54%) or was still similar to preplant levels at nursery C (17%). In contrast, root colonization was lower in all three fumigant treatments (3-7%) at both nurseries ($P = 0.041$). There were no differences in seedling root colonization by *Fusarium* among the three fumigant treatments at either nursery ($P = 0.367$).

There was no effect of any of the three biocontrol treatments on either *Pythium* or *Fusarium* soil populations, or on seedling root colonization by *Pythium* or *Fusarium* at harvest

(data not shown). Seedlings treated with any of the three biocontrol treatments were similar in size to the respective control seedlings treated only with water, regardless of which fumigation treatment they were applied into (data not shown). There were also no visible differences in seedling health (chlorotic versus not) between biocontrol treated seedlings and water control seedlings within each fumigation treatment.

3.2 Weeds

Nurseries differed in their responses with each measurement, so data are presented separately by site. The dominant weed species at nursery B was creeping yellowcress (*Rorippa sylvestris* (L.) Besser), a rhizomatous perennial species. Common annual weed species at that site included common chickweed (*Stellaria media* (L.) Vill.), common groundsel (*Senecio vulgaris* L.), horseweed (*Conyza canadensis* (L.) Cronq.), shepherd's-purse (*Capsella bursa-pastoris* (L.) Medik.), and annual bluegrass (*Poa annua* L.). Weeds observed at nursery C included the annuals common chickweed and horseweed, and the perennial birdseye pearlwort (*Sagina procumbens* L.). Preliminary analysis indicated that weed species did not differ by treatment, so weed species data were pooled into a single total weed cover percentage per quadrat prior to analysis.

Weed cover was reduced by soil fumigation at both nurseries, but there was no difference among fumigant treatments (Table 3). At nursery B, NF plots were infested with creeping yellowcress in July 2011, resulting in 58.3% total weed cover, contrasted with only 9.2% weed cover at nursery C. The August 2010 fumigation controlled weeds to less than 2% cover by July 2011 at both nurseries. Hand-weeding at nursery B reduced total weed cover to 7.7% in NF plots in September 2011, compared to < 1% cover among fumigation treatments. By January 2012, weed cover had returned to 36.6% in NF plots at nursery B, while fumigated treatments had only

increased to about 2 to 3% cover. At nursery C, September 2011 weed cover ranged from 2 to 4% in fumigated plots, compared to 11.7% in NF plots, while January 2012 weed cover was 25.3% in NF plots, and 4.7 to 8.8% in fumigated plots.

Fumigation reduced weeding time at nursery B by up to 90%, with an average weeding crew member able to clear just over 2 m per min in NF plots, contrasted with 17.91 to 22.64 m per person per min in fumigated plots (Table 3). While there were no differences in weeding times among fumigation treatments, there was a trend for DPC to be slightly more efficacious than MBC or MSC.

3.3 Seedling morphology, cull parameters, and cost analyses

At planting, seedlings were uniform in size within each nursery. Seedlings at nursery B were slightly taller (17 cm) with a larger stem diameter (3.3 mm) than seedlings at nursery C (15 cm and 2.5 mm, respectively) ($P < 0.001$). Root weight and shoot weight followed a similar distribution with seedlings having a greater biomass at nursery B (data not shown).

At harvest, seedlings in all three fumigant treatments appeared robust and dark green in color compared to those in the NF control treatment which were chlorotic and stunted. Seedling height, stem diameter, root weight, and shoot weight were always greater in the three fumigant treatments than in the NF control treatment ($P \leq 0.019$) (Table 4). All seedling morphological characteristics were positively correlated to each other ($R \geq 0.608$, $P \leq 0.001$) and gave similar results among treatments for all analyses. Seedlings from MBC and MSC plots were similar in size ($P > 0.050$) and consistently the largest at both nurseries, followed by those from the DPC treatment ($P \leq 0.019$). The only exception was root weight, which was similar across all three fumigant treatments ($P = 0.115$). Seedlings from NF plots were consistently the smallest in size, regardless of the parameter measured ($P = 0.019$).

Based on harvested seedling heights alone, a greater percentage of seedlings would be culled from the NF treatment than from any of the fumigant treatments, regardless of which of the six culling height parameters that the seedlings were evaluated against (Fig. 2). The percentage of culls from the NF treatment ranged from 1.7 to 5.4× greater than those from the DPC treatment, which itself was only slightly greater than the MBC and MSC treatments. Regardless of fumigation treatment, however, the percentage of culls increased as the stem height that the seedlings were evaluated against also increased. Results from the stem diameter and root:shoot ratio culling parameters were similar (data not shown).

Based on the combined cull parameters with seedling height < 30.5 cm, stem diameter < 6 mm, and a root:shoot ratio < 0.33, more seedlings would be culled from the NF treatment than from any of the fumigant treatments (Table 5). When applied to an average of 421,994 seedlings produced per ha at both nurseries, this would result in 1.6 to 2.3× the number of culls and about half the number of salable seedlings coming from NF plots than any of the three fumigant treatments. In terms of fumigant application costs, MSC was over \$500/ha cheaper to apply than either DPC or MBC (Table 5). However, because of the slightly smaller size of seedlings produced in DPC treatment plots, this treatment ended up costing almost as much to produce 1000 seedlings as the NF treatment. Despite being less expensive to apply initially, the NF treatment was the most expensive option per 1000 seedlings because of the large number of culls that resulted from this treatment.

4. Discussion

This study represents the first operational scale trial of reduced rates of MBC, MSC, and DPC under TIF in forest nurseries of the western U.S. All three fumigant treatments were

effective in reducing soilborne pathogens in the soil, in root debris, and from Douglas-fir seedling roots, as well as reducing weed cover and weeding times. However, while *Fusarium* soil populations remained low in all three fumigant treatments for the duration of the study, *Pythium* soil populations increased steadily over time from fumigation to harvest with DPC providing the most effective *Pythium* control, followed by MBC then MSC. In comparison to our previous study that utilized the standard rate of MBC at 392 kg/ha (Weiland et al., 2011), the reduced rates of MBC, MSC, and DPC used in this study appear as effective in reducing *Pythium* and *Fusarium* soil populations to at or near undetectable levels following fumigation. Although the formulation of MSC was different than that used in our previous study (468 l/ha + 137 kg/ha previously versus 253 l/ha + 168 kg/ha in this study), this fumigant combination also continued to be effective against both soilborne pathogens and weeds. Nevertheless, MSC efficacy has been reported to decrease with repeated use (Triky-Dotan et al., 2010) and it was the least effective fumigant treatment for controlling *Pythium* soil populations in this study. Therefore, the long-term performance of MSC must be evaluated further. Despite DPC being the most effective in reducing soil *Pythium* populations for the entire experiment, and similarly effective as the other two fumigant treatments in reducing *Fusarium* soil populations, the seedlings produced in the DPC plots were consistently smaller than those produced in either the MBC or MSC treatments. This reduction in size was not observed in previous studies with 1,3-dichloropropene on Douglas-fir (Anderson, 1963) or with a higher rate of DPC (448 kg/ha) on loblolly pine in the southeast U.S. (Enebak et al., 2011; Enebak et al., 2012). Thus, some further optimization of the formulation or application rate may be needed to minimize this effect in Douglas-fir seedling production.

Even though previous research showed that the combined application of multiple biocontrol agents has potential to improve disease control, we did not observe any effect of combining biocontrol agents in reducing either soil populations or root colonization by *Pythium* and *Fusarium*. One explanation might be that the biocontrol agents used were not compatible with each other. A recent review found that biocontrol agents were more likely to be antagonistic to each other when applied in combination with other agents than to work synergistically together to improve disease control (Xu et al., 2011). Therefore, disease control with this method may actually be more difficult to achieve than using biocontrol agents individually. An alternative explanation for the failure of biocontrol to work in our study may be that the biocontrol agents were not equally active against all members of the soilborne pathogen community at each nursery. For example, we know that the *Pythium* community at each nursery is unique (Weiland, 2011) and that the biocontrol agent *S. lydicus* WYEC108 does not inhibit all *Pythium* species and isolates from forest nurseries to the same degree (Weiland, 2014). *Pythium* species such as *P. ultimum* were inhibited less by *S. lydicus* than other species like *P. irregulare* (Weiland, 2014). Therefore, disease control may be compromised in soils where both pathogens are present. If the same holds true for the other biocontrol agents used in this study, there may be pathogen species or isolates that are present in the soil that are not adequately controlled by any of the applied biocontrol products.

Setting the combined cull parameters at < 30.5 cm seedling height, < 6 mm stem diameter, and < 0.33 root:shoot ratio for the morphology grade resulted in a high percentage of culls for the fumigant treatments (29-42%). However, these values were similar to those obtained in our previous study (21-34%) where we used the same combined cull parameter set to estimate the percentage of culls from treatments with the label rate of MBC or with reduced rates of MSC,

dimethyl disulfide, and methyl iodide (Weiland et al., 2011). By setting the cull parameters at a lower cutoff point, nurseries can increase the number of seedlings retained. If, for example, culls were based solely on seedling height and the cull parameters were lowered from 30.5 cm to 25.4 cm, the percentage of seedlings culled from the three fumigant treatments decreases from 8-18% to $\leq 5\%$ and a larger percentage of seedlings are retained (Fig. 2). However, because the retained seedlings are smaller, the price that consumers are willing to pay and the ensuing profit would be correspondingly less. Regardless of the parameter(s) selected, the number of seedlings retained at each cutoff level was always lower from NF plots than fumigant-treated plots. Similar curves can be created for the other cull parameters and can be used to help growers estimate losses and gains in order to maximize profits.

Application costs associated with the NF treatment were comparable in value to the fumigant treatments because the NF treatment included TIF application. However, even if TIF had been excluded from the NF treatment and the resulting application cost was \$0, the approximate 50% loss in yield with the combined cull parameter set (Table 5) would be unacceptable to growers who would experience a similar loss in profit. In this case, the grower could adjust the cull parameters downwards in order to keep a larger percentage of seedlings for sale (Fig. 2). However, the percentage of culls from NF plots is still unacceptably high in comparison to fumigated plots and the resulting seedlings are often compromised in size and health leading to poor survival and growth when outplanted. Leaving production fields without soilborne pathogen and weed control is not an option, particularly when other costs not addressed in this study (field preparation, irrigation, fertilization, etc.) are accounted for.

Based on the results of this study, reduced rates of MBC, MSC, and DPC applied under TIF are promising alternatives for the conventional rate of MBC in forest nurseries. The DPC

formulation may need some additional adjustment to minimize the small negative effect on seedling quantity and size, but otherwise it was comparable to MBC and MSC for soilborne pathogen and weed control. In the meantime, lower rates of MBC may also be used under TIF to conserve supplies of the fumigant and to reduce buffer zone requirements while achieving effective disease and weed control. Additional studies are needed to evaluate whether reduced rates of these alternative fumigants continue to perform well when applied to the same field over several production cycles.

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manage plant diseases in theory and practice. Phytopathology 101, 1024-1031.

493 **Table 1.** Biocontrol products applied during the study.

Biocontrol product	Biocontrol agent	Pathogen targets	Application rate
Actinovate SP ^a	<i>Streptomyces lydicus</i> WYEC108	<i>Fusarium</i> and <i>Pythium</i>	0.45 g/l
Cease ^b	<i>Bacillus subtilis</i> QST 713	<i>Fusarium</i> and <i>Pythium</i>	5 ml/l
RootShield WP ^b	<i>Trichoderma harzianum</i> T-22	<i>Fusarium</i> and <i>Pythium</i>	0.37 g/l
SoilGard 12G ^c	<i>Gliocladium virens</i> GL-21	<i>Pythium</i> , and damping-off and root rot pathogens	2.40 g/l

494 ^aMonsanto BioAg Inc., St. Louis, MO.495 ^bBioWorks Inc., Victor, NY.496 ^cCertis USA, L.L.C., Columbia, MD.

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Table 2. Means $\pm$ standard error of Douglas-fir root debris^a and seedling root colonization^b by *Pythium* and *Fusarium* among four fumigation treatments at two nurseries.

Treatment ^c	Root debris colonization (%)				Seedling root colonization (%)			
	<i>Pythium</i>		<i>Fusarium</i>		<i>Pythium</i>		<i>Fusarium</i>	
	Prefum	Postfum	Prefum	Postfum	Preplant	Harvest	Preplant	Harvest
NF	22 $\pm$ 4.1 a	5 $\pm$ 2.4 a	3 $\pm$ 1.7 a	13 $\pm$ 6.0 a	24 $\pm$ 1.6 a	71 $\pm$ 2.0 a	14 $\pm$ 2.9 a	36 $\pm$ 7.6 a
MBC	28 $\pm$ 3.9 a	0 $\pm$ 0.0 b	3 $\pm$ 2.3 a	1 $\pm$ 0.6 a	20 $\pm$ 1.5 a	35 $\pm$ 2.3 b	10 $\pm$ 2.3 a	3 $\pm$ 0.8 b
MSC	25 $\pm$ 4.3 a	0 $\pm$ 0.0 b	0 $\pm$ 0.0 a	0 $\pm$ 0.0 a	16 $\pm$ 1.6 a	36 $\pm$ 2.2 b	13 $\pm$ 2.8 a	6 $\pm$ 1.7 b
DPC	27 $\pm$ 3.7 a	0 $\pm$ 0.0 b	0 $\pm$ 0.0 a	0 $\pm$ 0.0 a	28 $\pm$ 1.9 a	26 $\pm$ 2.4 b	14 $\pm$ 3.3 a	5 $\pm$ 0.5 b

^aPercent root debris colonized by *Pythium* and *Fusarium* at prefumigation (Prefum, July 2010) and postfumigation (Postfum, September 2010). Means followed by the same letter in the same column are not statistically different ($P < 0.005$).

^bPercent seedling roots colonized by *Pythium* and *Fusarium* just prior to planting (Preplant, May 2011) and at harvest (December 2011). Means followed by the same letter in the same column are not statistically different ($P < 0.005$).

^c All treatments applied under totally impermeable film (TIF) in August, 2010. NF = nonfumigated; MBC = methyl bromide + chloropicrin; MSC = metam sodium + chloropicrin; DPC = dichloropropene + chloropicrin.

509 **Table 3.** Percent weed cover^a among four fumigation treatments at two forest nurseries and weeding times^c at nursery B.

Treatment ^b	Nursery B			Nursery C			Nursery B
	Jul	Sep	Jan	Jul	Sep	Jan	Weeding time
	2011	2011	2012	2011	2011	2012	(m per person per min.)
NF	58.3 a	7.7 a	36.6 a	9.2 a	11.7 a	25.3 a	2.04 b
MBC	0.3 b	0.3 b	2.0 b	1.1 b	3.1 b	4.7 b	22.64 a
MSC	0.5 b	0.5 b	2.3 b	1.8 b	4.1 b	8.8 b	22.26 a
DPC	0.5 b	0.5 b	3.4 b	1.1 b	2.0 b	6.6 b	17.91 a

510 ^aPercent weed cover within a 0.26-m² quadrat. Means followed by the same letter in the same column are not statistically different (P
511 < 0.05).

512 ^bAll treatments applied under totally impermeable film (TIF) in August, 2010. NF = nonfumigated; MBC = methyl bromide +
513 chloropicrin; MSC = metam sodium + chloropicrin; DPC = dichloropropene + chloropicrin.

514 ^cTime required to hand weed Douglas-fir nursery beds (m per person per min) at nursery B, July 28, 2011. This resulted in the
515 substantial reduction of weed cover between July and September 2011 at that nursery. Weed cover was not sufficient at nursery C on
516 July 28, 2011 to justify hand weeding. Means followed by the same letter in the same column are not statistically different (P < 0.05).

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Table 4. Means $\pm$ standard error of Douglas-fir seedling morphological traits^a at harvest (December 2011) among four fumigation treatments at two forest nurseries.

Treatment ^b	Height (cm)	Diameter (mm)	Root weight (g)	Shoot weight (g)	Root:shoot ratio
NF	31 $\pm$ 0.6 c	5.8 $\pm$ 0.11 c	4.0 $\pm$ 0.14 c	6.8 $\pm$ 0.28 c	0.64 $\pm$ 0.012 c
MBC	42 $\pm$ 0.6 a	7.3 $\pm$ 0.12 a	5.2 $\pm$ 0.16 a	11.5 $\pm$ 0.37 a	0.47 $\pm$ 0.008 a
MSC	43 $\pm$ 0.6 a	7.2 $\pm$ 0.12 ab	5.2 $\pm$ 0.17 a	11.5 $\pm$ 0.39 a	0.46 $\pm$ 0.008 a
DPC	39 $\pm$ 0.6 b	6.8 $\pm$ 0.13 b	4.7 $\pm$ 0.17 a	10.0 $\pm$ 0.39 b	0.50 $\pm$ 0.008 b

^aMeans followed by the same letter in the same column are not statistically different ($P < 0.05$).

^bAll treatments applied under totally impermeable film (TIF) in August, 2010. NF = nonfumigated; MBC = methyl bromide + chloropicrin; MSC = metam sodium + chloropicrin; DPC = dichloropropene + chloropicrin.

527 **Table 5.** Estimates of the number of Douglas-fir seedlings produced, culled^a, and sold per hectare, including 2015 fumigant and film
 528 application costs ^b.

	Number	Number	Number seedlings	Application	Application	Cost relative
Treatment ^c	seedlings	seedlings	sold/ha	cost/ha (\$)	cost/1000 seedlings	to MBC
	produced/ha	culled/ha (%)			(\$)	
NF	421,994	286,956 (68)	135,038	3405	25.22	1.23
MBC	421,994	122,378 (29)	299,616	6148	20.52	1.00
MSC	421,994	139,258 (33)	282,736	5464	19.33	0.94
DPC	421,994	177,237 (42)	244,757	6047	24.71	1.20

529 ^aSeedlings culled/ha estimated on the number of seedlings lifted from each treatment that were < 30.5 cm tall, < 6 mm in stem
 530 diameter, and with a root:shoot ratio < 0.33.

531 ^bCosts to apply each treatment at 2015 prices.

532 ^cAll treatments applied under totally impermeable film (TIF) in August, 2010. NF = nonfumigated; MBC = methyl bromide +
 533 chloropicrin; MSC = metam sodium + chloropicrin; DPC = dichloropropene + chloropicrin.

Fig. 1. Means ($\pm$ standard error) of (A) *Pythium* and (B) *Fusarium* soil populations (propagules per gram (ppg)) before soil fumigation in late July 2010 (Prefum), six weeks after fumigation in late September 2010 (Postfum - *Pythium* only), just prior to planting in mid-May 2011 (Preplant), and at harvest in December 2011 (Harvest) among four fumigation treatments at two forest nurseries ($n = 8$). All treatments applied under totally impermeable film (TIF) in August, 2010. NF = nonfumigated; MBC = methyl bromide + chloropicrin; MSC = metam sodium + chloropicrin; DPC = dichloropropene + chloropicrin. Treatments labeled with the same letter

542 within each time period are not significantly different ($P < 0.05$) according to Mann-Whitney and
543 Kruskal-Wallis nonparametric statistical tests. Note differences in scales between Fig. 1A and
544 1B.

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Fig. 2. Percent seedlings culled ($\pm$ standard error) at 25.4, 27.9, 30.5, 33.0, 35.6, and 38.1 cm seedling height cutoff points among four fumigation treatments at two forest nurseries. All treatments applied under totally impermeable film (TIF) in August, 2010. NF = nonfumigated; MBC = methyl bromide + chloropicrin; MSC = metam sodium + chloropicrin; DPC = dichloropropene + chloropicrin.