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Evaluation of Fumigants, EPTC Herbicide, and *Paenibacillus macerans* in the Production of Loblolly Pine Seedlings

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Abstract: Chloropicrin fumigation, Eptam 7-E (EPTC) herbicide, and *Paenibacillus macerans* seed treatments were evaluated as alternatives to fumigation with methyl bromide/chloropicrin for loblolly pine (*Pinus taeda* L.) seedling production at three nurseries in the southern United States. A treatment of metam sodium/chloropicrin was also evaluated at one nursery. Seedling density was unaffected by fumigation and EPTC treatments at all nurseries. Seedling diameter and shoot weight were greater in some chloropicrin treatments during the first year at the southern Georgia nursery. The only measurable disease losses were caused by a nematode, *Longidorus americanus*, at the southern Georgia nursery. Soil-borne population densities of *Pythium* and *Fusarium* spp. did not significantly differ between methyl bromide and the other fumigant treatments at any nursery. Chloropicrin controlled nutsedge (*Cyperus* spp.) in the loamy sand soils at the southern Georgia nursery, but there were no differences between the control and the fumigant treatments at the Alabama nursery, and nutsedge was rarely found at the northern Georgia nursery. The herbicide EPTC had no effect on nutsedge when compared to the controls at all nurseries and the effects of the bacterial seed treatment were inconsistent among the nurseries. Chloropicrin and metam sodium/chloropicrin can be effective alternatives to methyl bromide for reducing soil-borne fungi and nematodes, but the effectiveness of chloropicrin for nutsedge control may be affected by soil type. FOR. SCI. 53(1):73–83.

Keywords: chloropicrin, methyl bromide, metam sodium, eptam, *sec*-ethyl dipropylthiocarbamate, *Longidorus*, *Pythiam*, *Fusarium*, *Trichoderma*, *Pinus taeda*

SOIL FUMIGATION IS USED by most forest tree nurseries in the United States to produce bareroot forest tree seedlings for reforestation (Smith and Fraedrich 1993). In the southern United States, 96% of bareroot nurseries routinely fumigate soil before pine seedling production and of those, 89% fumigate with methyl bromide in combination with chloropicrin (Smith and Fraedrich 1993, Cram and Fraedrich 1996). Fumigation is typically applied in nurseries before every other tree seedling crop, although a quarter of growers apply fumigation before every seedling crop (Cram and Fraedrich 1996).

In 1991, methyl bromide was listed as an ozone-depleting substance under the Montreal Protocol, and was to be phased out of production in developed nations in 2005. In the United States, most forest tree nurseries received critical use exemptions (CUE) in 2005 and 2006, and requests for CUE must be reapplied for yearly (Environmental Protection Agency 2005). The amount of the CUE granted to the United States in 2006 by the parties of the Montreal Protocol has dropped from the 2005 level and is expected to continue to drop each year (Masters 2005). This reduction of CUE supplies of methyl bromide will continue to put pressure on availability and price of this fumigant. The dependence of many forest tree nurseries on fumigation with methyl bromide and chloropicrin has led to the need to identify alternatives that can provide similar control of soil-borne pests.

Loblolly pine (*Pinus taeda* L.) is the primary species produced in southern forest nurseries (McNabb 2005), and many soil-borne pests can affect its production (Cordell et al. 1989). Forest nursery managers presently need soil treatments for control of soil-borne pests that are comparable to methyl bromide. Methyl bromide has been a valuable broad-spectrum fumigant, and a variety of pest control practices may be needed to manage the diverse soil-borne pest problems that occur in forest tree nurseries. Chloropicrin has been shown to be an effective alternative to MC33 (67% methyl bromide/33% chloropicrin) for control of soil-borne pathogenic fungi (Enebak et al. 1990), plant-parasitic nematodes (Csinos et al. 1997), insects (Breakey et

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al. 1945), and weeds (Haar et al. 2003). However, chloropicrin has not been considered as effective as methyl bromide for the control of nutsedge (*Cyperus* spp.) (Csinos et al. 2000, South et al. 1997a). The herbicide EPTC (Eptam 7-E) is registered for control of nutsedge in pine nurseries, although few nurseries have used this herbicide in the past (South 1986, 1995). The combination of chloropicrin and EPTC may provide broad-spectrum pest control similar to MC33 in some nurseries.

Plant growth-promoting rhizobacteria (PGPR) can be used as biological seed treatments in agricultural crops to promote plant growth (Ryder et al. 1994), and have shown some promise for enhancing growth of tree seedlings (Chanway 1997). The use of PGPR on pine and spruce seeds has increased seedling emergence and growth in British Columbia (Holl and Chanway 1992). In addition, treatment effects of bacterial strains applied to loblolly pine seeds are dose-related and can increase the speed of seedling emergence, improve seedling growth, and decrease the incidence of damping-off (Enebak et al. 1998). Plant growth-promoting rhizobacteria may provide an alternative to MC33 when applied in combination with EPTC or other fumigants.

Studies were established in 1998 and 1999 in three southern US forest nurseries to examine alternative treatments for control of soil-borne pests and production of pine seedlings. The nurseries selected for the study had fields that had not been fumigated for over 4 years or had never been fumigated. The fields had been in pine production over the past decade and had been in small grain cover crops or fallowed during the year before the study. Specific objectives of these studies were to evaluate the effects of chloropicrin, EPTC, and a PGPR seed treatment, alone and in combination, on loblolly pine production, soil-borne fungi, nutsedge, and plant-parasitic nematodes.

Materials and Methods

Study establishment and design.—The studies were designed as a randomized complete block design with replication by blocks. All treatments were replicated four times (four blocks) at each nursery, with split-plots (with and without bacteria seed treatment) in each soil treatment. The whole plot factor was soil treatments of fumigants and/or herbicide; the subplot factor was PGPR seed treatment. Studies were established in 1998 at the southern Georgia nursery (Byromville, GA, Lat. 32°11′59″, Long. 83°59′33″) and the Alabama nursery (Atmore, AL, Lat. 31°10′39″, Long. 87°26′18″). The field at the southern Georgia nursery had been fumigated in 1993 with MC33 and a sorghum cover crop was grown the year before establishment of the study. The study was continued in 1999 and 2000 at the southern Georgia nursery. The Alabama nursery field had never been fumigated and was fallowed with applications of the herbicide glyphosate in the year before the study. The study at the Alabama nursery was terminated after 1 year and a new study area was established at the northern Georgia nursery (Chatsworth, GA, Lat. 34°37′17″, Long. 84°42′49″) in 1999 and continued in 2000. The field selected had also never been fumigated, and the cover crop grown the year before the study was corn.

Plots for each soil treatment were three seedbeds wide (4 m), and plot lengths were 24 m at the southern Georgia nursery, 15 m at the Alabama nursery, and 27 m at the northern Georgia nursery. Seedbeds were prepared at all three nurseries by ripping the field with subsoil shanks to break up hardpans and tilled to break up clods. Fumigants and EPTC were applied according to the label specifications. Fumigant treatments consisted of MC33 at 392 kg/ha, and chloropicrin (100%) at 168 kg/ha (CP168) and 336 kg/ha (CP336). The fumigants were injected into the soil to a depth of 15–20 cm and covered with clear polyethylene tarps. An additional treatment of metam sodium at 90 kg/ha with chloropicrin at 163 kg/ha (MS/CP) was included at the northern Georgia nursery. Metam sodium was sprayed on the soil surface and immediately incorporated with a roto-tiller. Chloropicrin was then injected into the soil to a 15–20-cm depth, the soil surface was roller packed, and covered with a clear polyethylene tarp. The herbicide EPTC (*sec*-ethyl dipropylthiocarbamate) was applied before sowing at 8 l/ha, alone and in combination with chloropicrin at 168 kg/ha (CP168/EPTC) or 336 kg/ha (CP336/EPTC). Fumigant treatments were applied only in the first year of the study at each nursery, while EPTC was reapplied yearly at all nurseries.

The PGPR seed treatment, *Paenibacillus macerans* Schardinger, was applied at a rate of 1.5 g/kg seeds at sowing each year. Whole plots with soil treatments were split and the PGPR-treated seeds were applied to one half of the plots. Application, sowing, and lifting dates are provided in Table 1.

Seedling Densities and Characteristics.— Six permanent inventory plots (three per seed treatment subplot) were established in the center bed of each soil treatment plot to

Table 1. Dates treatments were applied, loblolly pine seed sown, and seedlings were lifted at each nursery

Nursery	Year	EPTC applied	Fumigant applied	Seeds sown	Seedlings lifted
Alabama	1998	April 13	April 15	April 27	December 14, 1998
North Georgia	1999	March 29	March 29	May 4	November 8, 1999
	2000	March 29	—	May 4	November 7, 2000
South Georgia	1998	April 23	April 13 (MC33) April 26 (CP)	May 12	November 16, 1998
	1999	April 2	—	April 14	January 12, 2000
	2000	April 6	—	April 17	January 17, 2001

evaluate seedling and weed densities. At the northern Georgia and Alabama nurseries, inventory plots were 0.3 m × 1.2 m (4 sq ft). At the southern Georgia nursery, soil erosion occurred along the bed edges, therefore inventory plots were placed in the center portion of the bed and were 0.3 m × 0.6 m (2 sq ft). Seedling counts were performed at 7- to 10-day intervals for 5 to 7 weeks after sowing, and then at mid-season, and before lifting.

At lifting, 90 seedlings per soil treatment plot (45 per seed treatment plot) were collected to determine seedling diameter at the root collar, and shoot and root dry weights. Seedling shoot lengths were also determined at the southern Georgia nursery, where top pruning was not practiced. Seedlings were clipped at the root collar, and roots and shoots were dried at 80°C for 48 hours before weighing. Seedling shoot lengths were measured from the root collar to the shoot apex.

Weeds, Plant-Parasitic Nematodes, and Soil-Borne Fungi.— Population densities of nutsedge and other weeds were evaluated within 6 to 9 weeks after seed sowing and before postsowing operational weed control practices affected nutsedge densities. Weeds were counted in inventory plots used for assessments of seedling densities. To evaluate nematode populations, composite soil samples were obtained from each soil treatment plot at all nurseries within 6 weeks after fumigation and at lifting. The composite samples consisted of 5 to 10 soil cores taken to a 15 cm depth. Plant-parasitic nematodes were assessed by the Cooperative Extension Service at the University of Georgia using a centrifugation and floatation procedure to extract nematodes (Jenkins 1964). Nematodes were identified to genus. Populations of *Pythium*, *Fusarium*, and *Trichoderma* spp. were evaluated within 6 weeks after fumigation and again before lifting using composite soil samples collected as previously described. Komada's (Komada 1975) and PARP (pimaricin, ampicillin, rifampicin, pentachloronitrobenzene) (Kannwischer and Mitchell 1981) agar media were used to isolate *Fusarium* spp. and *Pythium* spp., respectively. TSM (*Trichoderma*-selective medium) (Elad et al. 1981) was used to isolate *Trichoderma* spp. at the Alabama and northern Georgia nurseries. PDATAR (potato dextrose agar, tergitol, ampicillin, rifampicin) with 1 ml tergitol (Kannwischer-Mitchell et al. 1994) was used for isolation of *Trichoderma* spp. at the southern Georgia nursery. Soil samples were assessed within 3 to 5 days after collection. Soil samples were mixed and 10 g were diluted 1:200 for assessment of *Pythium* and *Fusarium* spp., and 1:600 for *Trichoderma* spp., although dilutions as great as 1:6,000 were used when *Trichoderma* spp. were exceptionally high. One ml of the appropriate soil dilution was placed on five plates of each medium. Plates were incubated at 25°C and evaluated after 2 to 3 days for *Pythium* spp., and after 5 to 10 days for *Fusarium* and *Trichoderma* spp. Soil moisture contents were determined and the average number of colony-forming units (cfu) for each fungal genus was determined on a gram dry weight soil basis.

Soil and Nutrient Sampling.— Soil samples were taken at lifting from each soil treatment plot at each nursery in the first year. The composite sample for each treatment consisted of 5 to 10 soil cores taken to a 15 cm depth. Soil was sent to the Soil Testing Laboratory at Auburn University for analysis of the minerals, total carbon, and nitrogen using standards set by the Southern Extension Research Activity Information Exchange Group, SERA-6 (Hanlon 2001). A separate composite soil sample was taken across the fields of each nursery for a particle size analysis (hydrometer). Results of the soil analyses are presented in Table 2.

Statistical Analyses.—The experimental design was a split-plot with replication of the whole-plot factor by means of four blocks. Data for each variable were analyzed separately by year and nursery using Proc GLM Tukey's studentized range (HSD) test at the $\alpha = 0.10$ probability level (SAS Institute, Inc. 1989). The 10% significance level was chosen to reduce Type II error due to low replication, and to increase the power to detect a treatment difference that could be used as a practical management alternative (Remington and Schork 1985). Population densities of soil-borne fungi were transformed with a square root transformation or log transformation prior to analysis to correct for heterogeneity of variance (Steel and Torrie 1980).

Results

Seedling Bed Densities and Characteristics.—Fumigant or EPTC treatments did not significantly affect seedling density at any of the nurseries (Table 3). These treatments also had no effect on seedling diameter or root and shoot weight at the Alabama and northern Georgia nurseries. At the southern Georgia nursery, seedling diameter and shoot weight were greater in some of the chloropicrin treatments than the control in the first year after fumigation. In the third year after fumigation, seedling diameter and shoot weight were greater in the CP336/EPTC treatment than the EPTC treatment, but not significantly different from the control.

The results of treating loblolly pine seeds with *P. macerans* were variable depending on the nursery and the year (Table 4). Interactions between soil and seed treatments were not significant except for seedling diameter in one instance (southern Georgia nursery, year 1) when

Table 2. Soil Carbon/Nitrogen ratio, minerals, and physical characteristics

Nursery	C/N ratio	C	N	K	P	Ca	Mg	Fe	Sand	Silt	Clay	Field soil texture	Soil series
Alabama	19:1	1.49	0.08	120	9	290	59	27	50	18	32	Sandy clay loam	Greenville
North Georgia	18:1	2.14	0.11	107	27	676	67	54	47	29	24	Loam	Shellbluff
South Georgia	9:1	0.45	0.05	41	29	147	27	19	86	9	5	Loamy sand	Eustis

Table 3. Effect of fumigants and EPTC herbicide treatment on final loblolly pine seedling density, size, and dry weight at three forest tree nurseries

Nursery and year	Treatment	Seedlings	Root collar diameter	Root weight	Shoot weight
		(m^2)	(mm)	(g)	
Alabama					
1998	CP168	251	4.6	0.69	3.0
	CP168/EPTC	248	4.5	0.75	2.7
	CP336	239	4.8	0.86	3.2
	CP336/EPTC	253	4.4	0.67	2.5
	MC33	247	4.6	0.76	2.8
	EPTC	256	4.4	0.79	2.6
	Control	247	4.6	0.79	2.8
	(P > F value)	0.7752	0.3763	0.2890	0.1373
North Georgia					
1999	CP168	211	5.1	1.01	3.3
	CP168/EPTC	209	5.0	0.94	3.1
	CP336	218	5.1	0.97	3.5
	CP336/EPTC	220	5.1	0.89	3.4
	MS/CP	242	5.0	0.83	2.9
	MC33	227	5.2	1.05	3.7
	EPTC	218	5.0	0.82	3.1
	Control	220	5.0	0.90	3.1
	(P > F value)	0.3559	0.3204	0.2401	0.3548
2000	CP168	256	5.2	0.94	3.3
	CP168/EPTC	253	5.1	0.93	3.4
	CP336	267	5.0	0.88	3.4
	CP336/EPTC	255	4.9	0.96	3.2
	MS/CP	269	5.0	0.85	3.4
	MC33	245	5.3	0.99	3.6
	EPTC	256	5.1	0.89	3.5
	Control	252	5.0	0.89	3.2
	(P > F value)	0.2949	0.1090	0.3680	0.2786
South Georgia					
1998	CP168	239	4.4. abc*	0.73	3.4 ab
	CP168/EPTC	231	4.5 ab	0.76	3.0 abc
	CP336	237	4.6 a	0.77	3.8 a
	CP336/EPTC	226	4.4 abc	0.73	3.1 abc
	MC33	242	4.1 abc	0.65	2.9 abc
	EPTC	243	4.0 bc	0.64	2.6 bc
	Control	242	3.9 c	0.63	2.3 c
	(P > F value)	0.1732	0.0099	0.2020	0.0080
1999	CP168	233	3.7	0.71	2.8
	CP168/EPTC	227	3.7	0.70	2.7
	CP336	242	3.6	0.69	2.7
	CP336/EPTC	240	3.5	0.67	2.6
	MC33	242	3.5	0.61	2.7
	EPTC	246	3.6	0.68	2.5
	Control	242	3.6	0.73	2.6
	(P > F value)	0.6177	0.9595	0.8742	0.9485
2000	CP168	243	4.2 ab	0.79	3.0 ab
	CP168/EPTC	238	4.2 ab	0.82	3.1 ab
	CP336	242	4.1 ab	0.88	3.0 ab
	CP336/EPTC	227	4.4 a	0.87	3.3 a
	MC33	251	4.1 ab	0.77	2.9 ab
	EPTC	242	3.9 b	0.92	2.6 b
	Control	230	4.0 ab	0.76	2.7 ab
	(P > F value)	0.7724	0.0979	0.4737	0.0779

*Means within columns for each year and location followed by the same letter are not significantly different according to Tukey's studentized range (HSD) test at the $\alpha = 0.10$ level.

the variable was marginally significant ($P = 0.0941$). Since the main effect of soil treatments on seedling diameter was highly significant and that for the seed treatment clearly nonsignificant, this interaction was ignored. The seed treatment at the southern Georgia nursery resulted in reduced seedling heights during 1998 and 1999. There were no other effects from seed treatment on the rate (data not shown), seedling densities, diameter, and root and shoot biomass. In contrast, the seed treatment at the Alabama nursery increased the rate of seedling emergence, had an additional 14 seedlings per m^2, but a decrease in seedling shoot weight. At the northern Georgia nursery the seed treatment did not affect the rate of seedling emergence or diameter in either 1999 or

Table 4. Mean seedling density, size, and dry weight of loblolly pine seedlings treated with *Paenibacillus macerans* at the time of sowing at three forest tree nurseries

Nursery and year	Seed treatment	Seedlings	Root collar diameter	Seedling height	Root weight	Shoot weight
		(m^2)	(mm)	(cm)	(g)	
Alabama						
1998	Non-treated	242b*	4.6	ne†	0.79	2.9a
	Treated	256a	4.4	ne	0.72	2.7b
	(P > F value)	0.0155	0.1477		0.1430	0.0905
North Georgia						
1999	Non-treated	232a	5.0	ne	0.89b	3.1b
	Treated	208b	5.2	ne	0.96a	3.4a
	(P > F value)	0.0044	0.3204		0.0527	0.0449
2000	Non-treated	252b	5.1	ne	0.90	3.4
	Treated	261a	5.1	ne	0.90	3.3
	(P > F value)	0.0718	0.3827		0.2786	0.3680
South Georgia						
1998	Non-treated	241	4.3	21.4a	0.68	3.1
	Treated	233	4.2	19.7b	0.72	3.0
	(P > F value)	0.0173	0.2120	0.0024	0.1441	0.1851
1999	Non-treated	233b	3.6	25.3a	0.71	2.7
	Treated	244a §	3.6	24.5b	0.66	2.6
	(P > F value)	0.0215	0.3458	0.0915	0.1117	0.2860
2000	Non-treated	237	4.2	27.0	0.85	2.9
	Treated	241	4.1	26.6	0.81	2.9
	(P > F value)	0.6314	0.3929	0.4927	0.2834	0.9821

* Means within columns for each year and nursery followed by a different letter are significantly different according to Tukey's studentized range (HSD) test at the $\alpha = 0.10$ level.

† ne = variable not evaluated at the nursery because seedlings were top pruned.

§ A seed count at the time of sowing showed that one extra seed was sown per 0.093 m^2.

2000; however, seedling densities were significantly less in plots with bacterial-treated seeds in 1999 and significantly greater in 2000.

Weeds.—At the southern Georgia nursery, nutsedge densities (mean plants per m^2) were significantly different between treatments in 1998 ($P = 0.0162$) and 1999 ($P = 0.0270$). In the first year, all fumigation treatments (0 to 0.4 nutsedge/m^2) were less compared to the control (18 nutsedge/m^2). In the second year, nutsedge remained less in treatments with CP 336 (0.9 and 1.4 nutsedge/m^2) compared to the control (21 nutsedge/m^2). In the third year, nutsedge densities were not different between the control and the fumigant treatments. The herbicide EPTC did not have an effect on nutsedge counts in any year at the southern Georgia nursery.

Nutsedge was also the primary weed pest at the Alabama nursery, and although there were differences among treatments ($P = 0.0002$) in May 1998, none of the soil treatments was different than the control. Nutsedge was absent in the MC33, EPTC, and chloropicrin treatments with EPTC, but averaged 6 and 10 nutsedge plants per m^2 in the control and chloropicrin treatments without EPTC, respectively. Operational weed control practices (wick application of glyphosate) controlled the nutsedge, and by June there were no differences between plots.

Nutsedge occurred at low densities (<3 per m^2) at the northern Georgia nursery and there were no significant differences between treatments. A variety of broadleaf weeds was present in plots during the first 6 to 9 weeks after sowing and differences among the treatments ($P = 0.0284$) were found for total weed counts. In June 1999, weed densities (mean plants per m^2) were greater in the control (39 weeds/m^2) than the CP336/EPTC treatment (15 weeds/m^2) or the MC33 treatment (15 weeds/m^2). In June 2000, weeds were significantly different among treatments ($P = 0.0014$). Weeds were more prevalent in control plots (74 weeds/m^2) than plots of other treatments (25 to 43 weeds/m^2) with the exception of CP168 (52 weeds/m^2). By the ninth week in 1999 and the sixth week in 2000, the operational weed control program at the northern Georgia nursery effectively controlled weeds in all treatments and no differences were found.

Plant-Parasitic Nematodes.—Plant-parasitic nematodes were rare in the plots at the Alabama nursery, and therefore there were no treatment differences. At the southern Georgia nursery, the nematode population densities were also so low that there were no statistically significant differences in populations or seedling responses. However, during the third year of the study at the southern Georgia nursery, stunted seedlings were observed in four nonfumigated (control or EPTC-treated) plots. Patches (3 to 9 m long) of diseased seedlings were restricted to single beds, and stunted seedlings lacked lateral and feeder roots. A plant-parasitic nematode, *Longidorus americanus* (Handoo et al. 2005), was consistently associated with the stunted seedlings, and information on this nematode and the damage it caused were reported by Fraedrich and Cram (2002).

At the northern Georgia nursery, population densities of all plant-parasitic nematodes combined were significantly different ($P = 0.0046$) among the treatments. Plant-parasitic nematodes were less at sowing in fumigation treatments (range = 12 to 30 nematodes/100 cc soil) than the control

(104 nematodes/100 cc soil). Population densities of ring nematodes (*Criconemoides* spp.) were significantly greater in the control (50 nematodes/100 cc soil) than the MC33, MS/CP, CP150/EPTC, and CP300/EPTC treatments (range = 2 to 8 nematodes/100 cc soil). Densities of other plant-parasitic nematodes did not differ among treatments. Population densities of all plant-parasitic nematodes combined did not differ among treatments in November of 1999 (range = 10 to 76 nematodes/100 cc soil) or December of 2000 (range = 34 to 153 nematodes/100 cc soil). Symptoms of nematode damage were never observed on seedlings in any treatment at the northern Georgia nursery.

Soil-Borne Fungi.—The effect of fumigation treatments on soil-borne *Pythium*, *Fusarium*, and *Trichoderma* spp. varied among nurseries. With a few exceptions, differences among treatments occurred in the first year of the study at each location. At the Alabama nursery, the response of soil-borne fungi to the chloropicrin fumigants were not statistically different from the population densities found in MC33 with the one exception of *Pythium* spp. in the November sample of CP168/EPTC (Table 5).

At the southern Georgia nursery, all fumigants reduced populations of *Pythium* and *Fusarium* spp. and there were no significant differences in these population densities between MC33 and the chloropicrin treatments (Table 6). *Trichoderma* spp. were significantly greater in the MC33 treatment than all other treatments except the CP336 treatment after fumigation in May 1998. *Trichoderma* populations were greater in the MC33 treatment at the end of the second and third years compared to some of the chloropicrin-fumigated and nonfumigated treatments.

At the northern Georgia nursery, population densities of *Pythium* spp. were lower in the MC33, MS/CP, and CP336 treatments than the control in April 1999 (Table 7). By the end of 1999, there were no differences between the control and fumigant treatments. Fumigation had little effect on *Fusarium* populations at the northern Georgia nursery, and differences between the control and fumigation treatments were not detected. *Trichoderma* population densities increased greatly following fumigation with either MC33 or chloropicrin treatments. Population densities did not differ among treatments for any of the soil-borne fungi by the end of the second year of the study.

Table 5. Effect of fumigants and EPTC herbicide treatment on soil-borne fungi over time at the Alabama nursery

Soil fungi and treatments*	May 1998	August 1998	November 1998
Pythium			
CP168	87ab*	105	96ab
CP168/EPTC	51b	75	117a
CP336	27b	90	96ab
CP336/EPTC	42b	66	93ab
MC33	51b	45	57b
EPTC	111ab	111	126a
Control	156a	99	135a
(P > F value)	0.0099	0.1172	0.0053
Fusarium			
CP168	72b	153ab	87ab
CP168/EPTC	204ab	138ab	87ab
CP336	138ab	84b	114ab
CP336/EPTC	222ab	42b	30b
MC33	78b	39b	156ab
EPTC	189ab	225a	162a
Control	325a	159ab	176a
(P > F value)	0.0344	0.0094	0.0342
Trichoderma			
CP168	1,557ab	3654a	3,555
CP168/EPTC	1,110ab	2,805ab	3,101
CP336	336b	3231a	3,795
CP336/EPTC	516b	2,586ab	3,829
MC33	348b	1848b	2,730
EPTC	1,488ab	2,931ab	3,158
Control	2,181a	2,754ab	3,116
(P > F value)	0.0050	0.0057	0.5757

*Means within columns for each year followed by the same letter are not significantly different according to Tukey's studentized range (HSD) test at the $\alpha = 0.10$ level.

Discussion

Most southern forest nurseries in the United States continue to rely on fumigation with methyl bromide by using CUE and Quarantine Preshipment supplies of the fumigant (Environmental Protection Agency 2005, Masters 2005). However, as the amount of methyl bromide permitted as CUE for the United States decreases the price for the fumigant is likely to rise, and therefore will increase the pressure for nurseries to use alternative methods to control pests. The results of this study indicate that chloropicrin can substitute for MC33 to reduce potential soil-borne pathogens and plant parasitic nematodes in all three nurseries. Chloropicrin at both the 168 and 336 kg/ha rate could also be substituted for MC33 for control of nutsedge at the southern Georgia nursery. The nutsedge densities were low at the Alabama and Georgia nurseries, and there were no treatment differences when compared to the control treatment. None of the fumigation or EPTC treatments affected seedbed densities, and at two of the three nurseries fumigation had no effect on seedling morphological characteristics. The only effect of fumigation on seedling morphology was at the southern Georgia nursery, where root collar diameter and shoot weight increased during the first year after treatment with chloropicrin.

Diseases caused by soil-borne fungi were not observed in our studies and have been rarely observed in other recent studies conducted in southern nurseries (Barnard et al. 1997, South et al. 1997b, Fraedrich and Dwinell 2003a, b). Damping-off, root rots, and mortality were routinely reported in some southern pine nurseries prior to the advent of fumigation with methyl bromide (Hodges 1962, Seymour and Cordell 1979). The lack of soil-borne diseases in recent studies may be related to changes that have occurred in nursery production practices since the advent of fumigation. For instance, Hodges (1962) observed that root rots became increasingly severe over a period of years when consecutive pine crops were produced in the same area. Presently, most nurseries rotate pine crops with cover crops or a fallow period on a regular schedule. The Alabama and northern Georgia nurseries produce only one pine seedling crop and then rotate to a grass cover crop for 1 year, while the southern Georgia nursery produces two pine crops and then rotates to cover crops for 2 years. These rotations of seedling crops with specific cover crops can help prevent the

Table 6. Effect of fumigants and EPTC herbicide treatment on fungi colony forming units per gram (cfu/g) of soil over time at the south Georgia nursery

Soil fungi and treatments*	May 1998	November 1998	April 1999	January 2000	June 2000	January 2001
Pythium						
CP168	0	22b†	22ab§	34	68	69
CP168/EPTC	0	0b	76ab	11	22	113
CP336	0	0b	43ab	22	55	68
CP336/EPTC	0	0b	55ab	46	34	102
MC33	0	22b	0b	77	78	192
EPTC	0	33ab	108a	102	34	102
Control	22	91a	97a	112	22	127
(P > F value)	—*	0.0045	0.0472	0.1520	0.7136	0.6394
Fusarium						
CP168	0b§	196bc	942	556	697	631
CP168/EPTC	0b	65c	610	363	523	362
CP336	32ab	347bc	1,468	89	575	293
CP336/EPTC	0b	99bc	1,889	293	780	575
MC33	0b	98bc	1,011	120	797	588
EPTC	330a	960a	1,111	383	1,339	852
Control	165a	663ab	1,747	451	730	619
(P > F value)	0.0058	0.0018	0.4679	0.4113	0.5300	0.4228
Trichoderma						
CP168	490b	3,445	10,291	3,170ab	ne¶	1,307ab
CP168/EPTC	97b	4,003	4,062	2,924ab	ne	1,528ab
CP336	879ab	2,115	4,522	1,442b	ne	1,019b
CP336/EPTC	268b	2,401	5,544	2,725b	ne	1,235ab
MC33	1,996a	5,783	6,975	7,485a	ne	2,470a
EPTC	132b	2,324	2,692	1,220b	ne	611b
Control	132b	1,879	2,808	2,956ab	ne	474b
(P > F value)	0.0015	0.2495	0.3205	0.0216		0.0148

* Pythium recovered from only one control plot.
† Means within columns for each year followed by the same letter are not significantly different according to Tukey's studentized range (HSD) test at the $\alpha = 0.10$ level.
§ Logarithmic transformation of data performed before analysis.
¶ ne, not evaluated

buildup of soil-borne pathogenic fungi (Smith et al. 1989) and nematodes (Ruehle 1972) by using nonhosts or poor hosts. Another change in nursery operations is related to soil characteristics within individual nurseries. Nurseries established before 1960 usually had finer-textured soils (Boyer and South 1984), while more recently established nurseries (e.g., southern Georgia nursery) have well-drained sandy soils that provide managers with better control over soil moisture, which can be important in disease development (Cordell et al. 1989). The Alabama and northern Georgia nurseries, which have finer-textured soils and historically have not fumigated, are managed with yearly rotations of pine to cover crops to reduce soil compaction and lessen the risk of pest buildup.

The lack of damping-off and root disease in nursery beds during the first year at the southern Georgia nursery indicates that factors other than soil-borne pathogens may have been responsible for differences in seedling size. Fumigation can influence soil nutrient availability, and in other studies has increased the availability of nitrogen (Hansen et al. 1990), manganese (Fraedrich and Dwinell 2003b), and phosphorus (Ingestad and Molin 1960). Fumigation also alters the soil microbiota (Danielson and Davey 1969, Ridge and Theodorou 1972). *Trichoderma* spp. increased significantly in soil following treatment with MC33 at the southern Georgia nursery, and MC33 and chloropicrin at the northern Georgia nursery. *Trichoderma* spp. often increase in soil following fumigation (Danielson and Davey 1969, Fraedrich and Dwinell 2003b), and various *Trichoderma* spp. may be beneficial for seedling growth (Bailey and Lumsden 1998).

The only soil-borne disease that occurred during the study was found in 2000 at the southern Georgia nursery. Patches of stunted seedlings, caused by the plant parasitic nematode *Longidorus americanus,* developed in some nonfumigated plots (Fraedrich and Cram 2002). Stunted seedlings had been observed in the field outside of the study area before initiation of the study, and fumigation with MC33 was found to provide control of this nematode for only 1 year (Cram et al. 2003). The delayed development of disease, along with the occurrence of *L. americanus* only in the nonfumigated plots, indicates that the nematode was probably moved into the study area from the adjacent infested areas during field preparation for soil treatments in 1998, and that the fumigant treatments controlled the nematode before it could become established in the plot. The difference in seedling size between the nonfumigated EPTC and CP336/EPTC treatments during the third year at the southern Georgia nursery was most likely due to a combination of nematode damage and nutsedge competition. These problems likely increased over time and had a discernible impact on seedlings during the third year of continuous production.

Nutsedge is a major pest problem in southern forest nurseries and methyl bromide is relied on by many nurseries

Table 7. Effect of fumigants and EPTC herbicide treatment on soilborne fungi over time at the north Georgia nursery

Soil fungi and treatments (kg/ha)	April 1999	November 1999	December 2000
Pythium			
CP168	115bc*	270b	434
CP168/EPTC	168bc	510b	437
CP336	74c	150b	419
CP336/EPTC	298abc	510b	291
MS/CP	43c	120b	425
MC33	14c	150b	461
EPTC	647a	1,050a	633
Control	503ab	510b	342
(P > F value)	0.0007	0.0001	0.8867
Fusarium			
CP168	3,810ab	180	1,949
CP168/EPTC	2,862ab	150	2,732
CP336	1,751b	150	2,311
CP336/EPTC	3,446ab	330	2,724
MS/CP	4,430ab	240	2,690
MC33	7,129ab	150	1,945
EPTC	7,391a	270	3,111
Control	6,861ab	330	2,896
(P > F value)	0.0286	0.4292	0.4386
Trichoderma			
CP168	236,507ab†	21,263a†	16,513
CP168/EPTC	207,853ab	13,668abc	15,015
CP336	306,798a	19,538abc	18,736
CP336/EPTC	507,112a	20,625ab	21,007
MS/CP	45,745bc	12,563abc	18,961
MC33	275,869a	18,750abc	23,586
EPTC	8,624c	9,038bc	18,535
Control	16,448c	7,725c	12,049
(P > F value)	0.0001	0.0098	0.1896

* Means within columns for each year and site followed by the same letter are not significantly different according to Tukey's studentized range (HSD) test at the $\alpha = 0.10$ level.

† Logarithmic transformation of data performed before analysis.

for control of this weed (Cram and Fraedrich 1996). The southern Georgia nursery had a greater population of nutsedge than the northern Georgia and Alabama nurseries, and the impact of fumigants on nutsedge densities was observed for several years. The northern Georgia and Alabama nurseries did not traditionally use fumigation, and they appeared to have postsowing operational weed control programs that effectively managed nutsedge as well as other weeds. These nurseries used a wick applicator to apply glyphosate to nutsedge once it grew above the pine. The southern Georgia nursery uses glyphosate outside of seedbeds, but they have applied the herbicide fomesafen in loblolly pine seedbeds to manage nutsedge that escapes fumigation (South 1997). Glyphosate can be effective for nutsedge control in nurseries (South 1986, Fraedrich et al. 2003) because the herbicide is systemic and readily translocated throughout plants, killing shoots as well as tubers (Doll and Piedrahita 1982). In contrast, fomesafen suppresses tuber sprouting for yellow nutsedge (*Cyperus esculentus* L.), but is not regarded as effective for purple nutsedge (*Cyperus rotundus* L.) control (Webster 2003). The species of nutsedge present in the study at the southern Georgia nursery was primarily purple nutsedge.

Soil texture and organic matter content can also influence fumigant performance and subsequent seedling development. In general, fumigation is more effective in coarse-textured soils than fine-textured soils that are higher in organic matter or clay soils (Munnecke and Van Gundy 1979). Differences in soil texture and the carbon nitrogen ratio may explain, in part, why chloropicrin provided excellent control of nutsedge at the southern Georgia nursery, whereas chloropicrin failed to control nutsedge in the finer-textured soils at the Alabama nursery. Other recent studies at the southern Georgia nursery and elsewhere have also found that chloropicrin can control nutsedge (Carey and South 1999, Carey 2000); therefore, supplementing fumigation with EPTC may not be necessary in some nurseries.

The interaction of *P. macerans* seed treatments with the seedling species and the soil environment are complex and can lead to variable results (Enebak et al. 1998). The use of the *P. macerans* seed treatments in the nurseries resulted in inconsistent effects on seedling density and quality, and sowing into fumigated or nonfumigated soil had no effect on seed treatment. Greenhouse research with *P. macerans* under different soil conditions indicates that edaphic factors play an important role in the manifestation of growth promotion by bacteria (Estes 2001). In these studies and others (Chanway and Holl 1991), bacterial performance was shown to be more pronounced in nutrient-limiting soils as a result of either

direct (increased nutrient uptake rates) or indirect (increases in root mass) mechanisms. Variability in growth response between the nursery soil types could also have been attributed to host-plant specificity. Bacteria often are adapted to specific niches that give them a competitive advantage over non-native rhizobacteria (McArthur et al. 1988) and important genotypic relationships between plants and rhizobacteria have been documented (Chanway et al. 1990). The rhizobacterium used in the present studies were not isolated from nursery soils or *Pinus* spp., and perhaps rhizobacteria isolated from native loblolly pine soils may prove more effective at growth promotion in forest tree nurseries. A better understanding is needed of the mechanisms by which PGPR seed treatments affect seed germination and seedling growth in southern pine nurseries if this technique is to be applied operationally.

Conclusions

Chloropicrin has been found to be a viable alternative to methyl bromide as a preplant soil fumigant for a variety of crops nationally (South et al. 1997b) and internationally (Gullino et al. 2003). The results of this study also indicate that chloropicrin can be an alternative to methyl bromide. However, the EPTC herbicide did not adequately control nutsedge and the *P. macerans* seed treatment resulted in a highly variable seedling response. Chloropicrin was an excellent alternative to MC33 for control of potentially pathogenic soil-borne fungi at the southern Georgia nursery. Although the lower rate (168 kg/ha) reduced soil-borne populations of *Pythium* and *Fusarium* spp., the higher concentration (336 kg/ha) also provided control of nutsedge in the second year. The effects of fumigation with chloropicrin and MC33 on *Pythium* and *Fusarium* spp. was not as dramatic in the heavier textured soils at the northern Georgia and Alabama nurseries. Unlike many other southern nurseries (Cram and Fraedrich 1996), the Alabama and northern Georgia nurseries have routinely produced loblolly pine seedlings without the use of fumigation and have developed alternate herbicide strategies for managing nutsedge. The lack of demonstrable benefits of fumigation on seedling density and size indicated that these nurseries could continue to grow pine seedlings without fumigation in the absence of definite soil-borne pathogens. However, if soil-borne pests were to become established, our studies indicate that chloropicrin applied at 336 kg/ha and MS/CP could be suitable options to reduce soil-borne pathogenic fungi and plant parasitic nematodes before producing seedlings.

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