Southern Forest Nursery Management Cooperative

RESEARCH REPORT 21-07

EVALUATION OF FIVE METHYL BROMIDE ALTERNATIVES ON SEEDLING PRODUCTION AND QUALITY OVER TWO GROWING SEASONS AT THE RAYONIER TREE NURSERY IN ELBERTA, ALABAMA 2016-2018

by
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INTRODUCTION

Southern forest-tree nurseries have the goal of producing high quality, pest- and disease-free seedlings (Jang et al. 1993, Enebak 2013). To meet this goal, soil fumigants are commonly used in most bareroot nurseries to control pathogens, nematodes, insects, and weeds (Carpenter 2013). Of particular concern in the southeastern U.S. is the control of yellow and purple nutsedge and control of fungal pathogens, namely *Fusarium* spp., *Pythium* spp. and *Rhizoctonia* spp. which are associated with seedling root and foliage damage (Quicke et al. 2009). For more than 50 years, due to its broad efficiency over many soil types, methyl bromide (MBr) has been the industry standard soil fumigant used to ensure increases in both seedling size and density (Enebak 2007, 2013) compared to non-fumigated nurseries.

In 1993, MBr was listed as a class 1 ozone-depleting substance and production of MBr was frozen at the 1991 levels by the Montreal Protocol and to be gradually phased out by 2005 (EPA 2020, Enebak 2007). Continued use of MBr has been granted for forest nurseries through Critical Use Permits or Quarantine Pre-Shipment guidelines for seedlings that are shipped across state boundaries (Enebak 2007). However, eventually these exemptions could cease and the use of MBr as a soil fumigant could end. This has resulted in an increased focus on finding MBr alternatives to use as effective soil fumigants to meet pest, disease, and weed management objectives across a broad spectrum of conditions without sacrificing seedling quality (Weiland et al. 2011).

Even before the official phase-out program began, the Southern Forest Nursery Management Cooperative (SFNMC) had been examining potential alternatives for MBr fumigation (Enebak 2013). In fact, the SFNMC was part of the 5-year USDA – ARS Area-wide Pest Management Project of Methyl Bromide Alternatives – South Atlantic Division. This required the evaluation of the efficacy of many soil fumigants, under normal nursery management practices, against common nursery pests and their effect on seedling quality (Enebak 2012). Because effective alternatives in one nursery may differ at another nursery, the SFNMC frequently tested alternative fumigants at different member nurseries. In 2016, a study was established in southern Alabama to examine five new alternatives to MBr, evaluating each chemistry's effect on nematode management, weed control, and seedling quality.

MATERIAL AND METHODS

Soil Fumigants

Included in this trial were five fumigation treatments, in addition to MBr, to test their efficiency in producing quality pest-free forest tree seedlings at the Rayonier Tree Nursery in Elberta, AL (Table 1). The fumigants were applied on November 17, 2016. The Dominus and Vapam treatments were covered with low-density polyethylene (LDP) tarp while all other treatments were covered with total impermeable film (TIF) (Table 1). The experimental area occupied approximately 3.8 ac and the trial was laid out in six nursery sections that consisted of nine seedling beds between the irrigation pipelines. The experiment was established as a randomized complete block design with each treatment plot being 238 x 29 ft (0.158 ac, 3 nursery beds) and replicated 4 times. After fumigation, a single family of slash pine and a single family of loblolly pine were sown in 2 replications each in 2017. For the 2018 growing season the study area was resown with a single family of loblolly pine across all four replications. Seedlings in the trial were grown using standard nursery cultural practices until lifting in the fall of each year. After lifting, the treatment area was left fallow and prepared for sowing the following spring.

Seedling Quality and Quantity

The effects of the soil fumigant treatments on seedling densities and growth characteristics were assessed in four and three subplots (3 ft^2) in 2017 and 2018, respectively, at the end of each growing season before lifting. From each subplot, 25 seedlings were collected and returned to the laboratory at Auburn University for analysis. Seedling root collar diameter (RCD), shoot height and seedling dry weight (biomass) were measured for each seedling as a measure of seedling quality. In 2017, the number of weeds were also counted within each subplot to determine the efficacy of the tested fumigants to controlling weed seeds.

Soilborne Trichoderma and Nematodes

Soil samples were collected from the center seedling bed of each 3-bed treatment plot pre-fumigation (October 2016), post fumigation (March 2017) and 1-year post fumigation (May 2018). Half of each soil sample was plated onto Trichoderma-selective media (Elad et al. 1981) to determine soilborne fungi levels and the remaining half was sent to the Plant Diagnostic Lab at Auburn University for a quantitative assessment of nematodes.

RESULTS AND DISCUSSION

Study Goal

Our primary objective was to identify possible alternatives to MBr using multi-year trials in various soil types and conditions throughout the southern U.S. Finding a replacement soil fumigant has proven difficult to match the unique ability of MBr to consistently control weeds, insects, nematodes and fungi across varying growing conditions and nursery soils. To date, no perfect MBr alternative has been found, through extensive studies conducted within the southern U.S., that meets all these characteristics (Enebak et al. 2011, 2012). When evaluating an alternative fumigant, comparisons become especially important during the second year after fumigation when treatment differences usually begin to appear as disease, weed and nematode pressures increase.

Seedling Quality and Quantity
Seedling size is a measure of seedling quality and has been linked to outplanting survival. Soil fumigation with MBr has been shown to increase both seedling quality and survival (Enebak 2017). For this trial, we found that seedling densities at the Rayonier Tree Nursery were similar across all soil fumigants tested with average seedling densities of 20.7 and 21.8 seedling/ft^2 for slash and loblolly pine in 2017, respectively, and 20.0 seedlings/ft2 for loblolly pine in 2018 (Table 2). As far as their effect on seedling density, all alternative fumigants were similar to the control standard MBr.

No differences were observed either year in seedling height between fumigation treatments (data not shown) and likely as a result of top clipping cultural practices. During the first year of fumigation (2017), no differences in RCD were observed with average RCDs of 4.42 and 3.89 mm for slash and loblolly pine, respectively (Table 3). RCD in the Dominus fumigation treatment was significantly smaller, 3.99 mm compared to 4.43 mm, in the MBr fumigation treatment. All other fumigation treatments had similar seedling RCD the second year after fumigation (Table 3). However, when considering SFNMC seedling grades (Grade 1 = seedlings RCD > 4.69 mm, Grade 2 = seedlings RCD 3.2 – 4.69 mm and Cull = seedling RCD < 3.2 mm), especially during the second-year post fumigation when differences in seedling quality are most likely to appear, differences in quality of seedling produced began to manifest. In the second crop post fumigation, seedlings produced in soils fumigated with MBr, 37% were Grade 1, 57.3% were Grade 2 and only 5.7% were Culls (Figure 1). The percent of Grade 1 seedlings decreased to an average of 16% in soil fumigated with Ally 33 and Dominus. The number of Cull seedlings increased to an average of 15.8% for soils fumigated with Dominus and PO and these treatments were significantly different from soils fumigated with MBr (Figure 1).

Seedling biomass in all alternative fumigation treatments during the first-year fumigation in slash pine were comparable to the MBr treatment for shoot mass, root mass and root weight ratio (defined as the weight of the roots divided by the total seedling weight) which averaged 3.21g, 0.53g and 14.1%, respectively (Table 4). Likewise, no differences were observed between any fumigation treatments the first year in loblolly pine (Table 4). All alternative fumigants performed equally as well as MBr in respects to root mass and root weight ratio during the second year after fumigation in loblolly pine, however, shoot mass was decreased by 19% in the PO fumigation treatment when compared to that of MBr (Table 4).

MBr has been shown to effectively control weeds and in this study, we examined the weed control ability of the alternate fumigates tested. From weed counts in 2017, the first year after fumigation, average number of weeds in the PO fumigation treatment was significantly higher (23.5 weeds/ft^2) compared to the MBr fumigation treatment (0.25 weeds/ft^2) (Table 7). All other alternate fumigation treatments averaged 1.25 weeds/ft^2 (Table 5).

In this study, fumigated treated plots with all alternative fumigants tested resulted in equivalent quantity of seedlings produced to those of plots fumigated with MBr. However, during the second year after fumigation, seedling beds fumigated with PO produced seedlings with significantly less shoot biomass, probably due to poor weed control exhibited by this fumigant, when compared to MBr. Dominus fumigated plots showed a decrease in average RCD when compared to that of seedlings produced in MBr treatments. Given that the production of quality seedlings is an essential characteristic of an MBr replacement, Ally 33, Dominus, and PO all exhibited weakness.

Soilborne Trichoderma and Nematodes

Trichoderma is a beneficial soilborne fungus that not only has been linked to increase growth of pine seedlings (Quicke et al. 2009) but 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 fumigates. One of the reasons MBr has been a successful fumigant is the limited impact it has on Trichoderma levels (Carey et al. 2005, Starkey et al. 2006, Starkey and Enebak 2008). This was evident in this current study as Trichoderma remained constant from pre to post fumigation with MBr while decreasing in the majority of the alternative fumigants tested. In 2017, the first year of fumigation, the Ally 33, Dominus and Vapam fumigation treated plots had significantly less Trichoderma levels in the soil compared to MBr (Table 5). While Trichoderma levels in the EDN fumigant treatment were low in 2017, it was not significantly different from MBr; however, Trichoderma levels were low in that treatment before fumigation was applied. By the second year after fumigation, Trichoderma levels in all fumigation treatments were similar to MBr (Table 6). Given the 97% reduction in Trichoderma levels the first year of fumigation in the Ally 33, Dominus and Vapam fumigated plots when compared to MBr, these fumigants are considered undesirable for a replacement of MBr as a soil fumigant.

Nematodes can cause significant damage to forest seedlings, and control of nematode populations is an important characteristic to consider when searching for an alternative fumigant to MBr. Soil from each soil fumigant plot was examined 3 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 this study had a range (0 - 152 nematodes / 100cc soil) in numbers and species for all soil fumigants used (Table 7). Even with this variability, plots treated with PO had significantly higher populations of nematodes than the plots treated by MBr (Table 7). Even though nematode counts increased and ranged from 8 to 28.5 per 100cc soil across all fumigation treatments the second year after fumigation, no differences were observed between all alternate fumigants compared to that of the control MBr (Table 7). Interestingly, nematode counts in the PO fumigant treatment plots remained relatively constant throughout the duration of the study and proved to be inefficient at controlling nematode populations.

MANAGEMENT IMPLICATIONS

Methyl bromide (MBr) fumigation has been the industry standard for over 50 years for forest-tree nurseries to control fungi, nematodes, and weeds to produce pest-free quality seedlings. However, a new alternative must be found due to the world-wide phase out of MBr mandated by the Montreal Protocol. The SFNMC has undertaken numerous trials to accomplish this goal, as with this current trial testing five alternative fumigants in a large-scale nursery operation. Main characteristics to examine is seedling quality and quantity, nematode control, Trichoderma sensitivity and weed control. While none of the alternative fumigants tested were perfect in all these characteristics, with the exception of Trichoderma sensitivity, Ally 33, Vapam and EDN performed reasonably well, although Ally 33 produced less Grade 1 seedlings in year 2. Each of these fumigants decreased Trichoderma levels the first year after fumigation but Trichoderma levels rebounded by the second year. Both Dominus and PO fumigants were unsuccessful at maintaining seedling quality by the second year after fumigation and PO demonstrated a lack of weed control making these products unacceptable as a possible replacement for MBr.

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Table 1. Methyl bromide alternative soil fumigants and rates used in the 2016-2018 trial in Elberta, AL.

Treatment	Rate	Plastic	Fumigant Components
MBr	350 lbs/ac	TIF	80% MBr & 20% Chloropicrin
EDN	500 lbs/ac	TIF	99% Ethanedinitrile & 1% Solvent
Dominus	500 gal/ac	LDP	96% Allyl isothiocyanate & 4% Solvent
Vapam	75 gal/ac	LDP	33% Sodium methyldithiocarbamate % 67% Solvent
Ally 33	500 lbs/ac	TIF	67% Dominus & 33% Chloropicrin
PO	600 lbs/ac	TIF	100% Propylene oxide

TIF = total impermeable film; LDP = low-density polyethylene

Table 2. Mean (±SE) seedling density at lifting over two growing seasons (2017-2018) for the trial in Elberta, AL.

	Seedling Density (ft2)		
	Slash	**Loblolly**	**Loblolly**
Fumigant	**2017**		**2018**
MBr	21.0 ± 0.04	21.8 ± 0.15	19.4 ± 1.63
Ally 33	19.8 ± 3.00	24.1 ± 1.20	21.3 ± 0.46
Dominus	20.6 ± 0.08	22.1 ± 3.55	19.3 ± 1.79
Vapam	20.8 ± 1.42	21.0 ± 0.63	19.7 ± 0.73
PO	21.3 ± 0.54	18.9 ± 1.13	20.2 ± 0.85
EDN	20.9 ± 0.29	22.6 ± 0.02	20.3 ± 0.33

Table 3. Mean (±SE) seedling root collar diameter (RCD) at lifting over two growing seasons (2017-2018) for the trial in Elberta, AL.

	RCD (mm)		
	Slash	**Loblolly**	**Loblolly**
Fumigant	**2017**		**2018**
MBr	4.39 ± 0.10	3.97 ± 0.12	4.43 ± 0.23
Ally 33	4.56 ± 0.06	3.66 ± 0.00	4.08 ± 0.12
Dominus	4.44 ± 0.25	3.89 ± 0.10	**3.99 ± 0.10**
Vapam	4.33 ± 0.00	4.04 ± 0.19	4.16 ± 0.20
PO	4.47 ± 0.26	3.90 ± 0.30	4.01 ± 0.24
EDN	4.32 ± 0.07	3.86 ± 0.14	4.07 ± 0.08

Values in bold represent a significant ($p<0.05$) difference between values for MBr.

Table 4. Mean (±SE) seedling shoot and root biomass and root weight ratio over two growing seasons (2017-2018) for the trial in Elberta, AL.

	Shoot Mass (g)			Root Mass (g)			Root Weight Ratio (%)		
	Slash	Loblolly	Loblolly	Slash	Loblolly	Loblolly	Slash	Loblolly	Loblolly
Fumigant	2017		2018	2017		2018	2017		2018
MBr	3.19 ± 0.07	2.73 ± 0.02	2.97 ± 0.25	0.52 ± 0.02	0.51 ± 0.08	0.47 ± 0.02	14.01 ± 0.82	15.83 ± 2.29	13.72 ± 1.02
Ally 33	3.25 ± 0.19	2.55 ± 0.18	2.63 ± 0.13	0.55 ± 0.05	0.46 ± 0.08	0.38 ± 0.04	14.45 ± 0.36	15.05 ± 1.48	12.57 ± 1.01
Dominus	3.21 ± 0.19	2.68 ± 0.04	2.48 ± 0.13	0.53 ± 0.01	0.48 ± 0.06	0.39 ± 0.03	14.22 ± 0.93	15.13 ± 1.84	13.80 ± 1.53
Vapam	3.06 ± 0.02	2.89 ± 0.18	2.69 ± 0.20	0.56 ± 0.09	0.46 ± 0.04	0.41 ± 0.01	15.43 ± 1.93	13.65 ± 1.17	13.45 ± 1.10
PO	3.41 ± 0.16	2.72 ± 0.34	**2.40 ± 0.18**	0.52 ± 0.01	0.43 ± 0.01	0.38 ± 0.03	13.26 ± 0.67	13.73 ± 1.17	13.71 ± 1.08
EDN	3.15 ± 0.08	2.61 ± 0.15	2.55 ± 0.08	0.52 ± 0.01	0.48 ± 0.08	0.39 ± 0.02	14.15 ± 0.29	15.63 ± 2.92	13.43 ± 1.02

Values in bold represent a significant ($p<0.05$) difference between values for MBr.

Table 5. Mean (±SE) number of weeds per square foot from 2017 survey for the trial in Elberta, AL.

	# Weeds/ft^2
Fumigant	**2017**
MBr	0.25 ± 0.25
Ally 33	2.5 ± 1.25
Dominus	0.5 ± 0.5
Vapam	1.25 ± 1.25
PO	**23.5 ± 2.75**
EDN	0.75 ± 0.75

Values in bold represent a significant ($p<0.05$) difference between values for MBr.

Table 6. Mean (±SE) number of Trichoderma colony forming units (CFUs) from soil collected pre-fumigation and over two growing seasons (2017-2018) for the trial in Elberta, AL.

	Trichoderma (CFUs/mg soil)		
Fumigant	**Pre**	**2017**	**2018**
MBr	20.00 ± 4.74	29.30 ± 10.24	49.05 ± 7.54
Ally 33	14.45 ± 3.61	**0.15 ± 0.10**	38.40 ± 9.03
Dominus	20.25 ± 4.34	**2.85 ± 1.08**	39.85 ± 13.01
Vapam	16.65 ± 2.79	**0.15 ± 0.10**	49.65 ± 9.29
PO	13.25 ± 5.24	18.80 ± 9.12	58.20 ± 6.71
EDN	**0.75 ± 0.57**	6.60 ± 5.61	55.35 ± 12.03

Values in bold represent a significant ($p<0.05$) difference between values for MBr.

Table 7. Mean (±SE) number of nematodes per 100 cubic centimeters (cc) of soil from soil collected pre-fumigation and over two growing seasons (2017-2018) for the trial in Elberta, AL.

	Nematodes per 100 cc soil		
Fumigant	**Pre**	**2017**	**2018**
MBr	59.5 ± 26.29	1.0 ± 1.0	28.5 ± 6.99
Ally 33	88.0 ± 27.99	3.0 ± 1.91	8.0 ± 3.16
Dominus	85.0 ± 27.20	0.0 ± 0.0	12.5 ± 5.12
Vapam	47.0 ± 9.29	0.5 ± 0.5	16.5 ± 6.13
PO	30.0 ± 15.87	**23.0 ± 8.58**	21.5 ± 6.13
EDN	75.5 ± 25.72	4.5 ± 3.30	20.0 ± 12.94

Values in bold represent a significant ($p<0.05$) difference between values for MBr.

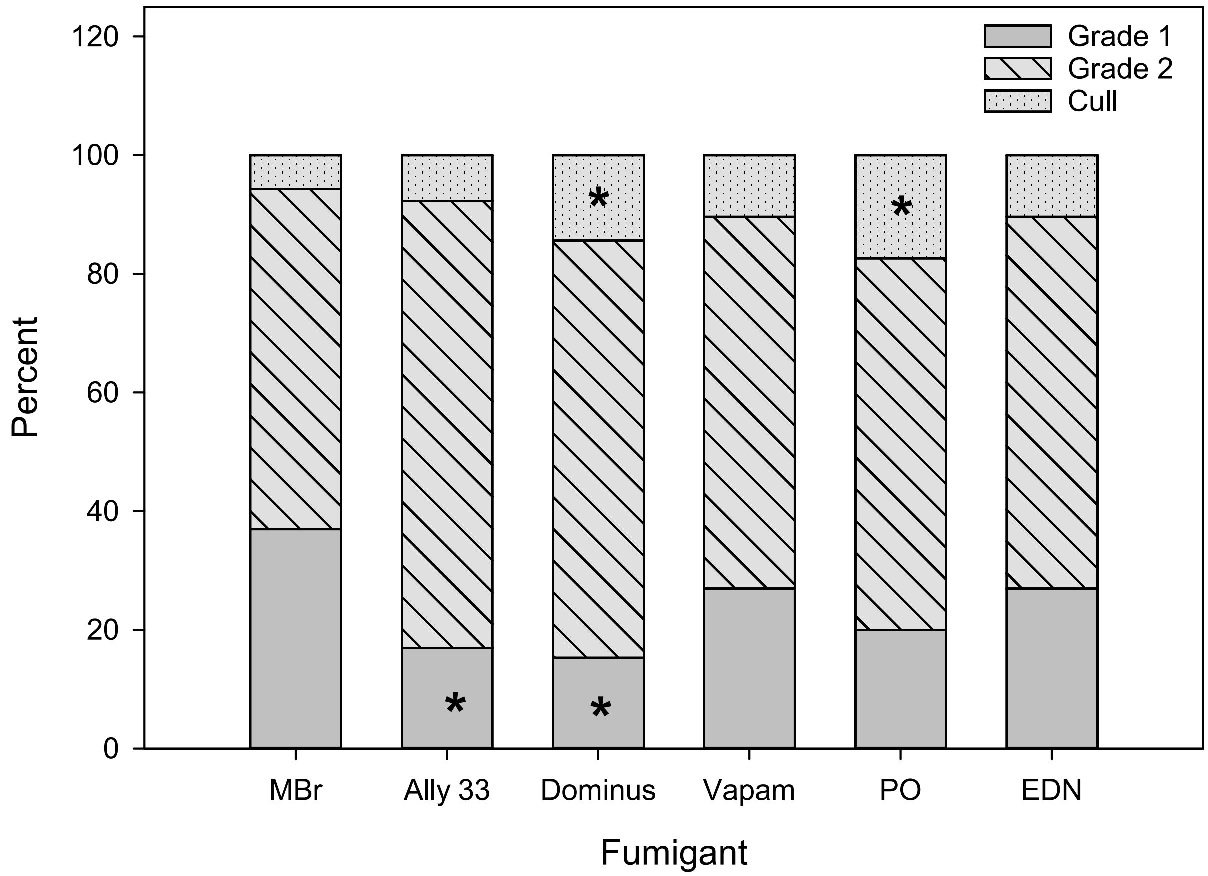

Figure 1. Percent of seedlings in each Grade class at lifting the second year after fumigation for the trial in Elberta, AL. (Grade 1 = seedlings RCD > 4.69 mm, Grade 2 = seedlings RCD 3.2 – 4.69 mm and Cull = seedling RCD < 3.2 mm). * denotes a significant differences between that fumigant and MBr in the grade class.