The effects of soil fumigation on pine seedling production, weeds, foliar and soil nutrients, and soilborne microorganisms at a south Georgia (U.S.A.) forest tree nursery

Stephen W. Fraedrich and L. David Dwinell

Abstract: Pine seedling production and pest problems were evaluated in plots fumigated with methyl bromide and nonfumigated plots over a 6-year period at a Georgia nursery. Fumigation increased bed densities for loblolly pine (*Pinus taeda* L.) in 1996 and slash pine (*Pinus elliottii* Engelm. var. *elliottii)* in 1998; differences were not observed between treatments in other years. The root collar diameter, height, and root and shoot masses of loblolly pine seedlings were often greater in fumigated plots during the first 3 years. Morphological characteristics rarely differed between treatments for slash pine. The primary pest problem was nutsedge (*Cyperus* spp.); most other weeds were controlled with herbicides used operationally at the nursery. Plant-parasitic nematode populations did not increase over time and were not a problem. Although *Fusarium* and *Pythium* spp. were more common in soil and on roots in nonfumigated plots, evidence of disease was rare. Fumigation increased the abundance of and changed the composition of *Trichoderma* spp. in soil and on roots. Soil manganese and iron, and foliar manganese, phosphorus, and nitrogen were greater in the fumigation treatment in some years. A better understanding of the risks of soilborne diseases may facilitate the development of pest management programs that arc more cost-effective.

Résumé : La production de semis de pins et les dommages cause's par les ravageurs ont été évalués dans des parcelles fumigées ou non au bromure de méthyle sur tme période de 6 ans dans une pépinière de la Géorgie. La fumigation a augmenté la densité des semis dans les planches pour le pins à encens (*Pinus taeda* L.) en 1996 et le pin de Floride (*Pinus elliottii* Engelm. var. *elliottii*) en 1998. Il n'y avait pas de difference entre les traitements les autres années. Le diamètre au collet, la hauteur et la masse dcs racines ct des pousses des semis de pin à encens Ctaient souvent plus élevés dans les parcelle fumigées pendant les trois premières années. Dans le cas du pin de Floride, les caractéristiques morphologiques différaient rarement entre les traitcments. Le principal ravageur était le souchet comestible (*Cyperus* spp.); la plupart des autres mauvaises herbes Ctaient contrôlées par l'utilisation opérationnelle d'hcrbicides. Les populations de nematodes phytoparasites n'ont pas augmenté avec le temps et ne causaient pas de problème. Bien que *Fusarium* et *Pythium* spp. aient été plus communs darts le sol et sur les racines dans les parcelles non fumigées, il y avait rarement des signes de maladie. La fumigation a augmenté la présence et changé la composition des espèces de *Trichoderma* dans le sol et les racines. Certaines années, le contenu en manganèse et cn fcr dans le sol ainsi qu'en manganèse, en phosphore et en azote dans le feuillage était plus élevé dans le traitement avec fumigation. Une meilleure comprehension des risques associés aux maladies présentes dans le sol peut faciliter le développement de programmes de gestion des ravageurs qui sont plus rentables.

[Traduit par la Rédaction]

Introduction

Reforestation on private lands throughout the southern United States depends on forest tree nurseries for production of high quality seedlings. In recent years, southern pine nurseries have produced as many as 1. l-l .65 billion seedlings annually, which was 69–78% of all forest-tree seedlings produced in the United States (Fraedrich and Smith 1994). Soil fumigation with methyl bromide has long been regarded as a key to controlling weeds, pathogenic fungi, nematodes, and insects. By the early 1970s, soil fumigation had become an almost universal operation for nursery soil management (Davey 1972). According to Dnvey (1972), many nurserymen at that time would have fumigated their soil if weed control was the only benefit. Prevention of soilborne diseases was regarded as an additional benefit. Today, many southern nursery managers still routinely fumigate fields with methyl bromide before every pine crop or before every other crop (Fruedrich and Smith 1994).

Methyl bromide has been identified as an ozone-depleting chemical, and a phase out on its production is scheduled to he completed by 2005 in accordance with the *United States Clean Air Act* and the *Montreal Protocol* (Environmental Protection Agency 1999). The loss of this chemical will require a better undcrstantiing of pest problems that affect pine production in southern nurseries to develop cost-effective alternatives for managing pests.

Received 28 January 2003. Accepted 18 March 2003. Published on the NRC Research Press Web site at http://cjfr.nrc.ca on IS August 2003.

S.W. Fraedrich' and L.D. Dwinell. Southern Research Station, USDA Forest Service, Athens, GA 30602. U.S.A

'Corresponding author (e-mail: sfraedrich@fs.fed.us)

 doi: IO. 1139/X03-084

Seedling production practices have changed substantially in southern nurseries since the practice of soil fumigation began more than 40 years ago. Since that time, there has been a steady increase in knowledge about seedling nutrition, soil water management, seed quality, and cultural practices that affect production. Weed control is an area of pest management where there have been substantial developments. Selective herbicides are now available that control many weeds in nursery beds at less cost than fumigation (South 1984). Although fumigation with methyl bromide provides excellent control of soilborne diseases and plant-parasitic nematodes, its routine use lessened the need to determine the prevalence of diseases, understand factors that favor disease development, and develop alternative practices to prevent and manage diseases.

A study was initiated in 1995 at a south Georgia forest tree nursery to determine seedling losses and the types and extent of pest problems if fumigation was not used. The objectives of this study were to (i) compare pine seedling production in soils fumigated with methyl bromide and nonfumigated soils; (ii) identify the types of pest problems that would be encountered without routine fumigation; (iii) evaluate the effect of continuous pine production with no fumigation on plant-parasitic nematode populations and potentially pathogenic and beneficial soilborne fungi; and (iv) determine the effect of fumigation on the availability of soil nutrients and subsequent seedling nutrition.

Materials and methods

Study design

The study was established in a 4.0-ha field at a forest tree nursery near Byromville, Ga. (Dooley County; 32°07′N, 83°52′W) in the spring of 1995. The field was regarded as a problem area because seedling losses were observed there in the late 1980s and early 1990s. The precise cause of the losses was not known, but nursery personnel suspected the problem was caused by fungi and nematodes. The field had not been fumigated since its removal from operational production in the early 1990s. Three blocks were established in 1995 and an additional three blocks in 1996. Each block consisted of the area between two irrigation risers. Blocks were divided into two plots, and each plot was 76.2 m long by 4.9 m (three seed beds) wide. Plots within blocks were randomly assigned to either the fumigation or no fumigation (control) treatment. Soil in fumigated plots was treated with methyl bromide – chloropicrin (67%/33%) at 393 kg/ha yearly between 1995 and 1998 (Table 1); the other six plots were not fumigated. Those blocks established in 1995 were designated for production of loblolly pine (*Pinus taeda* L.); those established in 1996 were for slash pine (*Pinus elliottii* Engelm. var. *elliottii*). Seeds were operationally sown, and seedlings were lifted each year on dates provided in Table 1.

All seedlings were grown under operational conditions. Standard weed control practices included presowing applications of fomesafen (REFLEX@) and oxyfluorfen (GOAL@) and weekly applications of oxyfluorfen after the seedlings were 6 weeks old until mid-August. Seedlings were undercut and laterally root pruned prior to lifting. Seedlings were not top pruned. Seedling measurements and samples were obtained from the center bed in each study plot. The outer two seed beds of each plot served as buffers.

Table 1. Dates for soil fumigation, seed sowing, and seedling lifting for the study area at the south Georgia nursery.

Year	Fumigation	Seeds sown	Seedlings lifted
1995	04/05	04/28	12/07
1996	04/09	04/24	12/10
1997	03/19	05/01	11/18
1998	04/13	05/14	10/05
1999	NF*	04/14	10/20
2000	NF*	04/17	12/05

*NF, no fumigation.

Seedling bed density and morphological characteristics

Three sample plots, each 0.3 x 1.2 m (1995 and 1996) or 0.3 x 0.6 m (1997–2000), were established in each plot immediately after seed sowing to evaluate seedling bed density. Each year, live and dead seedlings were counted in sample plots several times during the 8 weeks following sowing. A final assessment was conducted during the fall or winter just prior to lifting.

Seedlings were lifted from three 0.3 x 0.6-m sample plots selected at random locations over the length of each plot, and root collar diameter, shoot height, and shoot and root mass were determined. In 1995, six to seven seedlings were randomly selected and evaluated from each sample plot (20 seedlings per plot). In other years, 15-25 seedlings were measured in each of three sample plots (45-75 seedlings per plot). Seedling diameters were measured at the root collar. Seedling heights were measured from the root collar to the apex of the shoot. Seedlings were dried at 80 °C for 48-72 h prior to weighing roots and shoots.

Foliar nutrients

The foliar nutrient contents of seedlings were evaluated yearly at lifting from 1995 to 1998. Assessments were not conducted in 1999 or 2000. Needles were removed from a sample of 25 seedlings on each plot, dried, and ground. Samples were forwarded to Ward Laboratories (Kearney, Nebr., U.S.A.) for determination of nitrogen, phosphorus, potassium, calcium, magnesium, zinc, iron, and manganese.

Soil nutrients

Soil was collected from each plot at the end of each growing season from 1995 to 1998 and assayed for nutrients by Ward Laboratories. Assessments were not performed in 1999 or 2000. A composite sample consisting of approximately 10 soil cores was collected to a 15-cm depth over the length of the plot. Soil pH, organic matter, nitrate, phosphorus, potassium, calcium, magnesium, zinc, iron, manganese, and boron concentrations were determined.

Weed assessments

Population densities of weeds were determined at least once yearly with the exception of 1997, when evaluations were not conducted because of constraints on time and technical assistance. Weeds were counted in the same sample plots used to determine seedling density.

Mycoflora associated with roots

Root systems were evaluated yearly, except in 1999, for the presence *of Pythium, Fusarium,* and *Trichoderma.* Evaluations were conducted once at midseason (July or August) and again at lifting during 1995 through 1998. Evaluations were conducted a final time at the end of the study in December 2000. A sample of seedlings was collected at three locations on all plots at each evaluation period. Roots **were** rinsed with tap water, surface sterilized in a 0.5% sodium hypochlorite solution for 60 s, rinsed three times with sterile water, blotted dry, and plated on agar media. In 199.5, roots were plated on potato dextrose agar (PDA) with tergitol, ampicillin and rifampicin (PDATAR, Kannwischer-Mitchell et al. *1994)* for *Trichoderma* spp., water agar for *Pythium spp.*, and Nash-Snyder's (Nelson et al. 1983) agar medium **for** *Fusarium* spp. In other years, roots were plated on pimaricin–ampicillin-rifampicin-pentachloronitrobenzene (PARP) medium (Singleton et al. 1992) for *Pythium* spp., Komada's medium (Nelson et al. 1983) for *Fusarium* spp., and PDATAR. At midseason, the entire root systems of 10–12 seedlings/plot were plated for each of three agar media (30-36 seedlings (total)/plot). At the end of the season, roots of IO seedlings were cut into sections that were approximately 5-8 cm in length, and three sections were plated on each of the three **agar** media.

Agar plates with root samples were incubated in growth chambers at 25 "C. Roots on water agar or PARP plates were evaluated after 48-72 h. Roots on the other agar media were evaluated after 5-10 days. Roots were scored for the presence of *Fusarium*, *Pythium,* and *Trichaderma.* In 1995, we classified *Trichodermu* spp. only to genus, but in other years we accounted for types of *Trichoderma* spp. based on cultural morphology. Aerial mycelium of Type A *Trichoderma* isolates was blue-green and was clumped into isolated mounds. Hook-shaped sterile hyphae (Rifai 1969) were often observed on the margins of **these** isolates when viewed under a dissecting microscope. Mycelium of Type B *Trichoderma* isolates was dark green or yellow-green that became a darker green with age. Aerial mycelium of Type B isolates was continuous and not clumped. No sterile hyphae were observed in Type B isolates.

Fungi were evaluated to genus and to species when routinely isolated. Selected isolates of *Fusurium* spp. were transferred to KC1 agar, carnation leaf agar, and PDA and identified to species using the taxonomic key of Nelson et al. (1983). *Pythium* isolates were identified on corn meal agar and in grass-water culture and identified using the taxonomic keys of Middleton (1943) and Van der Plaats-Niterink (198 1). Selected isolates of each type of *Trichoderma* spp. were transferred to malt extract **agar,** and identification was based on the taxonomic key of Rifai (1969).

When observed, samples of dead and dying seedlings were collected, examined, and plated on PDA, Nash–Snyder's, water agar, or other appropriate agar media to determine fungal associations.

Soilborne myeoflora

Evaluations of soilborne populations of *Fusurium, Pythium,* and *Trichoderma* were initiated in May 1998 and were then evaluated yearly at lifting from 1998 to 2000. Soil samples were collected as previously described.

One millilitre of a 1:200 dilution was placed on each of five plates of PARP and Komada's media to estimate colony forming units (CFUs) of *Pythium* spp. and *Fusarium* spp., respectively. One millilitre of a 1:600 dilution was placed on each of five plates of PDATAR for estimation of the CFUs *of Trichoderma* spp. The CFUs of each genus were counted. Soil moisture content was determined for each soil sample, and CFUs were expressed on a gram dry mass basis. Selected isolates of *Fusurium* spp. and *Trichodermu* spp. were transferred to agar media and identified as previously described.

Plant-parasitic nematodes

Soil samples, collected as previously described, were evaluated monthly for plant-parasitic nematodes from May through December of 1995 and May through August of 1996. Thereafter, assessments were then conducted yearly at or near the time of lifting. Nematodes were extracted using the centrifugal-flotation technique (Hooper 1986) in 1995 and 1996, and the number of plant-parasitic nematodes per 100 cm^3 of soil was determined. Nematode samples were processed by the Cooperative Extension Services at the University of Georgia during 1997-2000. Plant-parasitic nematodes were evaluated to genus.

Statistical analyses

Comparisons between treatments were conducted yearly as a paired t test for each variable. Data for seedling morphological variables, foliar nutrients, and the mycoflora associated with roots was evaluated within pine species. Data for soil nutrients, plant-parasitic nematodes, soilborne fungi, and nutsedge (*Cyperus* spp.) were analyzed between pine species. The analyses were conducted with SAS (2000) and data analyzed as a randomized complete block design (Little and Hills 1978), with two treatments and three blocks for each pine species (or six blocks when combined between pine species). Data for the percentage of seedlings with specific fungi was transformed with the arcsin square root transformation (Steel and Torrie 1980). Soilborne fungal data and nutsedge data was transformed with the square root transformation prior to analysis (Steel and Torrie 1980). Non-transformed means are reported in **figures,** tables, and text. Because of the small number of replications per treatment for many variables, we established the significance level at 10% (a = 0.1; Steel and Torrie 1980).

Results

Seedling bed density and morphological characteristics

Seedling densities differed between fumigated and non-fumigated treatments for loblolly pine only in 1996 (Table 2). The difference was initially observed 3-4 weeks after seed sowing and was maintained through to the end of the growing season. In 1998, slash pine seedling density was slightly greater in the fumigation treatment (Table 3). There were no other significant differences between treatments for the density of slash pine in other years.

Table 2. Loblolly pine seedling density, root collar diameter (RCD), shoot height, and root and shoot mass in fumigation and control treatments at a south Georgia nursery during 1995-2000.

Year	Treatment	Seedlings/ 0.093 m^2	RCD (mm)	Shoot height (cm)	Shoot mass (g)	Root mass (g)
1995	Fumigation	25.6	4.4	29.9	3.3	0.96
	Control	25.2	3.4	26.1	1.7	0.68
	p value	0.4158	0.0097	0.0867	0.0049	0.0271
1996	Fumigation	24.4	4.1	32.2	3	0.45
	Control	20	4	27.8	2.1	0.57
	p value	0.0304	0.6815	0.0576	0.0513	0.2834
1997	Fumigation	28	4.3	36.2	3.7	0.61
	Control	26.6	3.4	27.2	1.8	0.47
	p value	0.5258	0.0154	0.0036	0.0113	0.0164
1998	Fumigation	23.1	3.2	23	2.4	0.39
	Control	23.6	3.3	22.7	2.1	0.33
	p value	0.8200	0.7986	0.6265	0.3281	0.4912
1999	Fumigation	19.8	3.3	23.9	2.4	0.34
	Control	18.8	3.5	23	2.5	0.38
	p value	0.5961	0.6396	0.6457	0.8734	0.6537
2000	Fumigation	20.3	4.3	28	2.9	1
	Control	24.4	4.2	27.5	2.7	1.16
	p value	0.1836	0.4631	0.3522	0.0754	0.3367

Note: Plots were fumigated with a mixture of 67% methyl bromide and 33% chloropicrin in 1995, 1996, 1997, and 1998 but were not refumigated in 1999 and 2000. *p* values represent the probability that treatment means within a year do not differ. Treatment means with $p < 0.1$ were considered to be significantly different. Means are based on three replications.

Table 3. Slash pine seedling density, root collar diameter (RCD), shoot height, and root and shoot mass in fumigation and control treatments at a south Georgia nursery during 1995-2000.

Year	Treatment	Seedlings/ 0.093 m^2	RCD (mm)	Shoot height (cm)	Shoot mass (g)	Root mass (g)
1996	Fumigation	26.3	4.1	24.5	2.9	0.62
	Control	25.5	3.9	22.7	2.1	0.62
	p value	0.5352	0.6198	0.1074	0.2879	0.9688
1997	Fumigation	32.2	4.2	31.4	3.3	0.56
	Control	29.9	3.9	30.6	2.6	0.59
	p value	0.1886	0.3544	0.6734	0.0758	0.6412
1998	Fumigation	19.1	3.4	19.3	2.4	0.47
	Control	18.1	3.1	18.6	2.3	0.42
	p value	0.0591	0.2083	0.6472	0.6343	0.6395
1999	Fumigation	21.3	3.9	24.1	3.2	0.67
	Control	21.1	3.9	24.6	3	0.47
	p value	0.7943	0.4503	0.5624	0.0893	0.3447
2000	Fumigation	22.3	4.4	27.8	3.2	0.81
	Control	23.9	4.1	26.5	2.8	0.82
	p value	0.6144	0.5926	0.4797	0.5475	0.9464

Note: Plots were fumigated with a mixture of 67% methyl bromide and 33% chloropicrin in 1995, 1996, 1997, and 1998 but were not refumigated in 1999 and 2000. *p* values represent the probability that treatment means within a year do not differ. Treatment means with $p < 0.1$ were considered to be significantly different. Means are based on three replications.

Loblolly pine seedlings were smaller in the nonfumigated plots in 1995 (Table 2). In 1996, only shoot height and mass differed significantly between treatments. In 1997, seedlings were again significantly larger for all morphological variables in fumigated plots. In 1998 and 1999, there were no differences between treatments, and in 2000, only shoot mass was greater in the fumigated plots.

There were no differences between treatments for slash pine morphological characteristics in 1996 (Table 3). In 1997 and 1999, only shoot mass was greater for slash pine

Fig. 1. Mean foliar nitrogen concentrations of loblolly pine (A) and slash pine (B) seedlings in fumigation and control treatments from 1995 to 1998. Lines above bars represent one standard error of the mean. The p values for differences between treatments within a year are presented above the bars. Treatment means with $p < 0.1$ were considered to be significantly different.

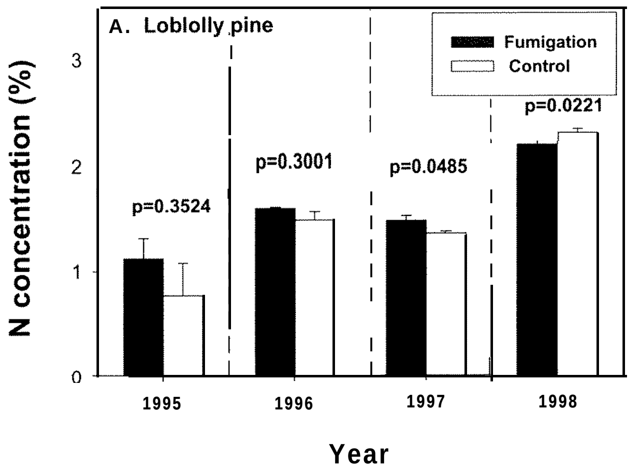
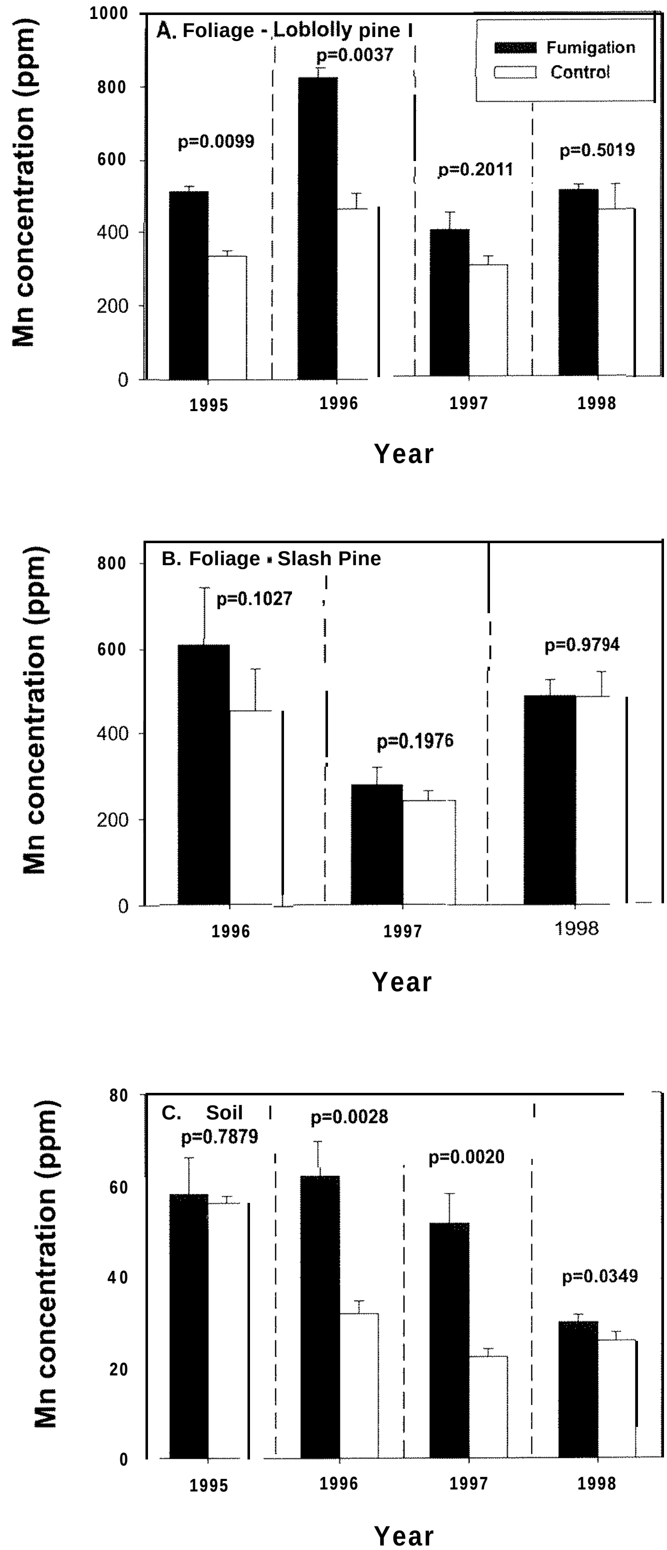


Fig. 2. Mean foliar manganese concentrations of loblolly pine (A) and slash pine (33) seedlings in fumigation and control treatments and soil manganese concentrations (C) in treatments from 1995 to 1998. Lines above bars represent one standard error of the mean. The p values for differences between treatments within a year are presented above the bars. Treatment means with $p < 0.1$ were considered to be significantly different.



seedlings in fumigated plots, and no differences were found between treatments for any of the morphological variables in 1998 and 2000.

Foliar nutrients

Foliar nitrogen concentrations were significantly greater in the fumigation treatment for loblolly pine in 1997 (Fig. 1A) and for slash pine (Fig. 1B) in 1996 and 1997, but in 1998, concentrations were slightly greater in loblolly pine foliage in the control treatment. Manganese concentrations were greater for loblolly pine seedlings in fumigated plots in 1995 and 1996 (Fig. 2A). Treatment differences for foliar manganese were not observed for loblolly or slash pine (Fig. 2B) in other years. In 1996, phosphorus was greater for both pine species in the fumigation treatment (loblolly — fumigation, 0.193%; control, 0.176%; p = 0.0878. slash fumigation, 0.184%; control, 0.162%; p = 0.0963). In 1997, phosphorus was greater for slash pine in the fumigation (0.107%) than in the control treatment (0.093%, p = 0.0572), and in 1998, phosphorus was greater for loblolly pine in the fumigation (0.193%) than in the control treatment (0.183%, p = 0.0001). In 1996, treatment differences were noted for calcium in loblolly pine and for zinc in slash pine, and in 1997, for potassium in loblolly pine, but there were no trends among years or pine species for these and other nutrients.

Soil nutrients

Soil nutrient concentrations did not differ between treatments in 1995. Soil manganese concentrations were significantly greater in the fumigation treatment in 1996, 1997, and 1998 (Fig. 2C). Soil iron concentrations were also greater in the fumigation treatment in 1996 (p = 0.0088), 1997 (p = 0.0002), and 1998 (p = 0.0045) and ranged among years from 15 to 16 ppm in the control and from 19 to 30 ppm in the fumigation treatment. Although differences were noted between treatments in some years for magnesium (1995), potassium (1996), nitrate (1998), and zinc (1998), there were no trends among years for these nutrients.

Weed assessments

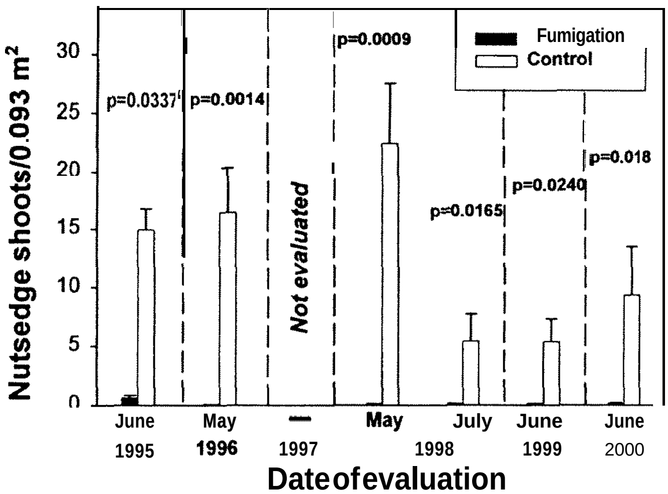
The nursery's operational weed management program controlled most weeds in nonfumigated plots, with the notable exception of nutsedge (Fig. 3). Nutsedge plants were rarely observed in fumigated plots. Nutsedge population densities in nonfumigated plots were 14.9 and 16.4 plants/0.093 m^2 in 1995 and 1996, respectively. Although not counted in 1997, visual observations indicated that nutsedge remained abundant and healthy throughout the growing season in nonfumigated plots. In May 1998, 22.3 nutsedge plants/O.093 m^2 were observed in nonfumigated plots, but the plants became increasingly chlorotic and eventually died during late May and June. Nutsedge was recounted in July 1998, and the population density was only 5.4 plants/0.093 m^2. Although nutsedge densities increased during 1999 and 2000, population densities in nonfumigated plots remained at low levels compared with densities that occurred prior to July of 1998. Purple nutsedge (*Cyperus rotundus* L.) was the primary weed in nonfumigated plots, although yellow nutsedge (*Cyperus esculentus* L.) was also noted.

Mycoflora associated with roots

Pythium spp. were rarely isolated from loblolly pine roots in either treatment at the midyear evaluation in 1995, but were frequently isolated from roots in nonfumigated plots at the end of the year (Table 4). In most other years, *Pythium* spp. were absent or infrequently isolated from slash and loblolly pine roots in fumigated plots, but they were normally present on some roots in the nonfumigated plots. *Pythium irregulare* Buisman was the primary *Pythium* sp. isolated from roots.

Fusarium spp. were more frequently isolated from roots of seedlings in the nonfumigated plots for both pine species. During evaluations conducted from 1995 to 1998, the isolation of *Fusarium* spp. from seedling roots ranged from 90 to 100% in nonfumigated plots, but only 17-67% in fumigated plots. The primary *Fusurium* sp. isolated was *Fusarium oxysporum* Schlectend., although *Fusarium solani* (Mart.) Sacc., and *Fusarium proliferatum* (Matsushima) Nirenberg were also encountered.

Fig. 3. Population densities of nutsedge (plants/0.093 m^2) in fumigation and control treatments during evaluations conducted on 29 June 1995, 16 May 1996, 21 May and 15 July 1998, 16 June 1999, and 21 June 2000. Lines above bars represent one standard error of the mean. The p values for differences between treatments within a year are presented above the bars. Treatment means with $p < 0.1$ were considered to be significantly different.



Trichoderma spp. were isolated more frequently from loblolly pine seedlings in the fumigation treatment during 1995. At the midseason assessment, *Trichoderma* spp. were isolated from 94% of seedlings in fumigated plots and 56% in control plots (p = *0.0138).* At the end of the year, *Trichoderma* spp. were isolated from 100% of seedlings in fumigated plots and only 63% in control plots (p = 0.0362). *Trichoderma* Type A isolates were more common on roots of loblolly and slash pine seedlings in the nonfumigated plots during 1997 and 1998. In most years, Type B isolates were more frequently associated with seedlings of both species in fumigated plots. The Type A isolates were primarily *Trichodermu hamatum* (Bon) Bain. The Type B isolates were primarily *Trichodermu harzianum* Rifai, although other unidentified species were also present.

Soilborne mycoflora

Pythium spp. were not isolated from soil of fumigated plots in May and October 1998, but occurred at low levels in nonfumigated plots (Table 5). *Pythium* spp. were infrequently isolated from fumigation and control treatments during 1999 and 2000, and there was no significant difference between treatments. *Fusarium* spp. were not isolated from fumigated plots in May 1998 and were less common in fumigated than in nonfumigated soil in October of that year. *Fusurium* spp. were again less common in fumigated plots in 1999, but no difference was observed between treatments in *2000.* Type A *Trichoderma* isolates were more common in control plots, and Type B *Trichoderma* isolates were predominant in the fumigated plots on all sample dates. The CFUs of Type B isolates appeared to decrease with time after the last fumigation in April 1998.

Plant-parasitic nematodes

Plant-parasitic nematodes were found infrequently in fumigated plots (Fig. 4), and although they were regularly found at low population densities in the nonfumigated control plots,

Table 4. Percentage of loblolly and slash pine seedlings from which selected fungi were isolated from roots in fumigated and nonfumigated soil at a south Georgia nursery during midyear and end-of-year evaluations from 1995–2000.

			Loblolly pine seedlings				Slash pine seedlings			
					Trichoderma spp.				*Trichoderma* spp.	
Year	Time period*	Treatment	*Pythium* spp.	*Fusarium* spp.	Type A†	Type B‡	*Pythium* spp.	*Fusarium* spp.	Type A†	Type B‡
1995	MID	Fumigation	3	17		—	—			—
	MID	Control	6	92	—			—		
		p value	0.4226	0.0041						
	EOY	Fumigation	7	40	—			—		
	EOY	Control	63	98		—	—		—	—
		p value	0.049	0.0736						
1996	MID	Fumigation	0	20	16	96	0	56	13	73
	MID	Control	4	91	24	33	4	96	16	36
		p value	0.4226	0.0059	0.0756	0.0419	0.4226	0.0343	0.7867	0.1857
	EOY	Fumigation	10	23	30	100	7	53	40	93
	EOY	Control	40	97	70	46	33	93	80	40
		p value	0.0187	0.0609	0.1942	0.1180	0.0850	0.0945	0.3660	0.0301
1997	MID	Fumigation	0	63	7	100	0	57	0	100
	MID	Control	7	100	80	10	10	83	40	10
		p value	0.1835	0.0925	0.0369	0.0064	0.4226	0.0675	0.040 1	0.0064
	EOY	Fumigation	0	67	37	100	0	80	20	100
	EOY	Control	20	97	83	17	7	97	63	17
		p value	0.0579	0.014	0.0721	0.0022	0.4226	0.2776	0.0324	0.0223
1998	MID	Fumigation	0	20	10	100	0	20	7	100
	MID	Control	13	90	53	10	17	83	50	23
		p value	0.047 1	0.0004	0.0828	0.0064	0.2776	0.0171	0.0126	0.028 1
	EOY	Fumigation	0	43	20	100	0	83	20	97
	EOY	Control	20	97	83	37	10	100	93	57
		p value	0.1435	0.1336	0.0039	0.0180	0.2187	0.0417	0.0995	0.0230
1999	No evaluations conducted									
2000	EOY	Fumigation	10	100	10	93	7	97	40	57
	EOY	Control	13	100	47	3	7	100	40	0
		p value	0.6657	1.0000	0.1579	0.002 1	0.9682	0.4226	0.9774	0.1407

Note: Plots were fumigated with a mixture of 67% methyl bromide and 33% chloropicrin in 1995, 1996, 1997, and 1998 but were not refumigated in 1999 and 2000. *p* values represent the probability that treatment means within a year do not differ. Treatment means with $p < 0.1$ were considered to be significantly different. Means are based on three replications. —, not applicable.

*MID, midyear assessment (July or August); EOY, end-of-year assessment (October to December).

†Isolates were predominately *Trichoderma hamatum*.

‡Isolates were predominately *Trichoderma harzianum*.

they were not believed to be problematic to pine seedlings. During monthly evaluations in 1995, population densities in nonfumigated plots ranged from 9 to 19 nematodes/100 cm^3 soil, but only O-6 nematodes/100 cm^3 soil in fumigated plots. In other years, population densities in nonfumigated plots ranged from 2.5 to 140 nematodes000 cm^3 soil. The ring nematode (*Criconemodes* spp.) was most common. In 1997, the root knot nematode (*Meloidogyne* spp.) was found in some nonfumigated plots at more than 100 nematodes/ 100 cm^3 soil, but it was absent in fumigated plots. Spiral (*Helicotylenchus* sp.) and stubby root (*Trichodorus* sp.) nematodes were occasionally found in fumigated and non-fumigated plots.

Disease of seedlings

Small patches of dead and dying loblolly pine seedlings were noted at scattered locations in June 1995. The disease occurred primarily in nonfumigated plots. Seedlings affected by the disease often lacked lateral and fine roots. Root tips or entire lateral roots appeared necrotic in some seedlings. The problem was of very short duration and involved relatively few seedlings. Spot mortality did not occur in sample plots that evaluated seedling bed densities. The problem did not recur in subsequent years. *Fusarium oxysporum* was isolated from the diseased seedlings, but this fungus was also isolated from roots of apparently healthy seedlings located in areas where there was no evidence of disease.

Discussion

Reductions in bed density and seedling size occurred primarily in nonfumigated plots with loblolly pine. Differences between treatments were observed less frequently for slash pine. When differences in the size of loblolly pine seedlings occurred between treatments, they often could be detected as early as 12 weeks after seed sowing (data not shown). Com-

Table 5. Colony forming units (CFUs) of specific fungi in fumigated and nonfumigated soils at a south Georgia (U.S.A.) nursery.

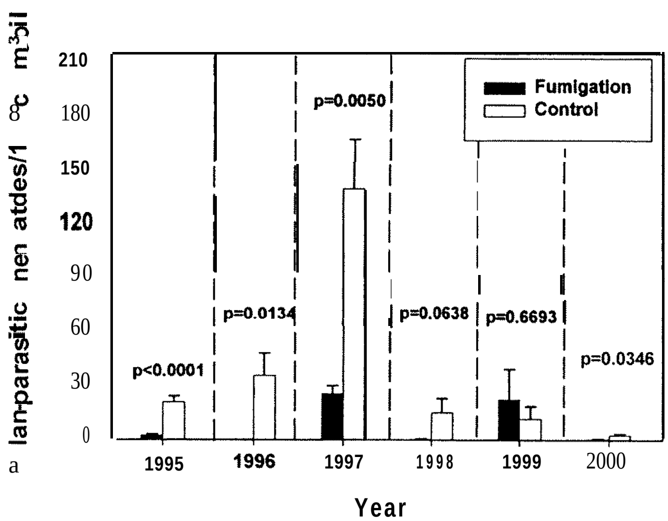
				Trichoderma spp.	
Time period	Treatment	*Pythium* spp.	*Fusarium* spp.	Type A*	Type B†
1998 (May)	Fumigation	0	0	22	31 996
	Control	22	840	1654	945
	p value	0.0756	0.0007	0.0010	0.0001
1998 (Oct.)	Fumigation	0	191	212	10 857
	Control	29	2209	1193	598
	p value	0.0833	0.0009	0.0139	0.0004
1999 (Dec.)	Fumigation	14	486	892	5 184
	Control	28	1322	2135	148
	p value	0.2066	0.0393	0.0186	0.0065
2000 (Dec.)	Fumigation	14	627	21	1 820
	Control	22	753	752	172
	p value	0.5490	0.2994	0.0038	0.0038

Note: Plots were fumigated with a mixture of 67% methyl bromide and 33% chloropicrin in 1995, 1996, 1997, and 1998 but were not refumigated in 1999 and 2000. *p* values represent the probability that treatment means within a year do not differ. Treatment means with $p < 0.1$ were considered to be significantly different. Means are based on six replications. Data was recorded as CFUs/gram dry mass soil.

*Isolates were predominately *Trichoderma hamatum*.

†Isolates were predominately *Trichoderma harzianum*.

Fig. 4. Population densities of plant-parasitic nematodes (per 100 cm’ soil) in fumigated and control treatments during evaluations from 1995 through 2000. Treatment means for 1995 are derived from eight monthly samples pooled from May to December, and in 1996, from four monthly samples pooled from May to August. Data for 1997 through 2000 are from one collection time when seedlings were lifted. Lines above bars represent one standard error of the mean. The *p* values for differences between treatments within a year are presented above the bars. Treatment means with $p < 0.1$ were considered to be significantly different.



petition from nutsedge was one likely cause for the smaller loblolly pine seedlings in nonfumigated plots, although we cannot fully explain why slash pine was not as affected by the nutsedge. The density of nutsedge plants was slightly greater in nonfumigated plots with loblolly pine compared with plots with slash pine in 1996 (20.0 and 12.5 plants/ 0.093 m^2, respectively), and therefore, nutsedge may have had a greater impact on loblolly pine. Similarly, the lack of treatment differences with respect to the size of loblolly pine seedlings beginning in 1998 may have been related to the dieback of nutsedge that occurred in this year.

No definite explanation can be offered for the decline of nutsedge that occurred in 1998. Personnel at the nursery indicated that they used fomesafen and oxyfluorfen operationally in all pine beds in 1998 as they had in previous years. According to its label, fomesafen can control yellow nutsedge but apparently not purple nutsedge. Effects of this herbicide on nutsedge had not been observed before 1998 in our study area, although nursery personnel indicated that the herbicide had been effective for nutsedge control in other fields. Oxyfluorfen was the only other herbicide reportedly used in this field, and its label does not list nutsedge among the weeds controlled by this herbicide.

Most weeds, with the exception of nutsedge, were controlled in nonfumigated plots by operational herbicide applications. Herbicides and other control measures are also available for managing nutsedge in southern pine nurseries (Hodges 1960; Rowan 1961; South 1979, 1984; Fraedrich et al. 2003), but at present, these alternative practices are underutilized and possibly not fully developed at many nurseries because of their reliance on regularly scheduled fumigation with methyl bromide. More effort is currently needed to integrate alternative practices for nutsedge control into nursery integrated pest management programs.

Increases in plant size following fumigation have been attributed to various factors other than weed control and include the elimination of soilborne pathogens (Hodges 1962*a*; Smith et al. 1989), increases in populations of beneficial soilborne microorganisms (Ingestad and Nillson 1964), and increases in availability of soil nutrients such as manganese (Alexander 1967) and nitrogen (Munnecke and Ferguson 1960; Rovira 1976; Millhouse and Munnecke 1979). In the present study, seedling mortality or evidence of root disease was rarely observed. Soil manganese concentrations were periodically greater in soil and foliage of seedlings in fumigated plots. Foliar nitrogen and phosphorus were also significantly greater for seedlings in the fumigated plots in some years. Fumigation with methyl bromide also consistently increased the relative abundance of *T. harzianum* in soil and its association with roots. However, *Trichodermu* spp., primarily composed of *T. hamatum*, were also common in soil and on roots of seedlings in nonfumigated plots. Fumigation with methyl bromide is known to change the microflora composition of soil and increase the abundance of *Trichodermu* spp. (Danielson and Davey 1969; Vaartaja 1967). Species of *Trichodermu* have been widely recognized as potential biocontrol agents (Papavizas 1985; Samuels 1996), and members of this genus can influence plant growth (Bailey and Lumsden 1998). The possible involvement of these fungi in the control of soilborne seedling diseases needs to be examined. Additional controlled studies are necessary to assess the influence of nutsedge, soil nutrients, and the soilborne mycoflora on pine seedling growth.

The lower density of loblolly pine seedlings in nonfumigated plots during 1996 may have been due to preemergence damping-off. The difference between treatments was evident within several weeks after seed sowing, but dead or dying seedlings were not observed, indicating that the losses occurred before or during germination. Historically, damping-off has not been as serious a problem at nurseries in the southern U.S.A. as elsewhere (Foster 1956, 1964). Guidelines (Hodges 1962*a*) for controlling this disease are generally practiced by managers. In addition, seeds used by the nursery are routinely treated with a thiram-based seed treatment, and captan is applied as a soil drench at the time of sowing. Both treatments are thought to be effective for controlling damping-off (Hodges 1962*a*). No evidence of damping-off occurred in subsequent years, and at this time, we do not understand the exact cause for this type of loss.

Root diseases have been cited as a primary reason for fumigation in southern pine nurseries. Charcoal root rot, caused by *Mucrophominu phaseolinu,* and a similar disease known as black root rot, caused by a complex of *M. phaseolina* and *F. oxysporum,* have been major problems in the past (Hodges 1962b; Smith et al. 1989). Although *F. oxysporum* was isolated routinely from roots of pine seedlings in the present study, *M. phaseolinu* was rarely encountered in soil or on roots (S.W. Fraedrich, unpublished data). Much has been learned about the biology of *F. oxysporum* over the last 30-40 years, and its routine isolation from roots of apparently healthy pine seedlings is now of questionable importance when assessing the potential for disease development. *Fusarium oxysporum* is commonly isolated from asymptomatic roots of crop plants, and nonpathogenic strains of *F. oxysporum* can be aggressive colonizers of the root cortex of plants (Gordon and Martyn 1997). Pathogenic and nonpathogenic isolates of *F. oxysporum* have been associated with roots of conifer seedlings in western North American nurseries (Bloomberg 1976; James et al. 1991), but research is needed to evaluate the pathogenicity and importance of this fungus in southern pine nurseries.

Numerous species of plant-parasitic nematodes have been found in southern nurseries (Hopper 1958), and some may pose a serious threat to pine seedling production (Ruehle 1973). Although there were generally more plant-parasitic nematodes in nonfumigated plots, their population densities remained at low levels and did not increase over time. Root-knot nematodes were found in nonfumigated plots in 1997, and some *Meloidogyne* spp. can infest roots of southern pines (Ruehle and Sasser 1962; Donaldson 1967); however, symptoms of nematode damage were not observed. Purple and yellow nutsedge are also known hosts of root-knot nematodes (Bird and Hogger 1973), and the presence of these nematodes in nonfumigated plots may have been linked with the presence of nutsedge. Ring nematodes were routinely found in nonfumigated plots, but their effect on southern pine seedlings is not known.

According to Hodges (1962*a*), root diseases generally become increasingly severe when several consecutive crops are grown in the same area. The present study was designed to encourage soilborne diseases by repeatedly growing pine crops in the same location. However, evidence of root disease in nonfumigated soils has been only rarely noted in the present study and other recent studies (Carey 1996, 1998; Barnard et al. 1997; South et al. 1997; Fraedrich and Dwinell 2003). One notable exception has been the association of a *Longidorus* sp. with loblolly pine seedlings in an adjacent field at the nursery where the present study was conducted (Fraedrich and Cram 2002). This problem may have resulted from continuous production of seedlings in portions of this field from 1990 to 2001 (Cram et al. 2003). Presently, many nursery managers rarely grow more than two successive pine crops in a field before rotating to cover crops. The use of specific cover crops or bare fallowing for 1-2 years after seedling production may prevent the buildup of pathogen populations (Fraedrich et al. 2002; Juzwik and Testa 1991; Hamm and Hansen 1990). Fertilization and water management regimes may also greatly influence the capability of fungi such as *M. phaseolina* and *F. oxysporum* to cause disease (Rowan 197 1; Hodges 1962*b*), and management practices in the past may have favored the development of root diseases in some nurseries (Barnard 1997). Over the last four decades, much has been learned about seedling production, and better production practices have been developed. These advancements may be important for the reduction of seedling stresses and the subsequent reduction in seedling susceptibility to many of the opportunistic, soilborne, pathogenic fungi that caused disease in the past.

Regardless of the reasons for the present lack of soilborne diseases, a better understanding is needed of soilborne pathogenic fungi and plant-parasitic nematodes and the risks that they pose for pine seedling production in the southern U.S.A. Many nurseries in the southern U.S.A. have likely fumigated on a schedule since their establishment without specific knowledge of soilborne diseases that may affect production at their location. Not all nurseries have the same pest problems, and some may be able to extend their current fumigation schedules. The establishment of unfumigated control plots in operational fields could provide information on the benefits of fumigation as well as the pest problems at individual nurseries. A better understanding of the risk of pest problems and the development of comprehensive integrated pest management programs may enable nursery managers to reduce pest control costs in the future.

Acknowledgements

The authors sincerely thank Johnny Branan, Wayne Belflower, and Greg Seabolt of the Georgia Forestry Commission for their help and advice in establishing the study; Tony Blalock, Jeff Fields, Susan Best, and Daniel Stuber for field and laboratory assistance; Dr. Jack Sutherland, Dr. Edward Barnard, Dr. George Kuhlman, and Dr. Kerry Britton for comments and suggestions on an earlier draft of the manuscript; and Michelle Cram for her comments and suggestions on a later version.

References

Alexander, M. 1967. Introduction to soil microbiology. John Wiley & Sons Inc., New York.

Bailey, B.A., and Lumsden, R.D. 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.

Barnard, E.L. 1997. Disease of seedlings in forest tree nurseries in Florida: history and status. In Proceedings of the Third Meeting of the IUFRO Working Party (Diseases and Insects in Forest Tree Nurseries), 19-24 May 1996, Orlando-Gainesville, Fla., U.S.A. *Edited by* R.L. James. USDA For. Serv. For. Health Protect. Rep. 97-4. pp. 13-21.

Barnard, E.L., Kannwischer-Mitchell, M.E., Mitchell, D.J., and Fraedrich, S.W. 1997. Development and field performance of slash and loblolly pine seedlings produced in fumigated nursery seedbeds and seedbeds amended with organic residues. *In* Proceedings of the Third Meeting of the IUFRO Working Party (Diseases and Insects in Forest Tree Nurseries), 19-24 May 1996, Orlando-Gainesville, Fla., U.S.A. *Edited* by R.L. James. USDA For. Serv. For. Health Protect. Rep. 97-4. pp. 86-91.

Bird, G.W., and Hogger, C.H. 1973. Nutsedges as hosts of plant-parasitic nematodes in Georgia cotton fields. Plant Dis. Rep. 57: 402.

Bloomberg, W.J. 1976. Distribution and pathogenicity of *Fusarium oxysporum* in a forest nursery soil. Phytopathology, 66: 1090-1092.

Carey, W.A. 1996. Testing alternatives to methyl bromide fumigation in southern forest tree nurseries. In National Proceedings: Forest and Conservation Nursery Associations, 25-27 June 1996, Gatlinburg, Tenn. *Edited by* T.D. Landis and D.B. South. USDA For. Serv. Gen. Tech. Rep. PNW-GTR-389. p. 28.

Carey, B. 1998. Loblolly seedling production, soil fungi and nematodes in the first two crops after fumigation at the Texas Supertree Nursery. Auburn University Southern Forest Nursery Management Cooperative, Auburn, Ala. Res. Rep. 98-3.

Cram, M.M., Fraedrich, S.W., and Fields, J. 2003. Stunting of southern pine seedlings by a needle nematode (*Longidorus* sp.). In National Proceedings: Forest and Conservation Nursery Associations — 2002, 16-18 July 2002. Gainesville, Fla. *Edited by* L.E. Riley, K.K. Dumroese, and T.D. Landis. U.S. Dep. Agric. For. Serv. Rocky Mt. Res. Stn. Gen. Tech. Rep. P-28. pp. 26–30.

Davey, C.B. 1972. Nursery soil management. *In* Proceedings, Southeastern Area Forest Tree Nurserymen's Conference, 7-10 August 1972, Wilmington, NC. USDA For. Serv. State and Private Forestry, Atlanta, Ga. pp. 182-l 87.

Danielson, R.M., and Davey, C.B. 1969. Microbial recolonization of a fumigated nursery soil. For. Sci. 15: 368–380.

Donaldson, ES. 1967. *Meloidogyne javanica* infesting *Pinus elliottii* seedlings in Florida. Plant Dis. Rep. 51: 455-456.

Environmental Protection Agency. 1999. Protection of stratospheric ozone: incorporation of Montreal Protocol adjustment for a 1999 interim reduction in Class I, Group VI controlled substances. Federal Register, **64**(104): 29 240 – 29 245.

Foster, A.A. 1956. Diseases of the forest nurseries of Georgia. Plant Dis. Rep. 40: 69–70.

Foster, A.A. 1964. Old and new disease problems in southern forest nurseries. In Proceedings: Region 8 Forest Nurserymen's Conferences, 19-20 August 1964, Morganton, N.C. *Edited by* E. Belcher. USDA Forest Service, Macon, Ga. pp. 89-91.

Fraedrich, S.W., and Cram, M.M. 2002. The association of a *Longidorus* species with stunting and root damage of loblolly pine (*Pinus taeda* L.) seedlings. Plant Dis. 86: 803–807.

Fraedrich, S.W., and Dwinell, L.D. 2003. An evaluation of dazomet incorporation methods on soilborne organisms and pine seedling production in southern nurseries. South. J. Appl. For. **27**(1): 41-51.

Fraedrich, S.W., and Smith, R.S., Jr. 1994. Soil fumigation in southern forest tree nurseries: current status and future needs for pest management. *In* Proceedings of the Second Meeting of the IUFRO Working Party S2.07.09 (Diseases and Insects in Forest Nurseries). 3-10 October 1993, Dijon, France. *Edited by* R. Perrin and J.R. Sutherland. Institut National de la Recherch Agronomique (INRA), Paris, France. (Les Colloques, n 68). pp. 267-282.

Fraedrich, S.W., Cram, M.M., and Handoo, Z.A. 2002. Host range and distribution of a *Longidorus* sp. associated with stunted loblolly pine seedlings. Phytopathology, 92: S26. [Abstr.]

Fraedrich, S.W., Dwinell, L.D., and Cram, M.M. 2003. Broadcast applications of glyphosate control nutsedge at a south Georgia forest tree nursery. South. J. Appl. For. 27. In press.

Gordon, T.R., and Martyn, R.D. 1997. The evolutionary biology of *Fusarium oxysporum.* Annu. Rev. Phytopathol. 35: 11 I-128.

Hamm, P.B., and Hansen, E.M. 1990. Soil fumigation, cover cropping, and organic soil amendments: their effect on soilborne pathogens and the target seedling. In Target Seedling Symposium: Proceedings, Western Forest Nursery Association, 13–17 August 1990, Roseburg, Oreg. *Edited by* R. Rose, S.J. Campbell, and T.D. Landis. USDA For. Serv. Gen. Tech. Rep. RM-200. pp. 174–180.

Hodges, C.S. 1960. Effect of soil fumigation in the nursery on growth of loblolly pine seedlings and control of weeds. Tree Planters' Notes, 42: 23-27.

Hodges, C.S., Jr. 1962*a*. Diseases in southeastern forest nurseries and their control. USDA For. Serv. Stn. Pap. SE-142.

Hodges, C.S., Jr. 1962*b*. Black root rot of pine seedlings. Phytopathology, 52: 210-219.

Hooper, D.J. 1986. Extraction of free-living stages from soil. In Laboratory methods for work with plant and soil nematodes. *Edited by* J.F. Southey. Ministry of Agriculture, Fisheries and Food, London. Ref. Book 402. pp. 5–30.

Hopper, B.E. 1958. Plant-parasitic nematodes in soils of southern forest nurseries. Plant Dis. Rep. 42: 308-314.

Ingestad, T., and Nillson, H. 1964. The effects of soil fumigation, sucrose application and inoculation of sugar fungi on growth of forest tree seedlings. Plant Soil, 20: 74-84.

James, R.L., Dumroese, R.K., and Wenny, D.L. 1991. Fusarium diseases of conifer seedlings. *In* Proceedings of the First Meeting of IUFRO Working Party S2.07-09, 22-30 August 1990, Victoria, B.C., Canada. *Edited hy* J.R. Sutherland and S.G. Clover. Forestry Canada, Victoria, B.C. pp. 181-190.

Juzwik, J., and Testa, F. 1991. Effect of cover crops on *Cylindrocladium* sp. in an Ontario bare-root nursery. Can. J. For. Res. 21: 724–728.

Kannwischer-Mitchell, M.E., Barnard, E.L., Mitchell, D.J., and Fraedrich, S.W. 1994. Organic amendments as a potential alternative to methyl bromide for control of soilborne pathogens in forest tree nurseries. *In* National Proceedings, Forest and Conservation Nursery Associations. 11-14 July 1994, Williamsburg, Va. *Edited* by T.D. Landis and R.K. Dumroese. USDA For. Serv. Gen. Tech. Rep. RM-GTR-257. pp. 12–15.

Little, T.M., and Hills, F.J. 1978. Agricultural experimentation. John Wiley & Sons Inc., New York.

McCain, A.H., and Smith, R.S., Jr. 1972. Quantitative assay of *Macrophomina phaseolina* from soil. Phytopathology, 62: 1098.

Middleton, J.T. 1943. The taxonomy, host range, and geographic distribution of the genus *Pythium*. Mem. Torrey Bot. Club, 20: 1-171.

Millhouse, D.E., and Munnecke, D.E. 1979. Increased growth of *Nicotiana glutinosa* as partially related to accumulation of ammonium-nitrogen in soil fumigated with methyl bromide. Phytopathology, 69: 793-797.

Munnecke, D.E., and Ferguson, J. 1960. Effect of soil fungicides upon soil-borne plant pathogenic bacteria and soil nitrogen. Plant Dis. Rep. 44: 552-555.

Nelson, P.E., Toussoun, T.A., and Marasas, W.F.O. 1983. *Fusarium* species. The Pennsylvania State University Press, University Park, Pa.

Papavizas, G.C. 1985. *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. Annu. Rev. Phytopathol. 23: 23-54.

Rifai, M.A. 1969. A revision of the genus *Trichoderma.* Mycol. Pap. 116: 1-56.

Rovira, A.D. 1976. Studies on soil fumigation. I. Effects on ammonium, nitrate, and phosphate in soil and on the growth, nutrition and yield of wheat. Soil Biol. Biochem. 8: 241-247.

Rowan, S.J. 1961. The effectiveness of EPTAM in controlling weeds in southern forest nurseries. Tree Planters' Notes, 48: 29-32.

Rowan, S.J. 1971. Soil fertilization, fumigation, and temperature affect severity of black root rot of slash pine. Phytopathology, **61: 184-187.**

Ruehle, J.L. 1973. Nematodes and forest trees — types of damage to tree roots. Annu. Rev. Phytopathol. **11: 99-118.**

Ruehle, J.L., and Sasser, J.N. 1962. The role of plant-parasitic nematodes in stunting of pines in southern plantations. Phytopathology, 52: 56-68.

Samuels, G.J. 1996. *Trichoderma:* a review of biology and systematics of the genus. Mycol. Res. **100: 923-936.**

SAS Institute Inc. 2000. The SAS system for windows, version 8.01. SAS Institute Inc., Cary, N.C.

Singleton, L.L., Mihail, J.D., and Rush, C.M. 1992. Methods for research on soilborne phytopathogenic fungi. APS Press, St. Paul, Minn.

Smith, R.S., Jr., Cordell, C.E., and Hodges, C.S. 1989. Charcoal root rot and black root rot. *In* Forest nursery pests. *Technical Coordinators:* C.E. Cordell, R.L. Anderson, W.H. Hoffard, T.D. Landis, R.S. Smith, Jr., and H.V. Toko. U.S. Dep. Agric. Agric. Handb. 680. pp. 112-113

South, D.B. 1979. Integrated weed management in southern forest nurseries. In Proceedings of the Southern Nursery Conference, 24-27 July 1978, Hot Springs, Ark. USDA For. Serv. Southeastern Area Tech. Pub. SA-TP-6. pp. 138-148.

South, D. 1984. Weed control. In Southern pine nursery handbook. *Edited by* C.W. Lantz. USDA Forest Service, Atlanta, Ga. Chap. 15.

South, D.B., Carey, W.A., and Enebak, S.A. 1997. Use of chloropicrin as a soil fumigant in pine nurseries. In Proceedings of the Third Meeting of the IUFRO Working Party (Diseases and Insects in Forest Tree Nurseries), 19-24 May 1996, Orlando–Gainesville, Fla. *Edited by* R.L. James. USDA Forest Service, Northern Region, USDA For. Serv. For. Health Protect. Rep. 97-**4.** pp. 70–73.

Steel, R.G.D., and Torrie, J.H. 1980. Principles and procedures of statistics: a biometrical approach. McGraw-Hill Book Co., New York.

Vaartaja, O. 1967. Reinfestation of sterilized nursery seed beds by fungi. Can. J. Microbiol. 13: 771-776.

Van der Plaats-Niterink, A.J. 1981. Monograph of the genus *Pythium*. Studies in mycology. Centradlbureau Voor Schimmelcultures, Baarn, Netherlands.