Auburn University
Southern Forest Nursery
Management Cooperative

RESEARCH REPORT 13-02

EVALUATION OF PLASTIC, REDUCED RATES AND LOW IMPACT APPLICATION METHODS ON LOBLOLLY PINE PRODUCTION AND SEEDLING QUALITY OVER THREE GROWING SEASONS

by
Scott A. Enebak, Tom Starkey, D. Paul Jackson and Barry Brooks

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). In 2006, the EPA began the process of reviewing the safety of soil fumigants in an attempt to mitigate bystander exposure. This process took into consideration application methods, soils, compounds, rates, crops, etc. and developed rules on usage and application methods as part of the reregistration of each soil fumigant. The compounds examined in this reregistration process included chloropicrin, dazomet, metam/potassium sodium, methyl bromide, 1,3-dichloropropene (Telone®), methyl isothiocyanate (MITC) and iodomethane. Similar risk assessment tools and methods were used for all fumigants and risk management approaches were consistent across all soil fumigants.

Using the newly proposed EPA rules for soil fumigants, a 4-ha block (10 acre) fumigated with 392 kg/ha (350 lb/a) chloropicrin under a High Density Plastic the best alternative to MBr (South and Enebak 2006, South et al 1997) would require a buffer zone of 1400 m (4600 ft or nearly 0.9 of a mile). The buffer zone footprint alone represents 585 additional hectares, an unfeasible amount to control for a 4 ha block of soil fumigation. Fortunately, new soil flux data, information on seedling production systems, identification of high barrier tarps, evaluation of new technologies, and a revised and amended Soil Reregistration Eligibility Decision (RED) were released in May 2009. These new rules include buffer zones, posting requirements, agricultural worker protection, applicator and handler training programs, tarp perforation and removal, good agricultural practices, application methods/practices and rate restrictions, new restricted-use designation for dazomet, site-specific fumigation plans, emergency preparation and response requirements, compliance assistance and assurance measures, and community outreach and education programs (EPA 2012).

These new rules will change the way nurseries use soil fumigants as they take into account buffer tables, new plastic tarp technologies that allow the gluing of high barrier plastics (virtually or totally impermeable films – VIF or TIF), and various soil credits that could allow nurseries to continue their use of soil fumigants in the production of forest-tree seedlings with minimal disruptions and loss of production acreage.

As part of the USDA ARS Areawide MBr alternative program, these trials were a large-scale study comparing soil fumigants using the new plastics, reduced rates and low impact coulter injection application techniques over three growing seasons to determine what effects these reduced rates and different plastics (required by the new soil fumigation rules) may have on seedling quality and quantity. Information gathered from these studies will be used in the next round of soil fumigation regulations as EPA plans to consider the soil fumigants again during the Registration Review that begins in 2013.

MATERIALS AND METHODS

Soil Fumigants. The forest-tree nursery was located in Wilcox County, Alabama, (32° 03' 53" N; 87° 20'57" W) that is primarily a Lenior silt loam. The nursery comprises 65 ha and has been in use since 1979 running a 3-1 rotation (3 seedling crops to 1 yr fallow/cover). Seedling production is 40 million plus conifer bareroot 1-0 seedlings annually. Included in this trial were four soil fumigation treatments that were selected from previous Southern Forest Nursery Management Cooperative fumigation studies under two rates and using two application methods which had never been tested previously in the production of forest-tree seedlings (Table 1). The new application technique was tested on one Chlor 60 treatment using the high and low rates was applied using a no-till coulter injection method that was covered with a 1 mm High Density Polyethylene (HDPE) as a broadcast/flat tarp. All other soil fumigants were shank injected and covered with Totally Impermeable Film (TIF). The experimental area occupied approximately 4 ha (10 a) and the trial was laid out in ten nursery production units that consisted of nine seedling beds between the irrigation pipelines with each bed being approximately 170 m x 1.2 m. The experiment was a randomized complete block design replicated four times with each treatment being 85 m long. Each 9-bed nursery unit included 2 soil fumigation treatments. In order to test different plastic requirements, the Chlor 60 H treatments could not be placed with the other soil fumigants. After fumigation and in each of the three growing seasons (2010, 2011 and 2012), a single family of loblolly pine (Pinus taeda L.) seed was sown at the same rate in the fumigated area. Seedlings in the trial area were maintained using standard nursery cultural practices (fertilization, irrigation, pest management, etc.) until lifting took place each fall. After lifting the seedlings in the winter (Nov-Dec), the treatment area was left fallow and prepared for sowing the following spring (April).

Seedling Quality and Quantity. The effect of the soil fumigants on seedling densities and growth characteristics was assessed in four subplots (1.2 m x 0.3 m) per treatment plot at 7 wk post-sowing, mid-summer (15 wk post-sowing) and just prior to lifting in the fall (26 wk post-sowing) in 2010, 2011 and 2012, respectively. Twenty to 35 loblolly pine seedlings per subplot were collected in the fall of each growing 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® software by Regents Instruments Inc., Quebec, Canada.

Soilborne Trichoderma and Nematodes. Throughout the three growing seasons, soil samples were collected from the center seedling bed of each treatment: at pre-sowing, post-sowing, mid-summer and just prior to seedling lifting in November of each growing season. Half of each soil sample was sent to the Soils Laboratory at Auburn University for a quantitative assessment of soil-borne pathogenic nematodes, the remaining half of the sample was used to determine soilborne fungi levels by plating onto Trichoderma-selective media (Elad et al 1981.

Analyses were carried out using the Statistical Analysis System (SAS 9.2). Analysis of variance (ANOVA) was performed using the PROC GLM function to test for treatment differences at an alpha level of 0.05. Both Duncan (within treatment) and Dunnett's paired T-test was performed using MBr 280 as the standard nursery treatment.

RESULTS

Seedling Quality and Quantity. When comparing the various treatments using Dunnett's paired T-test, none of the treatments were significantly better than the standard MBr 280 treatment with respect to seedling densities for the three growing seasons (Table 3). Seedling densities at the end of the first growing season in 2010 ranged from 190-261 seedling/m2 with some treatments out-performing others with respect to seedling numbers; for example, at the high rate, Chlor 60 under both HDPE and TIF produced significantly more seedlings than Chloropicrin and Pic+. Similar results were observed for the 2011 growing season, however, seedling densities in the Chlor 60 H treatment under HDPE plastic were significantly less than the Chlor 60 T at the lower rate (Table 3). At the end of the third growing seasons in 2012, the best soil fumigant for producing loblolly pine seedlings was Chloropicrin at 280 kg/h and MBr at 168 kg/h under TIF (Table 3).

Differences in seedling root collar diameters (RCD) among the soil fumigants tested at the Pine Hill nursery were observed only during the first growing season with Chloropicrin 168 kg/ha treated soils producing significantly larger seedling diameters over the standard MBr 280 soil treatment (Table 4). Chlor 60 treatments under both the HDPE and TIF produced some of the smallest root collar diameters of all the soil fumigants tested. These differences in RCDs were not maintained for the second and third growing seasons in 2011 and 2012 as all treatments resulted in similar RCDs (Table 4). Seedling RCDs declined for all treatments over the three-year rotation due to the buildup of soilborne pests and weeds over time.

Overall, seedling root architecture and root morphology as measured by root length, surface area, root diameter and root tips were similar to the standard MBr 280 treatment for all soil fumigants except for the Chlor 60 treatments under HDPE plastic in 2010 (Table 5). Both rates of Chlor 60 tended to have shorter roots and fewer root tips than MBr 280. Generally, first year soil fumigation results in larger seedlings, however, 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 2011. Like that of RCD and time since soil fumigation, root characteristics tended to decrease over the 2010 to 2011 growing season. Unfortunately, root architecture data was not collected in 2012.

The root weight ratio (RWR) of seedlings grown in the different soil treatments resulted in differences only during the first growing season; when comparing the lower rate of Chlor 60 H vs. Chloropicrin (Table 6). The RWR is defined as the weight of the roots divided by the total seedling weight; an optimum seedling has a root weight ratio of >27%. Seedlings with a higher RWR have better survival and growth after outplanting. While none of the treatments resulted in the optimum RWR, a number of factors affect RWR, including the time of lifting, growing density, the time of root pruning, irrigation and fertilization. For these trials, all of the MBr alternatives had similar RWR compared to the MBr control indicating that the alternatives were not detrimental to root growth that could affect seedling survival after outplanting.

In contrast, while RCD and RWR were similar (non-significant) among the 5 soil fumigants, there were significant differences among the rates used within a soil fumigant with respect to seedling grades. The number of Culls was less with the higher rates and there was a corresponding increase in the number of Grade 1 and Grade 2 seedlings for the high rate for each soil fumigant (Table 7).

Soilborne Trichoderma and Nematodes. The effect of the various soil fumigants on soilborne Trichoderma was dependent upon the compound and rate used. For example, a number of MBr alternatives were detrimental to Trichoderma when compared to the standard MBr 280 control (Table 8). This included a number of the Chlor 60 treatments and Pic +. By far, Chlor 60 under HDPE and TIF appeared to be detrimental to Trichoderma when compared to MBr 280 during the third growing season in 2012 (Table 7). Trichoderma is a beneficial soilborne fungus that is used to monitor the sensitivity of the soil micro-organisms to soil fumigation and therefore, suppression of Trichoderma is considered undesirable when screening soil fumigants.

Over the course of the 3-yr study, each soil fumigant plot was examined six 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 except for the pre-fumigation levels where trace levels of nematodes were recovered, all soil samples examined for nematodes were zero or non-recoverable in 100 cc soil samples (data not shown). Therefore, all soil fumigants were effective in eliminating nematode populations during the first growing season which were maintained during the 3-yr rotation.

DISCUSSION

The true test of an MBr alternative is its performance during the second and third growing season where treatment differences usually begin to appear as disease, weed, and nematode pressures increase. Based on these trials, those soil fumigants with chloropicrin were similar to MBr and appear to be the most useful in controlling pests and producing quality seedlings. Historically, the standard soil fumigation treatment was MBr applied at 448 kg/h (400 lb/ac) (98% MBr & 2% chloropicrin) under HDPE (South et all 1997). New rules governing the use of soil fumigants no longer allows either the amount (448 kg/h) or the formulation (98/2) so forest-tree nurseries will need to change their soil fumigation practices and applications rates.

Standard broadcast soil fumigation rigs use shanks to inject fumigants into the soil. One problem with shank injection rigs is that they can create chisel traces (openings or chimneys) in the soil that can allow fumigant gas to escape into the atmosphere. In an attempt to minimize fumigant loss from the chisel traces and increase fumigant efficacy, a coulter injected low disturbance fumigation rig was evaluated. The idea behind the low disturbance rig was to limit the upward movement of soil fumigants and decrease application rates to provide longer soil exposure rates and still achieve adequate pest (weeds, nematodes, insects, fungi) control as well as to achieve a high proportion of plantable seedlings at the end of the rotation. However, using this method, soil fumigant and plastic tarp, seedling quality and quantity was less in soils treated with Chlor 60 under the HDPE. Some possible reasons for the lack of fumigant efficacy may have been caused by one or a combinations: 1) A lower rate of fumigant (than normal) was used, 2) the fumigant injection was not deep enough on the low disturbance coulter injection rig, 3) Compacting (rolling) the soil before application may have prevented gas movement laterally through the soil or 4) the HDPE plastic did not contain the soil fumigant long enough to act on the soilborne pets. Based on the poor seedling performance and negative effect on soilborne Trichoderma, the low impact soil fumigation rig is not adaptable for broadcast soil fumigation methods that are currently used in forest-tree nurseries. This type of system works well in row crops where the plastic tarp remains in place and the fumigant dispersion needs only to be within the width of the tractor path (Chellemi and Mirusso 2004).

The soil type at the Pine Hill nursery (Lenior silt loam) is heavier than most forest tree nurseries and generally has not resulted in production issues to nematode populations (Cram and Fraedrich 2005). This was evident in the nematode populations monitored that were either trace or non-detectable throughout the three growing seasons (data not shown). Therefore, the lack of nematode pressure would not require the use of soil fumigants containing 1, 3-dichloropropene (Telone®) and could favor those with more chloropicrin compounds. Nurseries with a higher sand content may need to address the potential nematode pressures if moving away from MBr in their soil fumigation program.

One of the unique aspects of soil fumigants currently being tested in southern forest nurseries is that they do not completely eliminate 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, Mousseaux et al 1998). In these trials, the population levels of non-target soilborne fungi rebounded with all soil fumigants except for Chlor60 under HDPE (Table 8). Previous Nursery Cooperative research has shown that Trichoderma was not as sensitive to Chlor60 as to dazomet and methyl iodide (Carey et al 2005, Starkey and Enebak 2008). These two soil fumigants significantly reduced the levels of beneficial fungi which remained after two growing seasons (Starkey et al 2006). Why Chlor60 under HDPE suppressed Trichoderma in these trials is unknown, but may be due to the soil preparation process prior to treatment that allowed other soil fungi to out compete.

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). The effect of the soil fumigant on seedling growth was dependent upon the year and the fumigant, however, Chlor60 under HDPE resulted in significantly smaller root mass in 2010 when compared to the standard MBr280 control. Over the three year period, treatment differences were not observed, and in 2012 a miscommunication resulted in no data collected. While frustrating, based on previous work it is unlikely that treatment differences would have been observed among the soil fumigants after three years (Enebak et al 2011 and 2012). For the 2010 and 2011 growing seasons, the seedling densities and root characteristics with chloropicrin were encouraging using the reduced rates (280 and 168 kg/ha) as the previously recommended rate of 336 kg/ha (South et al 1997, Cram et al 2006) and the buffer zone restrictions under current soil fumigation practices in the US could limit the use of chloropicrin. These trials show that the use of chloropicrin at 280 and 168 kg/ha under TIF, a 16% reduction in active ingredient, can still produce plantable seedlings (Table 4 & 7).

One of the most important aspects of seedling quality is a plantable seedling with an RCD greater than 3.19 mm. Forest-tree seedlings are sometimes graded into Culls, Grade 1 and Grade 2 based on root collar diameter (Culls = Seedling RCD < 3.19 mm, Grade 2 = Seedling RCD > 3.20 mm but less than < 4.69 mm, Grade 1 = Seedling RCD > 4.70 mm). Generally, Grade 1 seedlings have increased survival and growth over Grade Two Seedlings and Culls are not planted (South et al. 1985). While there were no differences among treatments with respect to average RCD (Table 4), there were significant differences among the treatments with respect to rates within each soil fumigant. In all cases, the higher rate resulted in less Culls, and more Grade 2 and Grade 1 seedlings than the corresponding lower rate within a soil fumigant (Table 7). One of the more promising MBr alternatives was Pic+ at 280 kg/ha which had the greatest number of Grade 1 seedlings.

The wide-spread use of MBr has minimized extensive seedling losses due to soilborne pathogenic fungi (Cram et al 2006). Pythium still can cause damping-off problems in the early spring and is often limited to areas of poor drainage and standing water (Seymour and Cordell 1979). The soil-type at the Pine Hill nursery is conducive to damping-off caused by Pythium early in the growing season. Rhizoctonia can appear in nurseries both as root decay and as foliage blight, especially in second-year crops post fumigation as the fungus increases over the first growing season (Carey and McQuage 2004). What will happen to nurseries 8 years post MBr ban is unknown, but at least for this 3-yr trial, soilborne pathogens did not appear to affect seedling production.

MANAGEMENT IMPLICATIONS

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. 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 of the new soil fumigation rules have on each individual nursery. By far the best MBr alternatives tested were Pic+, Chloropicrin and Chlor 60 with all three soil fumigants controlling soilborne nematodes and producing quality seedlings for outplanting and reforestation programs. These trials at the Pine Hill Nursery in Camden AL, while not the perfect replacement, seedling production is still possible without MBr if compounds such as chloropicrin are used and the reduced rates of 250 under TIF if managers pay close attention to weed and nematode pests that are less susceptible to chloropicrin than MBr. The numbers of Culls were less with the higher rates with a corresponding increase in the number of Grade 1 and Grade 2 seedlings for each soil fumigant.

REFERENCES

Bailey BA, and Lumsden RD. 1998. Direct effects of Trichoderma and Gliocladium on plant growth and resistance to pathogens. In Trichoderma and Gliocladium, vol. 2. Edited by G.E. Harman and C.P. Kubicek. Taylor and Francis, Inc., Bristol, Pa. pp. 185–204.

Carey WA, McCraw D, and 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. 5 pp.

Carey, WA and McQuage K. 2004. Control of Rhizoctonia foliage blight by fungicides and fumigation: Lower application rates and fumigation effects. Auburn University Southern Forest Nursery Management Cooperative, Res. Rep. 04-03. Auburn, AL. 4 pp.

Chellemi, DO and Mirusso, J. 2004. An Apparatus to inject soil fumigants under raised, plastic-mulched beds. Applied Engineering and Agriculture 5:585-589.

Cram, M and Fraedrich SW. 2005. Management options for control of a stunt and needle nematode in southern forest nurseries. In: Dumroese, R. K.; Riley, L. E.; Landis, T. D., tech. coords. 2005. National proceedings: Forest and Conservation Nursery Associations; 2004 July 12–15; Charleston, NC; Proc. RMRS-P-35. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station .

Cram MM, Enebak SA, Fraedrich SW, Dwinell LD and Zarnoch SJ. 2007. Evaluation of fumigants, EPTC herbicide and *Paenibacillus macerans* in the production of loblolly pine seedlings. For. Sci. 53:73-83.

Davis AS, and Jacobs DF. 2005. Quantifying root system quality of nursery seedlings and relationship to outplanting performance. New Forests. 30:295-311.

Elad Y, Chet I, and Henis Y. 1981. A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. Phytoparasitica.9:59-67.

Enebak SA, Starkey TE and Quicke M. 2011. Effect of methyl bromide alternatives on seedling quality, nematodes and pathogenic soil fungi at the Blenheim and Trenton Nurseries in South Carolina: 2008-2009. Journal of Hort and Forestry. 3:379-487.

Enebak SA, Starkey TE and Quicke M. 2012. Effect of methyl bromide alternatives on seedling quality, nematodes and pathogenic soil fungi at the Jesup and Glennville Nurseries in Georgia: 2007-2008. Journal of Hort and Forestry. 4:1-7.

EPA 2012. Implementation of Risk Mitigation Measures for Soil Fumigant Pesticides. http://www.epa.gov/pesticides/reregistration/soil_fumigants/

Frampton J, Isik F, and 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, and 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. 277.

Jang E, Wood WS, Dorschner K, Schaub J, Smith D, Fraedrich S, and Hsu H. 1993. Methyl bromide phase out in the US: Impact and alternatives, In: USDA workshop on alternatives for methyl bromide. Crystal City, VA.

Mousseaux MR, Dumroese RK, James RL, Wenny DL, and Knudsen GR. 1998. Efficacy of *Trichoderma harzianum* as a biological control of *Fusarium oxysporum* in container-grown Douglas-fir. New Forests. 15:11–21.

Seymour CP and Cordell CE. 1979. Control of charcoal root rot with methyl bromide in forest nurseries. Southern J. Appl. For. 3:104-108.

South DB, Carey WA, and Enebak SA. 1997. Chloropicrin as a soil fumigant in forest nurseries. For. Chron. 73:489-494.

South DB, Boyer JN and Bosch L. 1985. Survival and growth of loblolly pine as influenced by seedling grade: 13 year results. South. J. Appl. Forestry 92:76-81.

South DB and Enebak SA. 2005. Integrated pest management practices in southern pine nurseries. New Forests. 31:1-19.

Starkey TE, and Enebak SA. 2008. Indian Mound Nursery, Texas: methyl bromide alternative study 2005-2007. Auburn University Southern Forest Nursery Management Cooperative Research Report 08-07. Auburn, AL. 11 pp.

Starkey TE, Enebak SA, and McCraw D. 2006. Seedling quality and weed control with methyl bromide and methyl iodide at the Glennville Regeneration Center 2005-2006. Auburn University Southern Forest Nursery Management Cooperative. Research Report 06-05. Auburn, AL. 5 pp.

Table 1. MBr alternative soil fumigants and rates used in the 2010-2012 reduced rate trial at Camden, AL.

Treatment	lb/a	Plastic[y]	Fumigant Components
Chlor 60 T	250 150	TIF	60% chloropicrin & 40% 1,3-dichloropropene (Telone®)
Chloropicrin	250 150	TIF	100% chloropicrin
MBr	250 150	TIF	80% MBr + 20% chloropicrin
Pic +	250 150	TIF	85% chloropicrin + 15% solvent
Chlor 60 H	250 150	HDPE	60% chloropicrin & 40% 1,3-dichloropropene (Telone®)

[y] HDPE = High Density Polyethylene; TIF = Totally Impermeable Film

Table 2. Site information for the 2010-2012 reduced rate trial at the Pine Hill forest-tree nursery in Camden, AL.

Trial Parameter	Pine Hill Nursery, Camden AL
Fumigation date	March 23, 2010
Fumigation types	A. Shank injected; broadcast/with TIF flat tarp B. No-till coulter injected; broadcast/with HDPE flat tarp
Experimental area	8 ac
Air temperature	55-73 °F
Wind speed	0-10 mph
Soil moisture	7.6%
Soil series	Lenoir silt loam
Plastic tarps in place	14 days

Table 3. Loblolly pine seedling density at lifting over three growing seasons (2009-2011) for the reduced rate trial at the Pine Hill forest-tree nursery in Camden, AL.

		Seedling Densities (ft^2)		
Treatment	**Rate (lb/a)**	**2010[X]**	**2011**	**2012**
Chlor 60 T	250	24.3 a	28.2 abc	22.1 ab
	150	20.8 bcd	29.4 a	22.5 ab
Chloropicrin	250	18.5 de	26.2 abc	23.9 a
	150	17.8 e	25.9 abc	21.6 ab
MBr	250	21.7 abc	26.7 abc	21.8 ab
	150	20.2 cde	25.3 abc	23.9 a
Pic +	250	23.5 ab	29.1 ab	21.8 ab
	150	17.7 e	24.9 bc	20.1 ab
Chlor 60 H	250	23.0 abc	24.3 c	16.8 b
	150	24.3 a	24.6 c	16.7 b
LSD (0.05)		*(6.8)*	*(5.3)*	*(5.9)*

[X] Means (within a column) followed by the same letter are not significantly different based on Duncan's Multiple Range Test ($p \leq 0.05$).

Table 4. Loblolly pine seedling root collar diameter at lifting over three growing seasons (2010-2012) for the reduced rate trial at the Pine Hill forest-tree nursery in Camden, AL.

		Root Collar Diameter (mm)		
Treatment	**Rate (lb/a)**	**2010[X]**	**2011**	**2012**
Chlor 60 T	250	3.9 bcd	3.6 a	3.6 a
	150	4.2 abc	3.5 a	3.4 a
Chloropicrin	250	4.3 ab	3.6 a	3.6 a
	150	4.6 a*	3.5 a	3.5 a
MBr	250	3.9 bcd	3.6 a	3.6 a
	150	4.1 abc	3.7 a	3.4 a
Pic +	250	4.0 bcd	3.4 a	3.6 a
	150	4.2 abc	3.4 a	3.5 a
Chlor 60 H	250	3.8 cd	3.6 a	3.7 a
	150	3.5 d	3.2 a	3.6 a
LSD (0.05)		*(0.56)*	*(0.38)*	*(0.33)*

[*] Treatment mean (within a column) was significantly different from MBr 280 based on Dunnett's t-Test ($p \leq 0.05$).
[X] Means (within a column) followed by the same letter are not significantly different based on Duncan's Multiple Range Test ($p \leq 0.05$).

Table 5. Loblolly pine seedling root morphology at lifting over three growing seasons (2010-2012) for the reduced rate trial at the Pine Hill forest-tree nursery in Camden, AL.

		Root Morphology											
		Root Length (cm)			Root Surface Area (cm^2)			Root Diameter (mm)			Root Tips (#)		
Treatment	**Rate lb/a**	**2010[X]**	**2011**	**2012**	**2010**	**2011**	**2012**	**2010**	**2011**	**2012**	**2010**	**2011**	**2012**
Chlor 60 T	250	231 ab	143 a	-	63.0 ab	45.1 a	-	0.88 b	1.00 ab	-	562 ab	296 a	-
	150	231 ab	145 a	-	66.5 a	47.2 a	-	0.91 ab	1.04 a	-	540 ab	296 a	-
Chloropicrin	250	232 ab	149 a	-	69.5 a	46.2 a	-	0.96 ab	0.98 ab	-	514 abc	298 a	-
	150	244 a	143 a	-	71.5 a	45.2 a	-	0.93 ab	1.01 ab	-	611 a	281 a	-
MBr*	250	239 ab	143 a	-	66.5 a	44.0 a	-	0.88 b	0.97 b	-	585 ab	291 a	-
	150	215 abc	153 a	-	60.7 ab	48.0 a	-	0.90 ab	1.00 ab	-	483 bc	334 a	-
Pic +	250	221 abc	162 a	-	62.5 ab	49.5 a	-	0.89 ab	0.97 b	-	519 abc	331 a	-
	150	203 bc	144 a	-	61.5 ab	45.7 a	-	0.97 a	1.00 ab	-	491 bc	287 a	-
Chlor 60 H	250	219 abc	155 a	-	61.5 ab	47.8 a	-	0.91 ab	0.98 ab	-	419 cd*	311 a	-
	150	189 c*	142 a	-	53.0 b	43.4 a	-	0.90 ab	0.98 ab	-	358 d*	296 a	-
LSD (0.05)		*(46)*	*(37)*	-	*(10.5)*	*(7.3)*	-	*(0.07)*	*(0.05)*	-	*(104)*	*(55)*	-

*Treatment mean (within a column) was significantly different from MBr 280 based on Dunnett's t-Test ($p \leq 0.05$).
[X]Means (within a column) followed by the same letter are not significantly different based on Duncan's Multiple Range Test ($p \leq 0.05$).

Table 6. Loblolly pine seedling root weight ratios at lifting over three growing seasons (2009-2011) for the reduced rate trial at the Pine Hill forest-tree nursery in Camden, AL.

		Root Weight Ratio (%)		
Treatment	**Rate lb/a**	**2010**[x]	**2011**	**2012**
Chlor 60	250	0.15 ab	0.16 a	0.13 a
	150	0.15 ab	0.16 a	0.13 a
Chloropicrin	250	0.15 ab	0.15 a	0.13 a
	150	0.14 b	0.15 a	0.13 a
MBr	250	0.16 ab	0.15 a	0.13 a
	150	0.16 ab	0.16 a	0.13 a
Pic +	250	0.15 ab	0.16 a	0.13 a
	150	0.15 ab	0.16 a	0.15 a
Chlor 60[y]	250	0.16 ab	0.17 a	0.13 a
	150	0.17 a	0.16 a	0.14 a
LSD (0.05)		*(0.024)*	*(0.020)*	*(0.177)*

[x]Means (within a column) followed by the same letter are not significantly different based on Duncan's Multiple Range Test ($p \leq 0.05$).

Table 7. Seedling grade by treatment for the 2012 growing season for the reduced rate trial at the Pine Hill forest-tree nursery in Camden, AL.

		Seedling Grades 2012 Growing Season			
Treatment	**Rate (lb/a)**	**Culls[X]**	**Grade 2**	**Grade 1**	**Plantable**
Chlor 60 T	250	29%	62%	9%	71%
	150	35%	57%	8%	65%
Chloropicrin	250	28%	63%	9%	72%
	150	36%	57%	6%	61%
MBr	250	34%	57%	9%	63%
	150	41%	56%	3%	59%
Pic +	250	28%	62%	10%	72%
	150	35%	59%	6%	65%
Chlor 60 H	250	21%	73%	6%	79%
	150	32%	61%	9%	70%

[X]Culls = Seedling RCD < 3.19 mm, Grade 2 = Seedling RCD > 3.20 mm but less than < 4.69 mm, Grade 1 = Seedling RCD > 4.70 mm. Grade 1 seedlings survive 10% better than Grade Two Seedlings. Culls are not planted. Plantable = Grade 1 and Grade 2 seedlings.

Table 8. Number of *Trichoderma* colony forming units (CFUs) from soils collected over three growing seasons (2009-2011) for the reduced rate trial at the Pine Hill forest-tree nursery in Camden, AL.

		Trichoderma spp (CFUs / mg soil)					
		2010		2011		2012	
Treatment	**Rate lb/a**	**June[X]**	**Dec**	**June**	**Dec**	**June**	**Dec**
Chlor 60 T	250	168 abcd	52 abc	110 bc	77 ab	78 b	59 bc
	150	89 cd	16 cd*	74 c*	51 b*	70 b	44 bc*
Chloropicrin	250	186 abc	54 abc	109 bc	73 ab	93 ab	52 bc*
	150	146 abcd	39 bcd	91 c*	76 ab	82 ab	82 ab
MBr	250	209 ab	80 a	176 a	98 a	127 a	112 a
	150	178 abc	44 abcd	155 ab	99 a	87 ab	56 bc
Pic +	250	198 abc	64 ab	109 bc	72 ab	70 b	66 bc
	150	113 bcd	33 bcd*	76 c*	51 b*	76 b	55 bc
Chlor 60 H	250	245 a	34 bcd	131 abc	48 b*	66 b*	82 ab
	150	58 d*	12 d*	99 bc*	54 b*	64 b*	34 c*
LSD (0.05)		*(100)*	*(33)*	*(52)*	*(27)*	*(43)*	*(40)*

[*] Treatment mean (within a column) was significantly different from MBr 280 based on Dunnett's t-Test ($p \leq 0.05$).
[X]Means (within a column) followed by the same letter are not significantly different based on Duncan's Multiple Range Test ($p \leq 0.05$).