Auburn University Southern Forest Nursery Management Cooperative

RESEARCH REPORT 11-01

EFFECT OF METHYL BROMIDE ALTERNATIVES ON LOBLOLLY PINE SEEDLING QUALITY AT THE JOSHUA TIMBERLANDS FOREST TREE NURSERY IN ELBERTA, ALABAMA: 2009-2010

by
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INTRODUCTION

As part of the ongoing USDA – ARS Area-wide Pest Management Project for Methyl Bromide Alternatives – South Atlantic Region, the Southern Forest Nursery Management Cooperative at Auburn University continues to test alternative soil fumigants to methyl bromide (MBr). In the southeastern United States, fumigation with MBr is important for producing high quality forest tree seedlings in an environment that is conducive for soil-borne pathogens, nematodes, and weeds. In October 2008, a large-scale trial was established at the Joshua Timberlands forest tree nursery in Elberta, AL that compared soil fumigants using standard nursery management practices over two growing seasons (2009 -2010). The objective of the trial was to identify a soil fumigant that could serve as a replacement for MBr at this particular nursery for the production of loblolly pine seedlings. Information in this report should be used by nursery managers in the southern US to choose the most viable MBr alternative for forest tree seedling production in their particular nursery.

METHODOLOGY

The fumigation treatments included two rates of MBr and five other currently available soil fumigants that were selected from previous Nursery Cooperative fumigation studies (Table 1). Fumigants were shank-injected on October 22, 2008 and covered with a 1 mm High Density Polyethylene (HDPE) Tarp (AEP Industries Inc.) as a broadcast/flat tarp that occupied four out of 20 total production acres within the nursery (Table 2). The trial was laid out in five nursery sections that consisted of nine seedling beds between the irrigation pipelines with each bed being approximately 560 ft long x 4 ft wide. The experiment was a randomized complete block design with each treatment being 280 ft long and replicated four times. Each 9-bed nursery section included 3 soil fumigation treatments (3 nursery beds per treatment). After fumigation, the nursery sowed a single family of loblolly pine (*Pinus taeda*) seed on April 30, 2009, and seedlings were maintained using standard nursery cultural practices (fertilization, irrigation, pest management, etc.) until lifting took place on January 13, 2010. The second growing season of loblolly pine was sown on April 19, 2010 and lifted on December 1, 2010.

In both growing seasons, soil fumigant efficacy was determined by measuring seedling quality parameters and soil-borne fungi levels. Seedling and soil samples were collected from the center seedling bed of each three-bed treatment area. In 2009, soil samples were collected pre-sowing, post-sowing, and at lifting in the fall, while collections took place only post-sowing and at lifting in 2010. Half of each soil sample was sent to the soils laboratory at Auburn University for an assessment of nematode populations, and the remaining half was plated onto *Trichoderma* selective media (TSM) (Elad et al. 1981) to determine the effects of the soil fumigants on *Trichoderma* levels within the soil. In both study years, seedling densities were assessed in four (1 x 4 ft) subplots per treatment at approximately 7 weeks post-sowing, 15 weeks post-sowing (mid-summer), and 33 weeks post-sowing (prior to fall lifting). At the end of each growing season, 25 loblolly pine seedlings were lifted from each of four subplots per fumigation treatment. Seedling quality measurements included root collar diameter (RCD), shoot height, and shoot and root dry weight (biomass). In addition, root morphology (root length, root surface area, root diameter, and the number of new root tips) was measured on ten seedlings per subplot using WinRhizo® computer software and a scanner (Regents Instruments Inc., Quebec, Canada).

RESULTS AND DISCUSSION

Seedling densities for all soil fumigation treatments in 2009 were below the nursery target of 17 seedlings per square foot (Table 3). The low seedling densities in 2009 was due to a 4-inch rain event two days after sowing that resulted in seedling bed wash out. Despite the overall reduction in seedling bed densities soils fumigate with MBC 70/30 had significantly higher seedling densities than DMDS + Chlor or MBr #1. In these trials, soils fumigated with MBr #1 were considered the control treatment in the study, representing an operational standard. In the 2010 growing season, soils treated with Chloropicrin, MBC 70/30, and Chlor 60 treatments met the nursery's target seedling density. Of all the soil fumigants tested at Elberta, the Chloropicrin treatment had more seedlings per square foot than either MBr treatment (Table 3). Since most nurseries grow 2-3 seedling crops following fumigation, it is in the second year where true differences among fumigation treatments tend to surface.

In the 2009 growing season, the root collar diameter of loblolly pine seedlings grown in MBr #1, Chloropicrin, and DMDS + Chlor fumigated soil were larger than RCDs of seedlings grown in soils treated with MBr #2 (Table 4). The percentage of Grade 1 and 2 (non-cull) seedlings (RCD $\geq$ 3.2 mm) for all fumigation treatments averaged 98% in 2009 (Figure 1). Soils fumigated with MBr #1 did not produce one cull seedling during the first year. All other treatments had $\leq$ 5% cull seedlings. Because of the excessive rainfall and seed "wash out" two days after sowing, lower seedling densities in those plots inflated the RCD of the remaining seedlings which resulted in a smaller proportion of culls. In the second year, Chloropicrin seedlings had significantly smaller RCDs compared to Pic+ seedlings, which were similar in size to all other soil fumigants. The percentage of cull seedlings ranged from 8-15% between soil treatments in 2010 (Figure 2). MBr #1 and DMDS + Chlor treatments each produced 92% non-cull seedlings with an average of 89% among soil treatments.

A fibrous root system at lifting is a key component to initial outplanting establishment and survival (Hatchel and Muse 1990; Frampton et al. 2002; Davis and Jacobs 2005). Root morphology measurements among the soil fumigants tested yielded no significant difference in

root length among treatments at the end of the first growing season (Table 5). With respect to other root characteristics, MBr #1 seedlings had a larger root surface area and more new root tips than seedlings grown using Chlor 60 and, along with DMDS + Chlor, had larger root diameters than MBC 70/30 seedlings. Chloropicrin seedlings had more new root tips than seedlings grown in Pic+ fumigated soil. By the end of the second growing season, root length and the number of new root tips were similar among all fumigation treatments (Table 5). However, Chloropicrin seedlings had smaller root surface area than Pic+ and smaller average root diameter than Pic+, Chlor 60, and DMDS + Chlor seedlings. All of the soil treatments yielded fewer new seedling root tips and smaller root diameters in 2010 than in 2009. The difference in root production from the first growing season to the second may be attributed to larger seedlings producing more roots in 2009 or reduced fumigant effectiveness over time.

The soil-borne fungus *Trichoderma* is important for proper pine seedling growth in the nursery (Cary et al. 2005; Starkey et al. 2006; Starkey and Enebak 2008) and shares an antagonistic relationship to common damping-off pathogens such as *Pythium* (Sun 1996). Some soil fumigants cause long-term suppression of *Trichoderma* resulting in increased disease pressure and plant stunting. With the soil fumigants tested at Elberta, all soil fumigants in both growing seasons had more *Trichoderma* colony forming units (CFUs) at the time of fall lifting than six weeks post-sowing (Table 6). In 2009, soil fumigated with Chlor 60 had significantly less *Trichoderma* than soil treated with 235 lbs/acre of MBr (MBr #2), but at lifting, Chlor 60 had 69 more CFUs than soil fumigated with 400 lbs/acre of MBr (MBr #1). The number of *Trichoderma* CFUs was similar among all soil fumigation treatments in 2010 (Table 6).

One of the more troublesome issues with the phase-out of MBr as a soil fumigant in forest tree nurseries is the lack of nematode control. With the exception of 14 total spiral nematodes detected in two plots in May 2009, only stubby root nematodes were detected in the first growing season. The highest average population was less than 17 per 100 cc of soil at lifting (Table 7). Of the alternatives tested, Chlor 60 had the fewest amount of nematodes (2.5/100 cc of soil), which is expected since that fumigant contains 40% 1, 3-dichloropropene (Telone®) and is labeled for use against nematodes. However, MBr #1 fumigated soil had similar levels (3/100 cc of soil) to Chlor 60 at the 235 lbs/acre rate. Stubby root nematodes were detected in June 2010, but at lifting, not a single nematode was discovered in any of the soil treatments (Table 7). These results indicate that the Elberta nursery may not have significant nematode issues to address in determining a potential MBr alternative.

MANAGEMENT IMPLICATIONS

The primary objective of the USDA Area-wide MBr Alternative Program is to identify possible alternatives to MBr using large-scale, multi-year trials throughout the southern US. The ability of MBr to consistently control soil-borne fungi, nematodes, and weeds across a range of growing conditions is what sets it apart from other potential alternatives. Of those with promise, 100% Chloropicrin at 300 lbs/acre demonstrated higher loblolly pine seedling density, similar seedling morphology and *Trichoderma* levels, and lower nematode populations than MBr #1 (400 lbs/acre of 98% MBr + 2% Chloropicrin). Fumigation with 100% Chloropicrin might be the best choice at the Elberta nursery once MBr becomes unavailable. The next best alternatives for the Elberta nursery might be DMDS + Chlor and Pic+, since many of the seedling parameters measured

were similar to those for MBr grown seedlings. However, the DMDS + Chlor fumigant has a significant odor problem that might limit its use as an alternative to MBr.

Loblolly pine seedlings grown in Chlor 60 (60% Chloropicrin + Telone) had less root surface area and new root tip growth after one growing season, but growth was similar to MBr #1 by the end of the second growing season. However, the potential is there for seedlings to have smaller root systems if Chlor 60 is used, which could jeopardize outplanting performance. The final decision on selecting a MBr alternative will vary by nursery, and each manager needs to consider the alternative soil fumigant's ability to work in their soils and the impact of the EPA's Re-registration Eligibility Decision (REDs) in order to continue growing quality forest tree seedlings.

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Table 1. Soil fumigants and treatment rates used in the 2009-2010 Area-wide demonstration trial at Elberta, AL.

Fumigant	Rate	Components
MBr #1	400 lbs/a	98% MBr + 2% Chloropicrin
MBr #2	235 lbs/a	98% MBr + 2% Chloropicrin
Chloropicrin	300 lbs/a	100% Chloropicrin
MBC 70/30	400 lbs/a	70% MBr (98/2) + 30% Solvent
Chlor 60	400 lbs/a	60% Chloropicrin + 40% 1,3-D (Telone)
Pic+	300 lbs/a	85% Chloropicrin + 15% Solvent
DMDS + Chlor	70 gal/a	79% Dimethyl Disulfide + 21% Chloropicrin

Table 2. Site information for the Area-wide demonstration trial at Elberta, AL.

	Elberta, AL
Fumigation Date	October 22, 2008
Fumigation Type	Shank injected; broadcast/flat tarp
Experimental Area	4 acres
Air Temperature	67-75°F
Wind Speed	3-10 mph
Soil Moisture	8.10%
Soil Series	Eustis loamy fine sand; Red Bay fine sandy loam
Soil Particle Size	11.2% clay; 9.3% silt; 79.5% sand
Plastic in Place	9 Days

Table 3. Seedling density at lifting for the Area-wide trial in the 2009 and 2010 growing seasons.

	Seedling Density (ft^2)	
	2009	**2010**
Fumigant	**38 wks***	**33 wks**
MBr #1	10.2 b	16.4 b
MBr #2	13.0 ab	15.6 b
Chloropicrin	12.8 ab	22.3 a
MBC 70/30	14.1 a	17.0 ab
Chlor 60	12.6 ab	17.2 ab
Pic+	11.2 ab	15.4 b
DMDS + Chlor	9.9 b	15.7 b
$lsd_{(0.05)}$**	*(3.5)*	*(5.1)*

* Weeks post-sowing
** Least significant differences are italicized

Table 4. Root collar diameter measurements from loblolly pine seedlings at lifting in the Area-wide trial at Elberta, AL.

	Root Collar Diameter (mm)	
	2009	**2010**
Fumigant	**38 wks***	**33 wks**
MBr #1	6.04 ab	4.41 ab
MBr #2	5.54 c	4.44 ab
Chloropicrin	6.02 ab	4.03 b
MBC 70/30	5.68 bc	4.48 ab
Chlor 60	5.78 bc	4.65 ab
Pic+	5.93 abc	4.81 a
DMDS + Chlor	6.21 a	4.59 ab
$lsd_{(0.05)}$**	*(0.37)*	*(0.62)*

* Weeks post-sowing
** Least significant differences are italicized

Table 5. Root morphology measurements from loblolly pine seedlings at lifting in the Area-wide trial at Elberta, AL.

	Root Morphology							
	Root Length (cm)		**Root Surface Area (cm^2)**		**Root Diameter (mm)**		**Root Tips (#)**	
	2009	**2010**	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**
Fumigant	**38 wks***	**33 wks**	**38 wks**	**33 wks**	**38 wks**	**33 wks**	**38 wks**	**33 wks**
MBr #1	173.9 a	177.4 a	70.5 a	63.0 ab	1.30 a	1.13 ab	504 abc	396 a
MBr #2	164.8 a	187.8 a	59.7 ab	67.6 ab	1.16 ab	1.13 ab	465 bcd	425 a
Chloropicrin	169.9 a	179.1 a	64.2 ab	58.0 b	1.21 ab	1.03 b	548 ab	370 a
MBC 70/30	168.8 a	196.0 a	59.5 ab	68.1 ab	1.13 b	1.10 ab	498 abcd	425 a
Chlor 60	149.3 a	174.4 a	57.2 b	64.8 ab	1.22 ab	1.17 a	413 d	400 a
Pic+	162.5 a	192.7 a	63.1 ab	71.1 a	1.25 ab	1.17 a	455 cd	432 a
DMDS + Chlor	166.0 a	178.6 a	67.7 ab	67.2 ab	1.31 a	1.19 a	565 a	388 a
$lsd_{(0.05)}$**	*(26.3)*	*(22.7)*	*(11.0)*	*(10.4)*	*(0.14)*	*(0.12)*	*(83)*	*(58)*

* Weeks post-sowing

** Least significant differences are italicized

Table 6. Number of *Trichoderma* colony forming units (CFUs) from soils collected over two growing seasons in the Area-wide trial at Elberta, AL.

	Trichoderma (CFUs/mg soil)			
	2009		**2010**	
Fumigant	**6 wks***	**38 wks**	**6 wks**	**33 wks**
MBr #1	10.0 bc	38.0 b	15.8 a	50.0 a
MBr #2	21.8 ab	63.7 ab	29.0 a	62.8 a
Chloropicrin	5.5 c	67.0 ab	18.3 a	81.3 a
MBC 70/30	24.0 a	65.3 ab	37.5 a	64.5 a
Chlor 60	6.0 c	107.0 a	25.5 a	35.0 a
Pic+	9.5 bc	72.8 ab	31.8 a	50.5 a
DMDS + Chlor	32.5 a	52.8 ab	26.8 a	40.8 a
lsd $_{(0.05)}$**	*(12.3)*	*(60.8)*	*(30.4)*	*(52.9)*

* Weeks post-sowing

** Least significant differences are italicized

Table 7. Average number of stubby root nematodes per 100 cubic centimeters (cc) of soil over two growing seasons in the Area-wide trial at Elberta, AL.

	Stubby Root Nematodes (#/100 cc of soil)			
	2009		**2010**	
Fumigant	**6 wks***	**38 wks**	**6 wks**	**33 wks**
MBr #1	0	15	6	0
MBr #2	0	3	6.5	0
Chloropicrin	0	7.5	15	0
MBC 70/30	0	6	8	0
Chlor 60	0	2.5	3	0
Pic+	0	8	21	0
DMDS + Chlor	0	16.5	3	0

* Weeks post-sowing

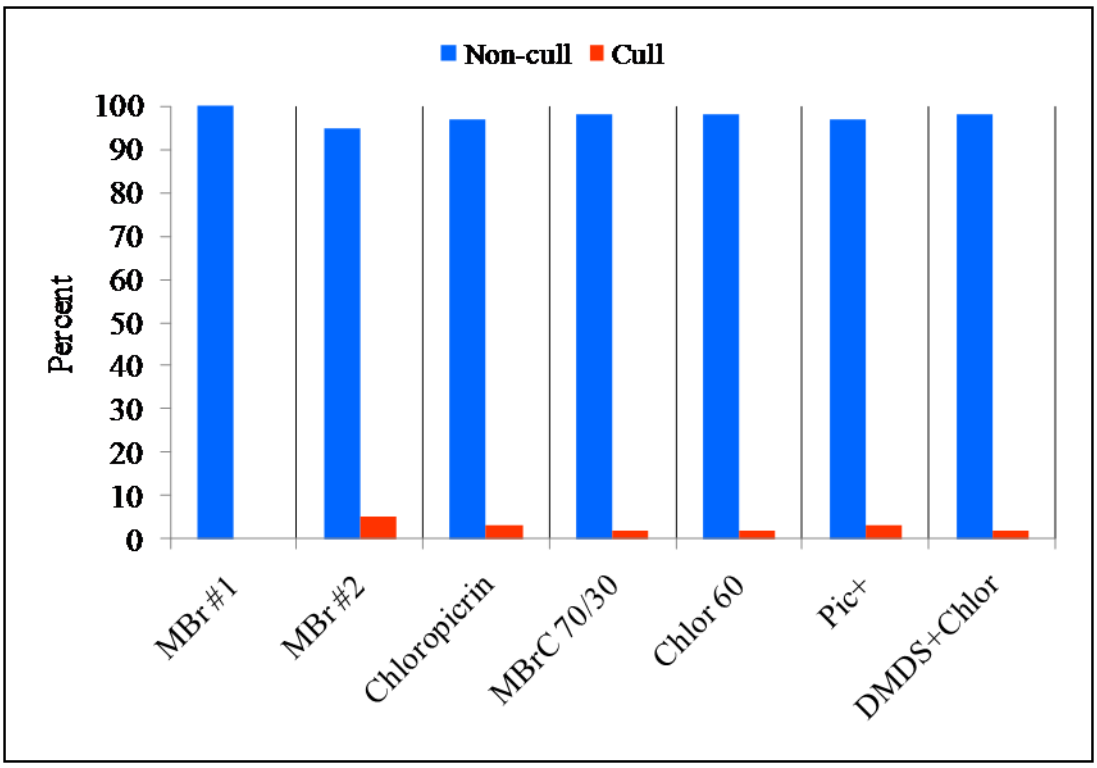

Figure 1. Percentage of cull vs. non-cull seedlings between soil treatments at Elberta, AL-2009.

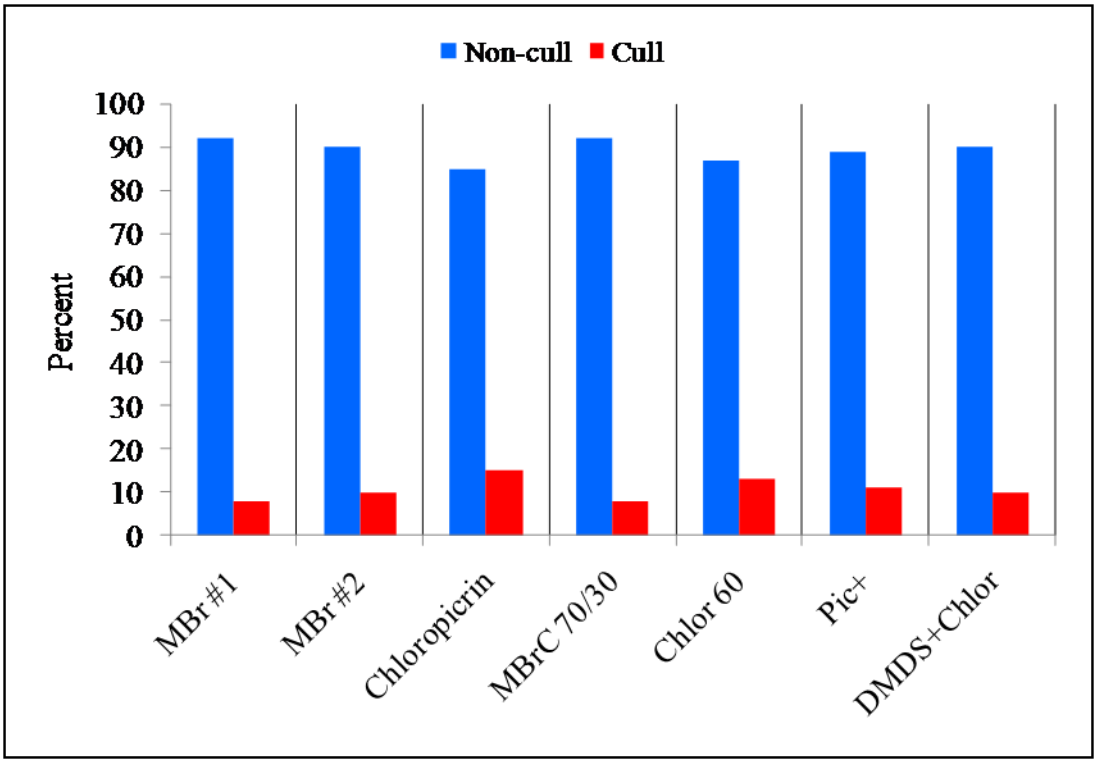

Figure 2. Percentage of cull vs. non-cull seedlings between soil treatments at Elberta, AL-2010.