Southern Forest
Nursery Management Cooperative

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EVALUATION OF FIVE METHYL BROMIDE ALTERNATIVES ON SEEDLING PRODUCTION AND QUALITY OVER TWO GROWING SEASONS AT THE WEYERHAEUSER MAGNOLIA NURSERY, ARKANSAS 2019-2020

by
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INTRODUCTION

Producing high quality, pest and disease-free seedlings is the goal of forest-tree nurseries (Jang et al. 1993, Enebak 2013). Many factors, such as pathogens, nematodes, insects, and weeds can hinder nurseries from achieving this goal and therefore most bareroot nurseries use soil fumigants to minimize these problems (Carpenter 2013). The control of several pathogens that include *Fusarium* spp., *Pythium* spp., and *Rhizoctonia* spp., that are associated with both seedling root and foliage damage, along with the control of yellow and purple nutsedge is of particular concern in forest nurseries in the southeastern U.S. (Quicke et al. 2009). Methyl bromide (MBr) has been the industry standard for more than 50 years due to its broad efficiency over many soil types which has resulted in increases in both seedling density and size (Enebak 2007, 2013).

In the early 1990s, concern over the ozone damage led to MBr being listed as a Class 1 ozone-depleting substance in 1993 (EPA 2020). By order of the Montreal Protocol, production of MBr was frozen to the 1991 levels and was to phase out by the year 2005 (Enebak 2007). Since there were no comparable replacements for MBr, nurseries were granted permission to use it first through Critical Use Permits and then the Quarantine Pre-Shipment guidelines since seedlings are shipped across state boundaries (Enebak 2007). However, there is still the possibility that these exemptions could cease resulting in no further use of MBr as a soil fumigant in forest nurseries. Thus, finding an effective replacement fumigant to MBr that meets pest, disease, and weed management objectives across a broad spectrum of conditions without sacrificing seedling quality has become a top priority for tree seedling nurseries (Weiland et al. 2011).

The Southern Forest Nursery Management Cooperative (SFNMC) has continued to examine potential alternatives for MBr fumigation, as it has done for numerous years. A five-year USDA – ARS Area-wide Pest Management Project of Methyl Bromide Alternatives, in which the SFNMC was involved, was set up in the South Atlantic Division and tasked to evaluate the efficiency of many soil fumigants under normal nursery management practices and against common nursery pests and their effect on seedling quality (Enebak 2012). Apart from this project, the SFNMC frequently tests alternative fumigants at different member nurseries because we have found that effective alternatives in one nursery may differ at another nursery. In 2019, a study was established in Arkansas to examine five new alternatives to MBr with the objective to evaluate each alternative’s effect on nematode management and impact on seedling quality for two years post fumigation.

MATERIAL AND METHODS

Soil Fumigants

In this trial, we tested five fumigation treatments, in addition to MBr as our standardized control for comparison, to test their efficiency in producing quality pest-free forest-tree seedlings at the Weyerhaeuser Magnolia Nursery in Arkansas (Table 1). The fumigants were shank-injected into the soil on March 22, 2019 and comprised of two passes per treatment. All fumigation treatments were broadcast tarped using total impermeable film (TIF) that remained in place for 7 days prior to being removed (Table 1). The experimental area occupied approximately 1.85 ac and the trial was laid out in three nursery sections that consisted of seven seedling beds between the irrigation pipelines. The experiment was a randomized complete block design with each treatment plot being 140 x 22 ft. (0.07 ac) and replicated 4 times (24 plots total). A single family of loblolly pine was sown across the trial in 2019 and 2020. Seedlings in the trial were maintained using standard nursery cultural practices until lifting occurred in the fall. Following 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 three subplots (3 ft^2) 7 weeks post sowing in 2019 and three subplots (4 ft^2) at the end of each growing season before lifting in 2019 and 2020. At time of lifting from each subplot, all seedlings were collected and returned to the laboratory at Auburn University for seedling counts and quality analysis. Seedling root collar diameter (RCD), shoot height, and seedling dry weight (biomass) were measured for each seedling.

Soilborne Trichoderma and Nematodes

Soil samples were collected from each treatment within each unit pre-fumigation (March 2019), post fumigation (May 2019), and 1-year post fumigation (August 2020). 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 present.

RESULTS AND DISCUSSION

Study Goal

The SFNMC has focused efforts in identifying possible alternatives to MBr by using multi-year trials in soils and conditions throughout the southern U.S. The unique ability of MBr to consistently control weeds, insects, nematodes, and fungi across varying growing conditions and nursery soils has made the effort to find a suitable soil fumigant to match these characteristics difficult. To date, no perfect MBr alternative has been found, through extensive studies conducted within the southern U.S., that adequately achieve all these criteria (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 pressure increase.

Seedling Quality and Quantity

Outplanting survival of nursery grown seedlings have been linked to both seedling size and quality. Using MBr as a soil fumigant increases seedling survival and quality (Enebak 2017). In this trial at the Weyerhaeuser nursery in Magnolia, AR, seedling densities were similar across all soil fumigants tested with average seedling densities of 20.2, 16.8, and 11.9 seedlings/ft^2 at 7-weeks post germination in 2019, at lifting 2019, and at lifting 2020, respectively (Table 2). The low seedling density in 2020 in all fumigation treatments was due to bed washing from excessive rain following sowing operations. In respect to their effect on seedling density, all alternative fumigants were similar to the industry standard MBr.

No observed differences in seedling height were observed either year between fumigation treatment (Table 3) and likely a result of top clipping cultural practices. RCD were on average 5.38 and 5.36 mm in 2019 and 2020, respectively, with no differences between any of the alternative fumigants tested compared to that of MBr (Table 3). However, while not statistically significant, RCD in the Pic80, PicDM425 and SFC400 treatments did decrease from 2019 to 2020 which could lead to longer term effects on average seedling size. While average size of seedlings was similar, we also wanted to look at the proportion of seedlings in each SFNMC grade class (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 following fumigation when differences were most likely to be present. For all alternative fumigants tested, along with MBr, the number of cull seedlings decreased from 2019 to 2020. On average in 2020, 78.1, 21.6 and 0.3% of seedlings were in grade 1, grade 2 and cull classes, respectively, and did not differ between treatments (Figure 1).

Seedling biomass was similar both years between MBr and all alternative fumigants tested. Shoot mass per seedling averaged 4.69 and 3.88g and root mass averaging 1.00 and 1.04g per seedling for 2019 and 2020, respectively (Table 4). In 2019, average root weight ratio (defined as the weight of the roots divided by the total seedling weight) increased in the SFC400 fumigant treatment compared to MBr, however in 2020 no differences were observed with an average root weight ratio of 21.4% across all tested fumigants (Table 4).

Production of quality seedlings in an essential characteristic of an MBr replacement. All alternative soil fumigants tested produced equal size seedlings when compared to that of MBr. Also, the percent of seedlings in Grade 1 (RCD > 4.69 mm) in all fumigation treatments was greater than 60 and 70% in 2019 and 2020, respectively. However, prolonged use of each fumigant is unknown and the trend in decreasing RCD in Pic80, PicDM425, and SFD400 from 2019 to 2020, especially when the decrease in seedling density (due to bed washing) should lead to increases in seedling RCD, may become a concern with repeated use of these soil fumigants and therefore need to be investigated further.

Soilborne Trichoderma and Nematodes

Not all soilborne fungi are detrimental to seedling quality. Trichoderma is a beneficial soilborne fungus that has been linked to increased growth of pine seedlings (Quiche et al. 2009). Measurements of Trichoderma levels in the soil is monitored to test sensitivity of the soil micro-organisms to soil fumigation and, therefore, suppression of Trichoderma is considered undesirable when screening soil fumigants. One of the reasons that MBr has been the industry standard in soil fumigation is due to its limited impact on Trichoderma levels (Carey et al. 2005, Starkey et al. 2006, Starkey and Enebak 2008). Trichoderma levels in this current study decreased in 2019 post fumigation by the PicDM375 and PicDM425 fumigation treatment compared to that of levels found with MBr, however, Trichoderma levels were already reduced in the PicDM375 fumigation treatment compared to MBr before fumigation. Pre-fumigation Trichoderma levels were not available for the PicDM425 pre-fumigation so ascertaining whether this alternative fumigant had negative effects on Trichoderma levels is difficult to establish. By 2020, Trichoderma levels in the MBr treatment were 16.3 colony forming units (CFUs)/mg soil and was decreased to 3.0 CFUs/mg soil in the SFC350 fumigation treatment. However, the SFC400 soil fumigation treatment increased soil Trichoderma levels in 2020 when compared to MBr. Trichoderma levels were relatively low in all treatments, including MBr, when available data compared (data not available in 2020 for Pic80 and PicDM425). However, the 3.0 and 5.0 CFUs/mg soil Trichoderma levels in the PicDM375 and SFC350 fumigant treatments may be concerning with repeated use of this soil fumigant.

The presence of nematodes in soil can cause significant damage to forest seedlings. MBr has been shown to adequately control nematode populations making the control of nematode populations 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 - 48 nematodes/100cc soil) in numbers and species for all soil fumigants used (Table 6). While no statistical differences (due to high variation between plots) in the number of nematodes was evident in this study between any alternate fumigant used and MBr for any sampling time, the relatively high number nematodes found in the PicDM425 and SFC400 fumigation treatments when compared to MBr could result in poor nematode control with these soil fumigants with repeated use.

MANAGEMENT IMPLICATIONS

When evaluating a new soil fumigant, seedling quality and quantity production, nematode control, Trichoderma sensitivity, and weed control are main characteristics to consider. Another, less detrimental characteristic to consider, is that of odor. It was reported by nursery staff in this trial that the fumigants containing dimethyl disulfide (PicDM375 and PicDM425) produced unpleasant odors that were even more prevalent when the plastic was removed. Statistically, with the exception of Trichoderma levels, all alternative soil fumigants tested performed well when compared to that MBr. However, concern over the trends for growth reduction, decreased nematode control and increased Trichoderma sensitivity, to some extent by each alternative tested, by the second-year post fumigation should lead to caution when selecting any one of these alternatives for a long-term replacement for MBr without further testing.

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Table 1. Methyl bromide alternative soil fumigants and rates used in the 2019-2020 trial in Magnolia, AR.

Treatment	Rate (lbs/ac)	Plastic	Fumigant Components
MBr	300	TIF	80% MBr & 20% Chloropicrin
Pic80	350	TIF	80% Chloropicrin & 20% Dichloropropene
PicDM375	375	TIF	60% Chloropicrin & 40% Dimethyl Disulfide
PicDM425	425	TIF	60% Chloropicrin & 40% dimethyl disulfide
SFC350	350 + 100	TIF	100% Sulfuryl Fluoride & 100 Chloropicrin
SFC400	400 + 100	TIF	100% Sulfuryl Fluoride & 100 Chloropicrin

TIF-total impermeable film

Table 2. Mean (±SE) seedling density over two growing seasons (2019-2020) for the trial in Magnolia, AR.

	Seedling Density (ft^2)		
Fumigant	**2019 - 7WK**	**2019 - LIFT**	**2020**
MBr	20.6 ± 0.15	15.4 ± 1.34	12.1 ± 0.77
Pic80	20.0 ± 0.16	17.9 ± 0.48	12.0 ± 0.67
PicDM375	20.5 ± 0.25	19.6 ± 0.53	13.4 ± 0.90
PicDM425	20.0 ± 0.15	14.8 ± 1.06	11.9 ± 0.41
SFC350	20.0 ± 0.30	15.9 ± 1.43	9.9 ± 1.03
SFC400	19.9 ± 0.17	17.1 ± 2.01	12.1 ± 0.78

Table 3. Mean (±SE) seedling root collar diameter (RCD) and height at lifting over two growing seasons (2019-2020) for the trial in Magnolia, AR.

	RCD (mm)		**Height (cm)**	
Fumigant	**2019**	**2020**	**2019**	**2020**
MBr	5.34 ± 0.10	5.48 ± 0.11	32.4 ± 0.55	31.0 ± 0.88
Pic80	5.60 ± 0.16	5.30 ± 0.14	33.5 ± 0.60	31.4 ± 1.51
PicDM375	5.04 ± 0.08	5.45 ± 0.18	32.9 ± 0.54	31.2 ± 1.07
PicDM425	5.58 ± 0.13	5.28 ± 0.10	33.1 ± 0.79	29.3 ± 0.56
SFC350	5.36 ± 0.04	5.43 ± 0.18	32.4 ± 0.33	28.6 ± 1.73
SFC400	5.36 ± 0.21	5.20 ± 0.04	32.1 ± 0.80	28.1 ± 1.15

Table 4. Mean (±SE) seedling shoot and root biomass and root weight ratio over two growing seasons (2019-2020) for the trial in Magnolia, AR.

	Shoot mass (g)		**Root Mass (g)**		**Root Weight Ratio (%)**	
Fumigant	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
MBr	4.62 ± 0.18	4.09 ± 0.21	0.96 ± 0.04	1.09 ± 0.03	17.31 ± 0.09	21.28 ± 0.71
Pic80	4.97 ± 0.30	3.99 ± 0.23	0.99 ± 0.09	1.02 ± 0.02	16.51 ± 0.57	20.72 ± 0.88
PicDM375	4.44 ± 0.23	4.11 ± 0.27	0.94 ± 0.04	1.06 ± 0.09	17.54 ± 0.52	20.55 ± 0.56
PicDM425	4.98 ± 0.28	3.69 ± 0.14	1.09 ± 0.04	1.05 ± 0.05	18.03 ± 0.47	22.23 ± 0.49
SFC350	4.56 ± 0.13	3.87 ± 1.06	0.99 ± 0.02	1.06 ± 0.09	17.85 ± 0.48	21.75 ± 1.29
SFC400	4.55 ± 0.35	3.50 ± 0.14	1.05 ± 0.06	0.97 ± 0.03	**18.88 ± 0.38**	21.99 ± 1.11

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

Table 5. Mean (±SE) number of Trichoderma colony forming units (CFU's) from soil collected pre-fumigation and over two growing seasons (2019-2020) for the trial in Magnolia, AR.

	Trichoderma (CFUs/mg soil)		
Fumigant	**PRE**	**POST**	**1 YEAR**
MBr	42.0 ± 7.36	23.3 ± 1.80	16.3 ± 4.22
Pic80	**16.6 ± 2.64**	21.9 ± 1.56	.
PicDM375	**20.2 ± 1.41**	**15.6 ± 1.63**	5.0 ± 1.96
PicDM425	.	**14.7 ± 2.23**	.
SFC350	**24.5 ± 3.53**	18.1 ± 1.82	**3.0 ± 1.82**
SFC400	26.4 ± 4.06	22.9 ± 1.68	**50.6 ± 2.94**

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

Table 6. Mean (±SE) number of nematodes per 100 cubic centimeters (cc) of soil from soil collected pre-fumigation and over two growing seasons (2019-2020) for the trial in Magnolia, AR.

	Nematodes per 100 cc soil		
Fumigant	**PRE**	**POST**	**1 YEAR**
MBr	2.5 ± 1.50	0.0 ± 0.00	7.0 ± 5.20
Pic80	1.0 ± 0.58	0.5 ± 0.50	5.5 ± 3.78
PicDM375	12.0 ± 5.48	0.0 ± 0.00	6.5 ± 2.87
PicDM425	14.0 ± 12.70	0.0 ± 0.00	34.5 ± 20.37
SFC350	8.0 ± 7.35	0.0 ± 0.00	12.5 ± 3.69
SFC400	9.0 ± 7.72	0.0 ± 0.00	48.5 ± 42.55

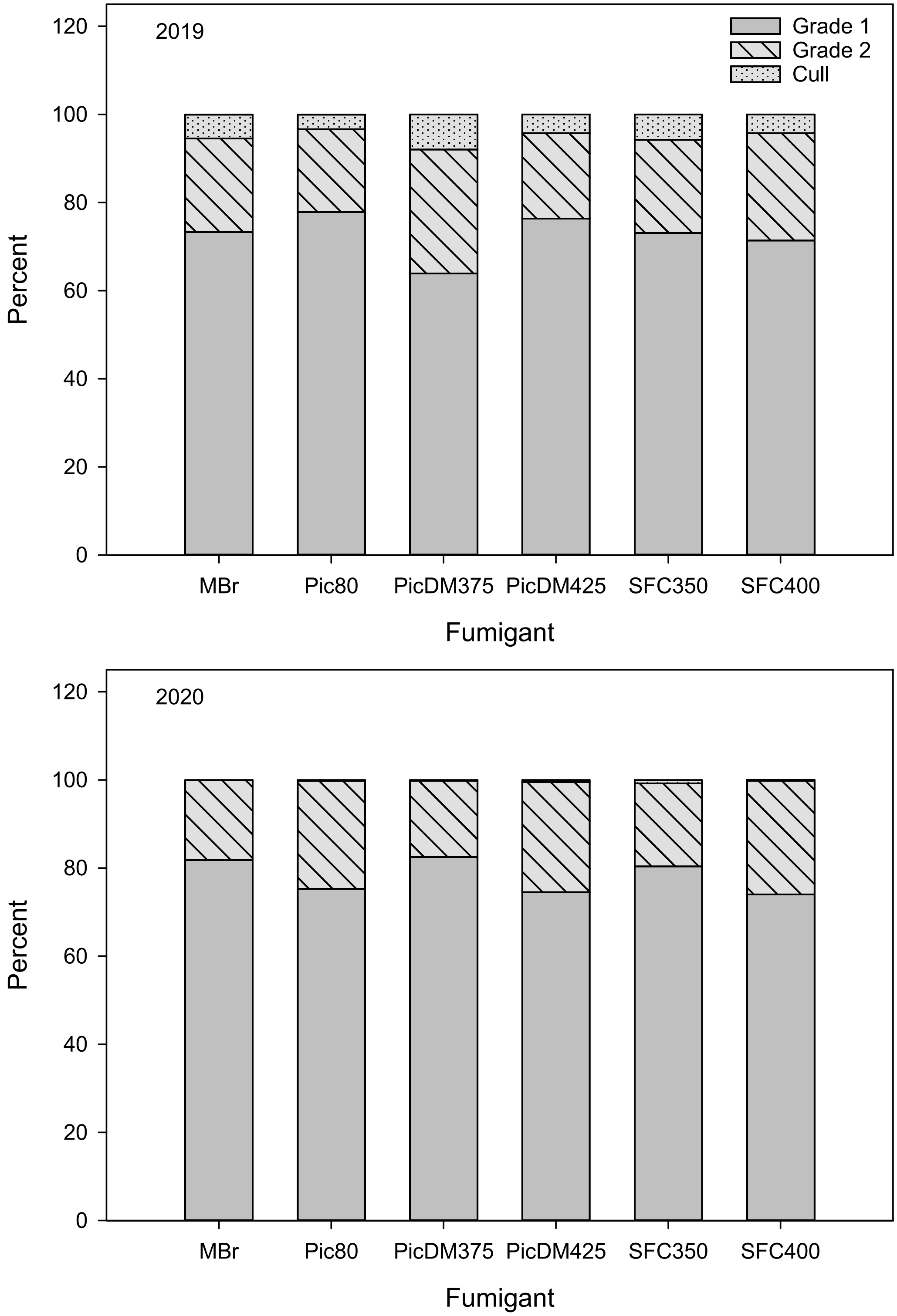

Figure 1. Percent of seedlings in each Grade class at lifting in 2019 and 2020 for the trial in Magnolia, AR. (Grade 1 = seedlings RCD > 4.69 mm, Grade 2 = seedlings RCD 3.2 – 4.69 mm and Cull = seedling RCD < 3.2 mm).