

Full Length Research Paper

# Effect of methyl bromide alternatives on seedling quality, nematodes and pathogenic soil fungi at the Jesup and Glennville Nurseries in Georgia: 2007 to 2008

S. A. Enebak<sup>1\*</sup>, T. E. Starkey<sup>2</sup> and M. Quicke<sup>2</sup>

<sup>1</sup>School of Forestry and Wildlife Sciences, 602 Duncan Drive, Auburn University, 36849, USA.

<sup>2</sup>School of Forestry and Wildlife Sciences, Southern Forest Nursery Management Cooperative, Auburn University, AL 36849, USA.

Accepted 8 April, 2013

For many years, forest-tree nurseries in the United States have relied on methyl bromide (MBr) soil fumigation to control weeds, pathogenic fungi, insects and nematodes. However, due to the concern over ozone depletion in the stratosphere, finding a soil fumigant alternative for MBr has been a priority for the forest nursery industry since 1991. A large-scale study comparing seven fumigants using operational application techniques and normal nursery management practices over two growing seasons was installed in two forest-tree nurseries in Georgia: the Plum Creek Nursery in Jesup, and the Rayonier Nursery in Glennville. Control of weeds, nematodes, and soilborne fungi was dependent upon the soil fumigant used and cropping season. By the end of the second growing season in 2008, there were no significant differences in seedling densities for any of the soil fumigants tested, as all soil fumigants were similar to MBr. While many seedling producers would prefer to use MBr to produce forest-tree seedlings in perpetuity, each nursery manager will need to identify the best alternative for their nursery conditions. Based on these 2 year trials, the soil fumigants with 100% chloropicrin and Pic + at 336 kg/ha (300 lbs/a) appear to be suitable alternatives for MBr in controlling forest-nursery pests and producing high quality seedlings at the Jesup and Glennville, GA Nurseries.

**Key words:** Soil fumigation, chloropicrin, soilborne fungi, nematodes, loblolly pine.

## INTRODUCTION

Fumigation with methyl bromide (MBr) mixtures has been the most commonly used method for producing high quality, pest-free forest-tree seedlings in the southeastern United States (Jang et al., 1993). Forest nurseries in the United States have relied for many years on MBr soil fumigation to control yellow and purple nutsedge, soil insects, nematodes and soilborne pathogenic fungi. In the southern United States, there are three fungal genera that are of primary concern in the production of pine seedlings. These genera are: *Fusarium*, *Pythium* and *Rhizoctonia*, each of which is associated with seedling

root and foliage diseases. Nursery managers have long recognized the importance of MBr to control these soil-borne pathogens in the production of forest-tree seedlings (Henry, 1953; South et al., 1997). Due to the concern over ozone depletion in the stratosphere, the Montreal Protocol under the Clean Air Act has begun a phase-out program for MBr use. Since 1991, finding alternatives for MBr has been a priority for the forest nursery industry. Other materials tested by the Southern Forest Nursery Management Cooperative, without much success, include basamid, hot pepper sauce, steam, hot water and biologicals. Although it may be difficult to find a soil fumigant alternative that is as broad-spectrum as MBr, the nursery industry realizes the importance of testing new compounds, rates and application techniques. Generally, forest-tree nurseries operate on a

\*Corresponding author. E-mail: [enebasa@auburn.edu](mailto:enebasa@auburn.edu). Tel: (334) 844.1028. Fax: (334) 844-1084.

**Table 1.** Soil fumigants and rates used in the 2007 to 2008 USDA area wide demonstration plots at the Plum Creek Nursery in Jesup, GA and Rayonier Nursery in Glennville, GA.

Soil fumigant treatment	Rate used	Chemical components
Methyl bromide + chloropicrin	392 kg/ha (350 lb/a)	67% MBr and 33% chloropicrin
Dimethyl disulfide + chloropicrin	113 l/ha (74 gal/a)	79% DMDS and 21% chloropicrin
MBrC 70/30	448 kg/ha (400 lb/a)	70% MBr (98/2) and 30% Solvent A
Pic+	336 kg/ha (300 lb/a)	85% chloropicrin and 15% Solvent A
New Pic+	336 kg/ha (300 lb/a)	85% chloropicrin and 15% Solvent B
Chloropicrin	336 kg/ha (300 lb/a)	100% chloropicrin
Chlor 60	448 kg/ha (400 lb/a)	60% chloropicrin and 40% 1,3-dichloropropene

Solvents A and B are proprietary compounds of Hendrix and Dail, approved by EPA for use in forest-tree nurseries and under full label considerations.

**Table 2.** Site information for the Jesup and Glennville, GA Nursery fumigation.

Application parameters	Jesup, GA	Glennville, GA
Fumigation	3-Apr-2007	20-Mar-2007
Fumigation type	Broadcast/shank injected	Broadcast/shank injected
Area in trial	2 ha	4 ha
Air temperature range	19 to 31°C	10 to 26°C
Wind speed	5 to 10 km/h	5 to 20 km/h
Soil moisture	8.5%	5.5%
Soil series	Norfolk loamy sand	Tifton loamy sand
Plastic in place	7 days	7 days

3 year cropping system with 2 production years per soil fumigation. Loblolly pine (*Pinus taeda* L.) is the primary species produced in southern forest nurseries (McNabb, 2006) and many soilborne pests can affect its production (Cordell et al., 1989). Despite the phase-out of MBr, forest nursery managers still need soil treatments that are comparable to MBr for control of soil borne pests.

The purpose of these studies is to look at the efficacy of a number of soil fumigants against common soil-borne fungi, weeds, and nematodes and their effect on seedling quality of forest-tree seedlings in the southern United States using operational application techniques under normal nursery management practices over a two year cropping cycle. These studies report a portion of the USDA – ARS Area-wide Pest Management Project for Methyl Bromide Alternatives and are part of a long-term continuing effort by the Southern Forest Nursery Management Cooperative to identify and evaluate soil fumigants. Information from these studies should be used by nursery managers in the southern US to select an MBr alternative that could be useful in the production of forest-tree seedlings in their nurseries.

## MATERIALS AND METHODS

Soil fumigation trials were established in two separate forest-seedling nurseries that differed in soil type and production systems in Georgia: the Plum Creek Nursery in Jesup and the Rayonier Nursery in Glennville. The soil fumigates used included MBr and six

alternatives that are currently available for large-scale use (Table 1). With the exception of New Pic+, soil fumigants were selected based on results of small plot studies previously conducted by the Southern Forest Nursery Management Cooperative (Starkey et al., 2006). The soil fumigation trial occupied 2 ha out of a total 20 production ha in Jesup and 4 ha out of 32 production ha in Glennville. For each nursery, the various soil fumigants were shank-injected to a depth of 20 to 25 cm using a Raven Flow Meter Control System<sup>®</sup> (Sioux Falls, SD) and immediately covered with 1 mm High Density Polyethylene Tarp (Cadillac Plastics Inc.) under the weather and soil conditions listed in Table 2. The trial was laid out in nursery sections consisting of nine seedling beds (3.5 m wide) between the irrigation pipelines (20 m) with each seedling bed approximately 182 m long. At the Jesup Nursery, each nursery bed was split into two plots of 90 m each. Each fumigated plot was 3.5 m wide x 90 m long with three seedling beds per treated plot. The experimental design at both nurseries was a randomized complete block that was replicated 5 times at Glennville and 4 times at Jesup. Each nursery sowed a single family of loblolly pine (*P. taeda*) seed in late April 2007 that were lifted in mid November 2007.

The area was fallow during the winter and in March 2008 the beds were prepared for sowing. The second seedling crops' sowing occurred in late April 2008 with seedlings lifted in October/November 2008. With respect to seedling production over the two growing seasons, standard nursery production systems used at each nursery (for example irrigation, fertilization, weed control, insect control, etc.) were allowed to continue within the experimental area.

## Seedling quality

The effect of the soil fumigants on seedling densities and growth

**Table 3.** Loblolly pine seedling density (No./m<sup>2</sup>), at the end of each cropping season at Jesup and Glennville, GA.

Soil treatment	Jesup, GA Nursery		Glennville, GA Nursery	
	Nov 2007	Nov 2008	Oct 2007	Oct 2008
MBr	205 b	215 a	144 a	166 a
Chloropicrin	258 a	226 a	147 a	168 a
Chlor 60	237 ab	237 a	142 a	156 ab
MBrC 70/30	205 b	226 a	135 a	139 b
DMDS + Chlor	205 b	226 a	147 a	139 b
Pic+	205 b	226 a	141 a	153 ab
New Pic+	205 b	237 a	150 a	155 ab
Isd <sub>(0.05)</sub>	43	43	39	23

Within column means followed by the same letter do not differ at P = 0.05 level using Duncan's Multiple Range Test. Target seedling density is 258 seedlings/m<sup>2</sup> at Jesup, GA and 192 seedling/m<sup>2</sup> at Glennville, GA.

characteristics were assessed in four subplots (1.2 x 0.3 m) per treatment plot at 7 wks post-sowing, mid-summer (15 weeks post-sowing) and just prior to lifting in the fall (26 weeks post-sowing) in 2007 and 2008, respectively. Twenty-five loblolly pine seedlings per subplot were collected in the fall of each cropping season and returned to the laboratory at Auburn University for analysis. Seedling root collar diameter (RCD), shoot height and seedling dry weight (biomass) was measured for each seedling as well as overall root growth. For root morphology, 10 seedlings per subplot were examined for root length, root surface area, average root diameter and the number of root tips using WinRhizo<sup>®</sup> software by Regents Instruments Inc., Quebec, Canada.

#### Soil trichoderma and nematodes

Throughout the two cropping seasons, soil samples were collected from the center seedling bed of each 3-bed plot: at pre-sowing, post-sowing, mid-summer and just prior to seedling lifting in November of each season. Half of each soil sample was plated onto *Trichoderma*-selective media (Elad et al., 1981) and the remaining half was sent to the Soils Laboratory at Auburn University for a quantitative assessment of nematodes.

#### Soilborne pathogenic fungi

In addition to seedling quality and nematode control, the ability of the soil fumigants to control common soilborne pathogens was examined. Three soilborne pathogens, *Pythium* spp, *Rhizoctonia* spp and *Fusarium* spp, were transferred from the culture collection in the laboratory onto potato dextrose agar (Difco<sup>®</sup>) and incubated for 2 weeks. Approximately 6 weeks prior to soil fumigation, agar plates of each fungus were used to inoculate bags containing moistened, sterilized oatmeal. The inoculated bags were mixed on a regular basis to encourage fungal growth throughout the oatmeal and left to grow on the laboratory bench at 22°C. Two days prior to soil fumigation, a 300 ml beaker of each oatmeal/fungus mixture was placed into 30 Hubco<sup>®</sup> soil sample bags (12 x 18 cm), the top folded over, stapled shut, and labeled to each fungus. As the soil fumigation treatments were being applied in the nursery, three Hubco<sup>®</sup> bags containing the inoculated oatmeal of each soilborne fungus were placed into the soil of each treated plot. To do this, a 45 cm slit was cut into the plastic tarps placed over the fumigated soil and the three bags were buried 15 cm deep. The slit was then sealed with fumigation tape. Six days after soil fumigation, the

Hubco<sup>®</sup> soil bags were removed from each of the soil fumigation plots, placed in a cooler and returned to Auburn University. In the laboratory, each Hubco<sup>®</sup> soil bag was thoroughly mixed and then opened in a laminar flow hood. From each fungal species, nine oatmeal pieces were placed in groups of three on the following selective media: 1) PARP media for *Pythium* (Kannwischer and Mitchell, 1981); 2) Komada's media for *Fusarium* (Nelson et al., 1993); and 3) Ko and Hora's media (1971) for *Rhizoctonia*. There were three agar plates for each treatment replication.

One week after plating the fumigated oatmeal onto selective agar media, each plate was examined for fungal growth that would indicate the target soilborne pathogens. In addition to the soilborne pathogens, the non-target fungi were also counted. As a control, bags of each soilborne pathogenic fungus not fumigated were also plated out on selective media as described earlier.

## RESULTS

### Seedling quality

At the Plum Creek Nursery in Jesup, seedling densities at the end of the first growing season in the 100% chloropicrin treatments were significantly greater than the MBr treatments. However, by the end of the second growing season in 2008, there were no significant differences for seedling densities for any of the soil fumigants tested, as all gave similar seedling densities as the standard MBr soil fumigation treatment (Table 3). With respect to the Rayonier Nursery in Glennville, GA, seedling densities for 2007 were far below target levels due to wind damage that occurred shortly after sowing. Taking this seedling reduction into account, there were no significant differences between seedling densities for any of the soil fumigants tested in 2007. While seedling densities for the 2008 seedling crop were generally higher than 2007, they were still below target levels for all soil fumigants tested (Table 4). Unlike the 2007 growing season, where all soil fumigants were similar to MBr, in 2008, soils treated with MBrC 70/30 and DMDS + Chloropicrin had significantly fewer seedlings when

**Table 4.** Loblolly pine seedling root collar diameter (mm) at the end of each growing season at Jesup and Glennville, GA.

Soil treatment	Jesup, GA Nursery		Glennville, GA Nursery	
	Nov 2007	Nov 2008	Oct 2007	Oct 2008
MBr	4.83 ab	4.43 ab	5.34 a	4.93 ab
Chloropicrin	4.65 b	4.70 a	5.33 a	4.62 b
Chlor 60	4.91 ab	4.16 ab	5.41 a	4.58 b
MBrC 70/30	4.99 ab	4.27 ab	5.66 a	4.88 ab
DMDS + Chlor	5.19 a	4.04 ab	5.49 a	5.14 a
Pic+	5.13 a	4.46 ab	5.69 a	4.95 ab
New Pic+	5.13 a	3.97 b	5.55 a	4.91 ab
lsd <sub>(0.05)</sub>	0.37	0.70	0.68	0.44

Means within columns followed by the same letter do not differ at P = 0.05 level using Duncan's Multiple Range Test.

**Table 5.** Loblolly pine seedling grade by soil fumigation in 2007 and 2008, Jesup, GA.

Soil treatment	2007 Season				2008 Season			
	Total	Grade 1	Grade 2	Culls	Total	Grade 1	Grade 2	Culls
MBr	205 b	129 ab	65 a	11 a	215 a	64 b	140 a	11 a
Chloropicrin	258 a	107 b	151 b	0 a	226 a	118 a	97 b	11 a
Chlor 60	237 ab	140 a	75 a	11 a	237 a	54 b	161 a	22 a
MBrC 70/30	205 b	129 ab	75 a	0 a	226 a	65 b	140 a	22 a
DMDS + Chlor	205 b	140 a	54 a	11 a	226 a	43 b	151 a	32 a
Pic+	205 b	140 a	43 a	22 a	226 a	75 b	140 a	11 a
New Pic+	205 b	151 a	54 a	0 a	237 a	54 b	161 a	22 a
lsd <sub>(0.05)</sub>	43	22	32	22	43	4	32	22

Means within columns followed by the same letter do not differ at P = 0.05 level using Duncan's Multiple Range Test. Numbers are seedlings per square meter of nursery bed. Nursery target for both growing seasons was 258 seedlings/m<sup>2</sup>.

compared to the standard MBr treatment (Table 3). Differences in seedling root collar diameters (RCD) among the soil fumigants tested at Plum Creek were observed (Table 4). Some of the newer chemistries, Pic+, New Pic+ and DMDS had seedlings with significantly larger RCD than seedlings grown in 100% chloropicrin. In 2008, soils fumigated with chloropicrin had significantly larger RCD than New Pic+ (Table 4). When considering seedling grades (Grade 1 = seedlings > 4.69 mm, Grade 2 = seedlings 3.2 to 4.69 mm and Cull = seedlings < 3.2 mm), the proportion of seedlings for each grade was similar across all soil fumigants in 2007 except for chloropicrin. In 2007, chloropicrin was the only soil fumigant tested at this nursery that had more Grade 2 seedlings than Grade 1 seedlings (Table 5).

In 2008 the proportion of Grade 1 seedlings was fewer than in 2007 for all soil fumigants except for chloropicrin (Table 5). Higher seedling densities typically result in a lower mean RCD yielding fewer Grade 1 seedlings per square foot. Chloropicrin-fumigated soils had higher

seedling density than other fumigants in 2007, producing a lower percentage of Grade 1 (42%) than Grade 2 (58%) seedlings; yet, with a lower seedling density in 2008, more Grade 1 (50%) than Grade 2 (45%) seedlings were produced. When considering seedling grades at the Rayonier Nursery in Glennville, GA, the proportion of seedlings grown in 2007 for each grade was similar across all soil treatments tested: 77% Grade 1, 22% Grade 2 and 1% Cull. Not surprising, in 2008 the proportion of Grade 1 seedlings declined for all soil treatments with 56% Grade 1, 41% Grade 2 and 3% Cull (Table 6). The lower number of Grade 1 seedlings in 2008 was due to the higher seedling densities. When comparing seedling densities and proportion of Grade 1 seedlings in 2008, two soil fumigants stand out. MBr has a high percentage of Grade 1 seedlings even though it has high seedling density. Soils fumigated with Chlor 60 had a relatively low percentage of Grade 1 seedlings even though those plots had a low seedling density (Table 6). In contrast, soils fumigated with chloropicrin

**Table 6.** Loblolly pine seedling grade by soil fumigation in 2007 and 2008 Glennville, GA.

Soil treatment	2007 Season				2008 Season			
	Total	Grade 1	Grade 2	Culls	Total	Grade 1	Grade 2	Culls
MBr	144 a	101 a	42 a	1 a	166 a	98 a	65 b	3 bc
Chloropicrin	147 a	110 a	35 a	2 a	168 a	79 ab	82 a	7 a
Chlor 60	142 a	101 a	40 a	1 a	156 ab	70 b	81 a	5 ab
MBrC 70/30	135 a	107 a	27 a	1 a	139 b	79 ab	56 bc	4 ab
DMDS + Chlor	147 a	118 a	28 a	1 a	139 b	93 ab	43 c	3 bc
Pic+	141 a	114 a	25 a	2 a	153 ab	90 ab	60 bc	3 bc
New Pic+	150 a	122 a	27 a	2 a	155 ab	85 ab	65 b	5 bc
Isd <sub>(0.05)</sub>	39	23	17	1	23	16	22	2

Within column means followed by the same letter do not differ at P = 0.05 level using Duncan's Multiple Range Test. Numbers are seedlings per square meter of nursery bed. Target seedling density was 192 seedlings/m<sup>2</sup>.

**Table 7.** Loblolly pine seedling root morphology at the end of each growing season Jesup, GA.

Soil treatment	Root length (cm)		Root surface area (cm <sup>2</sup> )		Avg root Dia (mm)		No. of root tips	
	2007	2008	2007	2008	2007	2008	2007	2008
MBr	315 a	243 a	91 a	54 a	0.92 ab	0.72 a	910 a	568 a
Chloropicrin	254 b	249 a	69 b	55 a	0.87 b	0.72 a	730 b	584 a
Chlor 60	274 ab	212 a	78 ab	46 a	0.91 ab	0.72 a	806 ab	532 a
MBrC 70/30	317 a	227 a	91 a	50 a	0.91 ab	0.72 a	943 a	560 a
DMDS + Chlor	299 ab	231 a	87 a	51 a	0.93 a	0.71 a	925 a	579 a
Pic+	277 ab	218 a	84 a	51 a	0.96 a	0.77 a	834 ab	545 a
New Pic+	313 a	232 a	91 a	49 a	0.92 ab	0.70a	941 a	636 a
Isd <sub>(0.05)</sub>	51	54	15	9	0.06	0.10	155	176

Means within columns followed by the same letter do not differ at P = 0.05 level using Duncan's multiple range test.

had a relatively low percentage of Grade 1 seedlings from those plots, but had the highest seedling density. Overall, seedling root architecture and root morphology indicated smaller seedlings in 2008 when compared to seedlings grown in 2007 at Jesup (Table 7). First year soil fumigation results in larger seedlings and these alternative soil fumigants are behaving as expected. In 2007, as far as an MBr alternative, all soil fumigant alternatives performed as well as MBr across all the root morphology measurements at this nursery.

In 2008, there were no significant differences in seedling quality and quantity among any of the soil fumigants that did not contain MBr in its formulation. Overall, the total seedling root length in these trials ranged from 212 to 317 cm, or about 2.1 to 3.0 m of total roots per seedling. Like seedlings at Jesup, seedling root architecture and root morphology indicated smaller seedlings in 2008 when compared to seedlings grown in 2007 in Glennville (Table 8). As far as an MBr alternative, Pic+ performed well across all the root morphology measurements at Glennville with root length and number of root tips significantly better than the other non-MBr fumigants tested.

### Soil trichoderma and nematodes

At the end of the first growing season in 2007, nursery soils at Jesup, GA fumigated with MBr had significantly lower levels of *Trichoderma* than Chlor 60 treatments (Table 9). Other soil fumigants, including MBrC 70/30 and chloropicrin, reduced *Trichoderma* levels like that of MBr. By the end of the second growing season (2008), the *Trichoderma* levels within the various soil fumigants tested were all similar to MBr. The effect of soil fumigants on soilborne fungi in 2007 at the Glennville Nursery indicated that soils treated with MBr had significantly lower levels of *Trichoderma* than DMDS + Chloropicrin (Table 9). By the end of the second growing season (2008), the *Trichoderma* levels within the various soil fumigants tested were similar to MBr. Over the course of the 2 year study, each soil fumigant plot was examined five times for both the number and species of nematodes within the soil/seedling interface. Nematode populations within the soil are rarely distributed uniformly across the nursery beds and these studies had a wide range (0 to 687 nematodes /100 cc soil) in numbers and species for all soil fumigants used (Table 10). Because of the

**Table 8.** Loblolly pine seedling root morphology at the end of each growing season, Glennville, GA.

Soil treatment	Root length (cm)		Root surface area (cm <sup>2</sup> )		Avg root dia (mm)		No. root tips	
	2007	2008	2007	2008	2007	2008	2007	2008
MBr	292 a	167 a	83 ab	45 ab	0.92 b	0.86 a	715 ab	366 a
Chloropicrin	218 b	152 ab	68 b	40 b	1.02 a	0.85 a	515 c	328 a
Chlor 60	289 a	148 ab	86 a	39 b	0.95 ab	0.87 a	738 ab	303 a
MBrC 70/30	304 a	157 ab	91 a	45 ab	0.98 ab	0.93 a	820 a	325 a
DMDS + Chlor	266 a	170 a	78 ab	48 a	0.95 ab	0.90 a	633 bc	342 a
Pic+	302 a	165 ab	88 a	48 a	0.93 b	0.93 a	735 ab	344 a
New Pic+	264 a	142 b	81 ab	41 ab	0.99 ab	0.94 a	680 b	310 a
lsd (0.05)	49	25	17	7	0.08	0.12	137	86

Within column means followed by the same letter do not differ at 0.05 level using Duncan's Multiple Range Test.

**Table 9.** Post-fumigation recovery of *Trichoderma* spp. from nursery soils in Jesup and Glennville, GA Nurseries in 2007.

Treatment	Jesup, GA Nursery			Glennville, GA Nursery		
	7 weeks	15 weeks	26 weeks	7 weeks	15 weeks	26 weeks
MBr	86 a	117 a	18 bc	137 a	75 bc	90 b
Chloropicrin	42 ab	71 ab	18 bc	56 b	91 b	118 ab
Chlor 60	53 ab	90 ab	64 a	44 b	71 bc	90 b
MBrC 70/30	27 b	42 b	6 c	94 ab	54 c	98 b
DMDS+Chlor	63 ab	84 ab	30 abc	84 ab	146 a	168 a
Pic+	34 ab	61 ab	20 bc	63 b	94 b	108 b
New Pic+	48 ab	93 ab	57 ab	78 ab	68 bc	119 ab
lsd(0.05)	55	71	44	67	38	57

Means within columns followed by the same letter do not differ at P = 0.05 level using Duncan's Multiple Range Test. numbers are in colony forming units (CFU) per mg of soil.

variability, there were no differences between treatments within a nursery for any of the soil fumigants tested. Overall, all soil fumigants were effective in reducing nematode populations the first growing season which then increased during the 2 year rotation. One of the more troublesome species on seedling production is the Stunt nematode (*Tylenchorhynchus claytoni*) which appeared during the second cropping season in all soil fumigants tested.

Of the soil fumigants tested, Pic+ had the lowest number of nematodes recovered in soil samples over the course of the study at the Plum Creek nursery. At the Rayonier Nursery, of the fumigants tested, soils treated with Chlor 60 had the fewest nematode numbers.

### Soilborne pathogenic fungi

All soil fumigants and rates tested at both nurseries were effective in eliminating target *Pythium*, *Fusarium* or *Rhizoctonia* that was present on the oatmeal in the Hubo<sup>®</sup> soil bags. Of the three soilborne pathogens tested, *Fusarium* was recovered more often than the other two fungi (Table

11). When non-fumigated control bags were plated onto their selective media, the individual soilborne fungus was recovered from 100% of all oatmeal groups on each plate. In addition to the soilborne pathogenic fungi, a number of non-target fungi were recovered from the oatmeal. The non-target fungi were primarily saprophytic fungi, *Penicillium*, *Trichoderma* or *Aspergillus*. DMDS + chloropicrin had the lowest recovery of non-target fungi compared to the other soil fumigants used. The difference in fungal recovery on all the DMDS agar plates for all fungi was apparent even after 7 days, with many agar plates void of any fungal growth. New Pic+ had significantly less non-target *Pythium* and *Rhizoctonia* than chloropicrin.

### DISCUSSION

The primary objective of the USDA Areawide MBr Alternative program is to identify possible alternatives to MBr using large-scale, multi-year trials in soils and

**Table 10.** Soil nematode levels at the end of each growing season in Jesup and Glennville, GA.

Soil treatment	Nematode	Jesup, GA		Glennville, GA	
		2007	2008	2007	2008
MBr	Stunt	8	140	1	39
	Stubby root	39	0	1	0
	Ring	0	0	0	0
Chloropicrin	Stunt	20	133	2	25
	Stubby root	29	1	0	0
	Ring	0	3	0	0
Chlor 60	Stunt	3	123	0	7
	Stubby root	34	0	0	0
	Ring	0	0	0	0
MBrC 70/30	Stunt	13	145	4	74
	Stubby root	19	0	2	0
	Ring	0	1	0	0
DMDS+Chlor	Stunt	33	65	3	18
	Stubby root	34	0	0	0
	Ring	0	1	0	0
Pic+	Stunt	46	47	4	16
	Stubby root	19	0	1	0
	Ring	0	1	1	0
New Pic+	Stunt	14	47	14	138
	Stubby root	36	0	0	0
	Ring	0	0	0	0

Due to the non-uniform distribution of nematodes, there were no differences observed for any treatment x nematode x year,  $P = 0.05$ . Numbers are the average of 4 replicates per treatment recovered in 100 cc of soil.

**Table 11.** Proportion of pathogenic soilborne recovered from inoculated oatmeal exposed to soil fumigants and planted onto selective agar media at Jesup and Glennville, GA, 2007.

Soil fumigant	Pythium		Fusarium		Rhizoctonia	
	Jesup	Glennville	Jesup	Glennville	Jesup	Glennville
MBr	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.02 a
DMDS + Chlor	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
MBrC 70/30	0.00 a	0.00 a	0.25 a	0.00 a	0.00 a	0.00 a
New Pic+	0.00 a	0.00 a	0.03 a	0.00 a	0.00 a	0.00 a
Pic+	0.00 a	0.00 a	0.04 a	0.00 a	0.00 a	0.00 a
Chloropicrin	0.00 a	0.00 a	0.00 a	0.02 a	0.00 a	0.00 a
Chlor 60	0.00 a	0.00 a	0.01 a	0.06 a	0.00 a	0.00 a
Isd	0.00	0.00	0.31	0.38	0.00	0.03 a

Means within columns followed by the same letter do not differ at  $P = 0.05$  level using Duncan's Multiple Range Test.

conditions throughout the southern U.S. One of the unique aspects of MBr as a soil fumigant is its ability to

consistently control weeds, insects, nematodes and fungi across many different growing conditions. Studies

conducted within the southern US in forest-tree nurseries have yet to find an MBr alternative that fits those characteristics, and these studies bear that out. For example, seedlings grown in soils fumigated with chloropicrin had the smallest RCD (4.6 mm) for 2007 and the largest RCD (4.7 mm) in 2008 in Glennville. Overall, seedling production and seedling characteristics were dependent upon the soil fumigant used and the cropping season. The true test of an MBr alternative is its performance during the second growing season where treatment differences usually begin to appear as disease, weed, and nematode pressures increase. Based on these trials, when MBr is eventually phased out, those soil fumigants with chloropicrin appear to be the most useful in controlling pests and producing quality seedlings in both Jesup and Glennville forest-tree nurseries. In both instances, seedling densities and root characteristics from plots treated with chloropicrin-based products were similar to MBr. One of the primary reasons for determining the effects of these soil fumigants on root architecture is that a more fibrous root system increases the chance of seedling survival in the field (Hatchell and Muse, 1990; Frampton et al., 2002; Davis and Jacobs, 2005). One soil fumigant, DMDS + chloropicrin, was comparable to MBr in RCD and root morphology characteristics and soilborne *Trichoderma* levels, but had a significant odor problem (garlic or strong propane) that lasted long into the growing season.

The lingering odor with this particular soil fumigant will limit its acceptance by growers as an alternative to MBr. By far the best MBr alternatives tested were Pic+ and New Pic+, with both soil fumigants controlling soilborne pathogens, nematodes, and weeds and producing quality seedlings. The difference between Pic+ and New Pic+ is the solvent used in the application/injection process that reportedly decreases the volatility of chloropicrin and keeps the soil fumigant in place longer. When used as a soil fumigant, both these treatments resulted in similar seedling characteristics (RCD, seedling densities, seedling root morphology, etc) and had similar effects on the levels of the important soilborne fungi, *Trichoderma*. However, it was observed at Glennville, GA that the New Pic+ formulation did not control annual sedge (*Cyperus compressus*). Because of the lack of weed control by New Pic+, this compound has been dropped from further trials in forest-tree nurseries. In addition to the weed problems at Glennville, New Pic+ also had a high incidence of nematodes the second growing season (Table 11). The Rayonier nursery has a long history of nematodes reaching levels that affect seedling production. For these reasons, 1, 3, dichloropropene (Telone<sup>®</sup>) may need to be used in nurseries with reoccurring nematode pressure. Chlor 60 (containing 40% 1, 3-dichloropropene) had the lowest nematode levels and may be an option for nurseries that have nematode issues in the second growing season. While

the seedling densities and root characteristics with chloropicrin were encouraging, one of the potential pitfalls with using 100% chloropicrin at 336 kg/ha (300 lbs/a) is the buffer zone restrictions under current nursery fumigation practices. If these restrictions limit the use of 100% chloropicrin, then Pic+ with 85% chloropicrin would be the best alternative at both nurseries. The final decision when selecting an MBr alternative needs to take into consideration the ability of the soil fumigant to work under individual nursery soil conditions and the impact the new soil fumigation rules that come into place in 2012 will have on each individual nursery.

Soil fumigation is an effective way to reduce pathogenic soilborne fungi in the nursery that affect seedling production and survival after outplanting. All soil fumigants tested at both nurseries were found to be effective in controlling *Pythium*, *Rhizoctonia* and *Fusarium* when inoculated onto oatmeal. The widespread use of MBr has minimized extensive seedling losses due to soilborne pathogenic fungi. Of the fungi tested, *Pythium* still can cause damping-off problems in the early spring and is often limited to areas of poor drainage and standing water. *Rhizoctonia* can appear in nurseries both as root decay and as foliage blight, especially in the second-year crops post fumigation as the fungus increases over the first growing season (Carey and McQuage, 2004). At best, the soil fumigants reduced levels of these pathogens for the two cropping seasons and longer rotations would need to be tested to see if a nursery could get three seedling crops from one fumigation. One of the unique aspects of soil fumigants currently being tested in southern forest nurseries is that they do not completely eliminate beneficial fungi which are needed for seedling growth. In these two nurseries, the soil fumigants tested did not completely eliminate all soilborne fungi. This is important since previous research has shown that *Trichoderma* is an important soilborne fungus necessary for proper pine seedling growth (Bailey and Lumsden, 1998; Dong et al., 1987; Papavizas, 1985; Mousseaux et al., 1998; Samuels, 1996). In these trials, the population levels of non-target soilborne fungi rebounded quickly with all soil fumigants used as previous Nursery Cooperative research has shown that *Trichoderma* is not as sensitive to MBr as other soil fumigants (Carey et al., 2005; Starkey and Enebak, 2008). In contrast, dazomet, a soil fumigant tested by the Nursery Cooperative for several years, significantly reduced the levels of beneficial fungi which remained after two growing seasons (Starkey et al., 2006). While many nursery managers would prefer to use MBr in perpetuity to grow forest-tree seedlings, MBr will eventually be unavailable and each nursery manager will need to identify the best alternative for their nursery conditions.

These trials and others indicate that, while not the perfect replacement, seedling production is still possible

without MBr if compounds such as chloropicrin are used and managers pay close attention to weed and nematode pests that are less susceptible to chloropicrin than MBr.

## REFERENCES

- Bailey BA, Lumsden RD (1998). Direct effects of *Trichoderma* and *Gliocladium* on plant growth and resistance to pathogens. In *Trichoderma and Gliocladium*, Edited by G.E. Harman and C.P. Kubicek. Taylor and Francis, Inc., Bristol, 2: 185–204.
- Carey WA, McCraw D, Enebak SA (2005). Seedling production by seed treatment and fumigation treatment at the Glennville Regeneration Center in 2004. Auburn Univ. South. For. Nursery Manage. Coop., Res. Rep., 05-04. Auburn, AL, p. 5.
- Cary WA, McQuage K (2004). Control of Rhizoctonia foliage blight by fungicides and fumigation: Lower application rates and fumigation effects. Auburn Univ. South. For. Nursery Manage. Coop., Res. Rep. 04-03. Auburn, AL, p. 4.
- Cordell CE, Anders R, Hoffard WH, Landis TD, Smith RS, Toko HV (1989). Forest nursery pests. Wash (DC): Agric. Handb., 680: 184.
- Davis AS, Jacobs DF (2005). Quantifying root system quality of nursery seedlings and relationship to outplanting performance. *New For.*, 30: 295-311.
- Dong LF, Mang XY, Mang HG (1987). Breaking seed dormancy of *Pinus bungeana* Zucc. with *Trichoderma*-4030 inoculations. *New For.*, 1: 245-249.
- Elad Y, Chet I, Henis, Y (1981). A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. *Phytoparasitica*, 9: 59-67.
- Frampton J, Isik F, Goldfarb B (2002). Effects of nursery characteristics on field survival and growth of loblolly pine rooted cuttings. *South. J. Appl. For.*, 26: 207-213.
- Hatchell GE, Muse, HD (1990). Nursery cultural practices and morphological attributes of longleaf pine bare-root stock as indicators of early field performance. USDA For. Serv. Res. Pap. SE., p. 277.
- Henry BW (1953). A root rot of southern pine seedlings and its control by soil fumigation. *Phytopathology*, 43: 81-88.
- Jang E, Wood WS, Dorschner K, Schaub J, Smith D, Fraedrich S, Hsu H (1993). Methyl bromide phase out in the US: Impact and alternatives. In: USDA Workshop on alternatives for methyl bromide. Crystal City, VA.
- Kannwischer ME, Mitchell DJ (1981). Relationship of numbers of spores of *Phytophthora parasitica* var. *nicotianae* to infections and mortality of tobacco. *Phytopathology*, 71: 69-73.
- Ko WH, Hora FK (1971). A selective medium for the quantitative determination of *Rhizoctonia solani* in soil. *Phytopathology*, 61: 707.
- McNabb KL (2006). Forest tree seedling production in the South for the 2005-2006 planting season. Auburn Univ. South. For. Nursery Manage. Coop., Tech. Note, 06-02: 10.
- Mousseaux MR, Dumroese RK, James RL, Wenny DL, Knudsen GR (1998). Efficacy of *Trichoderma harzianum* as a biological control of *Fusarium oxysporum* in container-grown Douglas-fir. *New For.*, 15: 11–21.
- Nelson PE, Toussoun TA, Marasas WOF (1993). *Fusarium* species: An illustrated manual for identification. The Pa. State Univ. Press. University Park.
- Papavizas GC (1985). *Trichoderma* and *Gliocladium*: Biology, ecology, and potential for biocontrol. *Annu. Rev. Phytopathol.*, 23: 23–54.
- Samuels GJ (1996). *Trichoderma*: A review of biology and systematics of the genus. *Mycol. Res.*, 100: 923–936.
- South DB, Carey WA, Enebak SA (1997). Chloropicrin as a soil fumigant in forest nurseries. *For. Chron.*, 73: 489-494.
- Starkey TE, Enebak SA (2008). Indian Mound Nursery, Texas: Methyl bromide alternative study 2005-2007. Auburn Univ. South. For. Nursery Manage. Coop., Res. Rep. 08-07. Auburn, AL, p. 11.
- Starkey TE, Enebak SA, McCraw D (2006). Seedling quality and weed control with methyl bromide and methyl iodide at the Glennville Regeneration Center 2005-2006. Auburn Univ. South. For. Nursery Manage. Coop., Res. Rep. 06-05. Auburn, AL, p. 5.