Detection and Management of Stunt and Stubby-Root Nematodes in a Southern Forest Nursery

Michelle M. Cram
Stephen W. Fraedrich

Michelle M. Cram is a Plant Pathologist, Forest Health Protection, USDA Forest Service, Athens, GA 30602-2044; Tel: 706.559.4233; E-mail: mcram@fs.fed.us. **Stephen W. Fraedrich** is a Research Plant Pathologist, Southern Research Station, USDA Forest Service, Athens, GA 30602-2044; E-mail: sfraedrich@fs.fed.us.

In: Riley, L. E.; Dumroese, R. K.; Landis, T. D., tech. coords. 2007. National proceedings: Forest and Conservation Nursery Associations—2006. Proc. RMRS-P-50. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. Online: http://www.rngr.net/nurseries/publications/proceedings

Abstract: Populations of stunt (*Tylenchorhynchus claytoni*) and stubby-root (*Paratrichodorus minor*) nematodes, as well as predaceous nematodes (*Mylonchulus* spp., *Mononchus* spp.), were monitored in 2005 for 8 months in three loblolly pine fields at a southern forest nursery. The fields were selected based on their 2004 management regimes and consisted of one that was fall fumigated with a combination of 67% methyl bromide and 33% chloropicrin, another that had been in a sorghum cover crop, and a third that had been used for longleaf pine production. In April 2005, the populations of the nematodes were higher in fields that had sorghum or pine than in the fumigated field. By December, the stunt nematodes had increased dramatically in some sections of the fumigated field and the field that had sorghum. Populations of the stubby-root nematode remained under 47/100 cc soil in all fields. The predacious nematode populations did not get above 40/100 cc soil and their effect on the plant-parasitic nematode populations remains undetermined. Host range tests for the stunt and stubby-root nematodes found that the fallow treatment yielded the lowest number of stunt nematodes, followed by a pearl millet (*Pennisetum americanum*) cover crop.

Keywords: nematode, stunt, stubby-root, *Tylenchorhynchus claytoni*, *Paratrichodorus minor*, *Pinus taeda*, pine, cover crops, host range, sorghum, rye, pearl millet, fallow

Introduction

The symptoms of nematode damage on tree seedlings in forest nurseries are often nonspecific and can resemble many other factors responsible for malfunctioning root systems (Ruehle 1973). In a forest nursery where seedlings receive adequate water and fertilizer, seedlings damaged by nematodes are often smaller and only occasionally off-color. These symptoms can be attributed to other factors (for example, poor fertility, seasonal effects, and so on), and, consequently, can go undiagnosed. Many southern forest tree nurseries use soil fumigation with methyl bromide and chloropicrin before every other pine crop. Fumigation is known to control nematode populations (Johnson and Feldmesser 1987), but the population densities can rebound and damage the second production crop (Sipes and Schmitt 1998; Fraedrich and Dwinell 2003). The use of non-host and poor host cover crops, as well as fallow, has been promising for assisting nursery managers in controlling a needle nematode (*Longidorus americanus*) and a stunt nematode (*Tylenchorhynchus ewingi*) (Fraedrich and others 2003; Cram and Fraedrich 2005; Fraedrich and others 2005). Forest nursery managers need more specific information on the host range of other nematodes in order to develop better pest management programs.

A southern forest nursery has had problems with smaller loblolly pine (*Pinus taeda*) seedlings in some fields during the second year of production following fumigation. The cause for the stunting was not known, and the manager was not aware of a problem with nematodes. The manager has been able to treat fields with extra fertilizer to grow seedlings to a merchantable size.

A survey to monitor soilborne pests was initiated at the nursery in April 2005 in three fields of loblolly pine. The first sampling revealed the presence of the stunt nematode, *Tylenchorhynchus claytoni,* and a stubby-root nematode, *Paratrichodorus* *minor*. Host range tests using common nursery cover crops were initiated for both of these nematode species.

Materials and Methods

Survey

Three fields of loblolly pine were sampled for fungi and nematodes. The soil texture of the three fields was a loamy sand. Each field had different management histories with respect to the sequence of crops grown and the year of fumigation. The fumigant used at the nursery was MC33 (methyl bromide 67%/chloropicrin 33%), which has traditionally been applied in the fall. The year of fumigation and crops produced for the 3 fields prior to the 2005 loblolly pine crop were as follows:

Field 1—Fall fumigated in 2004 (fumigated field);
Field 2—Fall fumigated in 2003, longleaf pine crop in 2004 (pine field); and
Field 3—Fall fumigated in 2001, two consecutive pine crops, and sorghum cover crop in 2004 (sorghum field).

Second-generation, rust-resistant loblolly pine (Piedmont) seeds were sown in the fumigated field, and second-generation, bulk collected loblolly pine (Piedmont) seeds were sown in the pine and sorghum fields. Three sections (delimited by risers) within each field were divided in half for a total of six sample areas (18 sample areas, total). A composite soil sample was taken from each sample area that consisted of nine cores taken systematically to a 15 cm (6 in) depth.

Soil samples were taken 4 April (prior to sowing), 6 June, 15 August, and 6 December 2005. The April soil samples were tested for nematodes, *Fusarium* spp., *Pythium* spp., and *Trichoderma* spp. Stunt and stubby-root nematodes were isolated and identified by Dr Zafar Handoo (ARS Nematology Laboratory, Beltsville, MD) as *Tylenchorhynchus claytoni* and *Paratrichodorus minor*, respectively. Predaceous nematodes (*Mylonchulus* spp., *Mononchus* spp.) were also found. Soil samples were taken again in June and August in order to evaluate the nematode populations over time. The June and December soil samples were also checked for *Pythium* spp. and *Fusarium* spp.

Nematodes were extracted from the soil with the centrifugal-flotation method modified by using 10 and 325 mesh soil screens (Jenkins 1964). Komada's (Komada 1975) and PARP (Kannwischer and Mitchell 1981) agar media were used to isolate *Fusarium* spp. and *Pythium* spp., respectively. PDA-TAR with 1 ml tergitol was used for isolation of *Trichoderma* spp. (Kannwischer-Mitchell and others 1994). Soils were assessed within 3 to 5 days after collection. Soil was mixed by hand and 10 g (0.4 oz) diluted 1 to 200 for *Pythium* and *Fusarium* spp., and 1 to 600 for *Trichoderma* spp. One ml of the appropriate soil dilution was placed on each of five plates for each medium. Plates were incubated at 25 °C (77 °F) 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 from the April sampling was potted and planted with 10 stratified loblolly pine seeds for each composite soil sample. The pots were placed in a growth chamber at 25 °C (77 °F) with a 12-hour photoperiod. Seedlings with symptoms of damping-off were washed in sterile water and placed on water agar. Cultures were observed after 2 to 10 days and the fungi identified.

Three seedling counts (2 ft^2 [0.6 m^2]) were systemically performed in the center row of each sample area at the end of the growing season. Five seedlings were lifted from each of nine locations within each sample area (45 seedlings/sample area), and 15 seedlings were selected randomly from each area to determine seedling diameter at the root collar, and shoot and root dry weights. Seedlings were clipped at the root collar, and roots and shoots dried at 80 °C (176 °F) for 48 hours before determining dry weights.

Nematode Host Range

Host range tests were conducted to evaluate the suitability of cover crops used in the South for stunt (*T. claytoni*) and stubby-root (*P. minor*) nematodes. The cover crops tested for the stunt nematode were sorghum-sudan (*Sorghum bicolor* 'SG Ultra'), rye (*Secale cerale*'Elbon'), corn (*Zea mays* 'Roundup Ready'), ryegrass (*Lolium multiflorum* 'TAM 90'), oats (*Avena sativa* 'Mora'), pearl millet (*Pennisetum americanum* 'ET-300'), and brown top millet (*Panicum ramosum* 'DW-01'). The same cover crops were tested for the stubby-root (*P. minor*) nematode, except corn and oats were replaced by buckwheat (*Fagopyrum esculentum* 'Mancan') and the 'Tiff' cultivar of pearl millet. Loblolly pine and bare fallow treatments were also included as controls in each test.

Soil with a loamy sand texture was microwaved for 8 minutes in 2000 g (70.5 oz) batches, and containers were filled with 1600 cc (98 in^3) of soil. There were four replications (containers) of each species, and five plants were established in each container (except the fallow containers). The stunt and stubby-root nematodes were extracted from stock cultures using a Baermann funnel method (Shurtleff and Averre 2000). Each treatment container was infested with 1,000 nematodes. Containers were placed in a growth chamber at 25 °C (77 °F) with a 15-hour photoperiod. Population densities were determined after 12 weeks for stunt nematodes, and after 18 weeks for stubby-root nematodes. Nematodes closely associated with roots were extracted by placing roots in approximately 1 l (0.3 gal) of water for 15 minutes, and then extracting nematodes on a 325 mesh screen. These nematodes were then placed in the soil, which was mixed well before removing 100 cc (6 in^3) of soil for determination of nematode population densities. Nematodes were extracted from soil samples using the centrifugal flotation method (Jenkins 1964). Roots were dried for 48 hours at 80 °C (176 °F) and dry weights subsequently determined.

Results

Surveys

Plant-parasitic nematodes were rarely found in any sample area of the fumigated field prior to sowing, but were common in many areas of the sorghum and pine fields (figure 1). Most nematodes in the sorghum and pine fields were located in sections along roads. This pattern of nematode occurrence

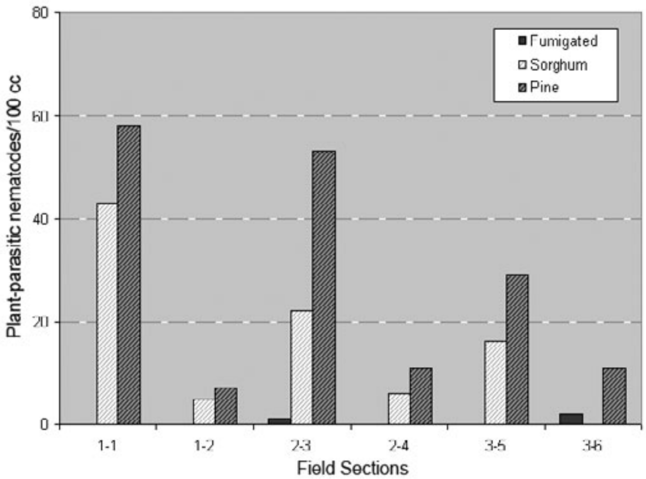

Figure 1—Plant parasitic nematode population densities by sampling areas in three fields prior to sowing loblolly pine (6 April 2005).

was consistent throughout the sampling period for these two fields (table 1).

The stunt nematode, *T. claytoni*, increased in all fields over time with the greatest gains in the sorghum field, followed by the fumigated and pine fields (figure 2A). The stubby-root nematode, *P. minor,* was primarily found in the pine field and did not increase from April to December (figure 2B). Predaceous nematodes were also isolated from the soil samples and were originally found only in the sorghum and pine fields (figure 2C). However, by August, the predaceous nematodes were found in the fumigated field. The predaceous nematodes did not increase as populations of stunt nematodes increased.

Table 1—Stunt and stubby-root nematode population densities by field and section on 6 December 2005.

Field	Section	Stunt nematodes per 100 cc soil	Stubby-root nematodes per 100 cc soil
Fumigated	1–1	136	12
	1–2	131	15
	2–3	235	14
	2–4	219	7
	3–5	5	8
	3–6	32	4
Sorghum	1–1	435	0
	1–2	159	1
	2–3	395	6
	2–4	41	24
	3–5	231	3
	3–6	33	3
Pine	1–1	148	23
	1–2	6	20
	2–3	91	37
	2–4	2	47
	3–5	29	22
	3–6	1	9

(100 cc = 6 in^3)

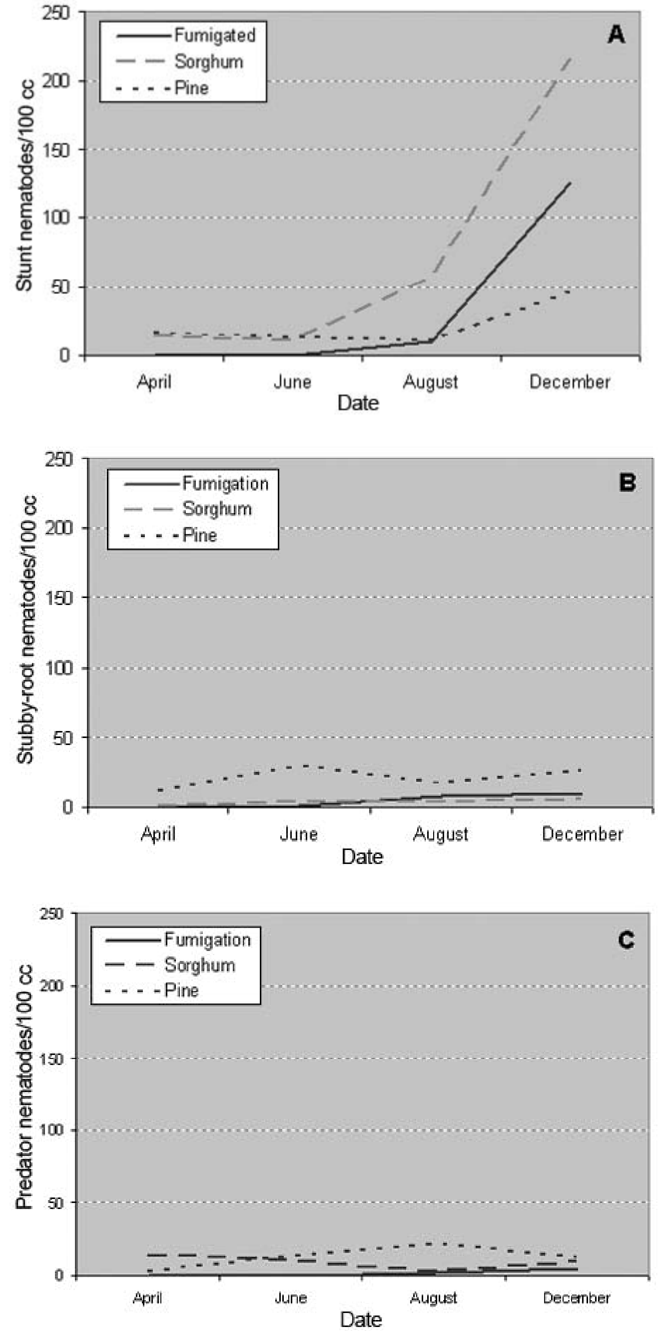

Figure 2—Population densities from April to December in three loblolly pine fields for: A) stunt nematode; B) stubby-root nematode; and C) predator nematodes.

The baiting test consisting of germinating loblolly pine seedlings placed in containers with soil from the fumigated, sorghum, and pine fields resulted in damping-off of 8.3%, 6.7%, and 11.7% of the seedlings respectively. *Pythium* was associated with the damping-off of five seedlings in the fumigated field. A *Rhizoctonia*-type sp., *Fusarium proliferatum*, *F. oxysporum*, and *Pythium* were associated with the damped-off seedlings in the pine field. *Fusarium proliferatum* and *Pythium* were associated with the damping-off in the sorghum field.

A check of the *Pythium* cfu/g of soil in June determined that *Pythium* was scattered in a few areas of all fields (figure 3A). The population levels of *Fusarium* did not vary much among the 3 fields prior to sowing (figure 4A). In December, the soilborne *Fusarium* populations were greater in some sections of the sorghum and pine fields than in the fumigated field (figure 4B). The average population densities of *Trichoderma* prior to planting were greater in the fumigated soil (34,952 cfu/g soil) than the sorghum (2,239 cfu/g soil) or pine (2,168 cfu/g soil) fields.

The average number of seedlings was greater in the fumigated field (25/ft^2 [277/m^2]), than in sorghum (20.5/ft^2 [228/m^2]) or pine (19/ft^2 [211/m^2]) fields, in December 2005. Seedlings in the sorghum field had smaller root collar diameters (3.94 mm) root (0.90 g [0.03 oz]) and shoot weights (2.75 g [0.09 oz]) compared to seedlings in the fumigated field (4.22 mm, 1.07 g [0.04 oz], 3.54 g [0.12 oz], respectively), which may be partially due to the different populations of stunt nematodes early in the growing season (figure 2A). Seedlings in the pine field received two additional applications of fertilizer in

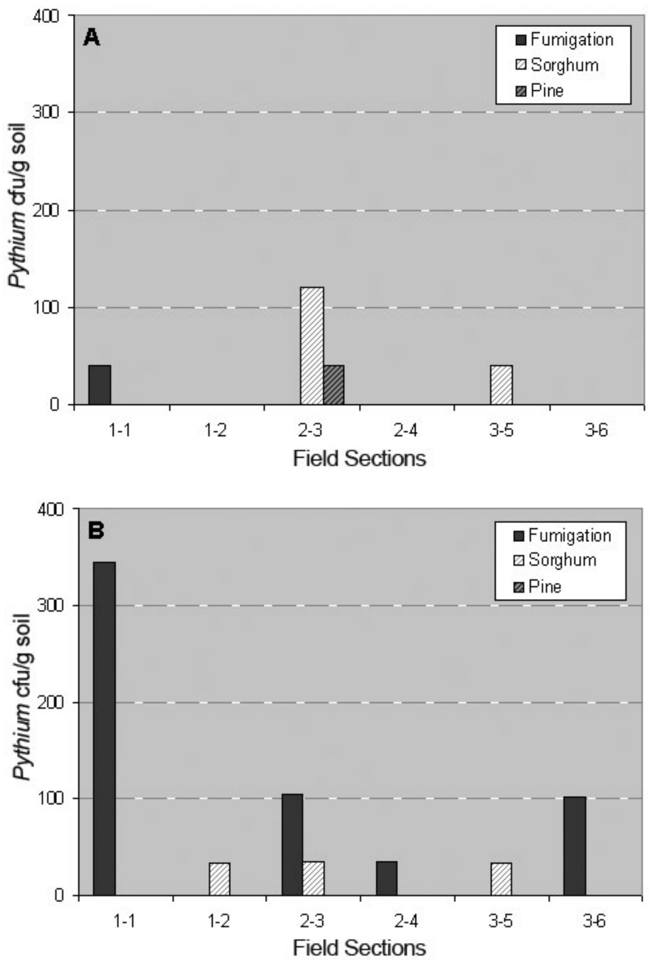

Figure 3—*Pythium* cfu/g of soil in three loblolly pine fields: A) on 6 June 2005; and B) on 6 December 2005.

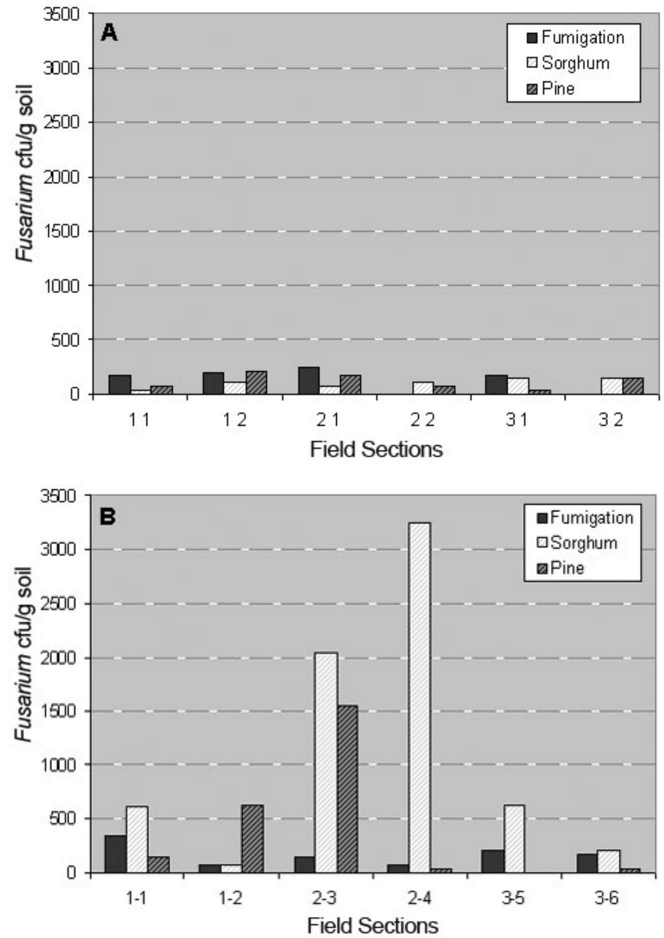

Figure 4—*Fusarium* cfu/g of soil in three loblolly pine fields: A) on 6 April 2005; and B) on 6 December 2005.

August at 100 lb/ac (112 kg/ha) each, and seedling root collar diameters (4.27 mm), and root (1.24 g [0.04 oz]) and shoot (3.39 g [0.12 oz]) dry weights were comparable to seedlings in the fumigated field.

Host Range Tests

An evaluation of common cover crops as hosts for the stunt nematode found that pearl millet was the poorest host, followed by brown top millet (table 2). The fallow treatment had the lowest number of stunt nematodes. The two cover crops used by the nursery, rye grain and sorghum sudan grass, were excellent hosts for the stunt nematode.

In a similar evaluation of cover crops as hosts for the stubby-root nematode, the two varieties of pearl millet tested appeared to be non-hosts for this nematode, and results did not significantly differ from the fallow treatment. Again, rye grain and sorghum sudan grass cover crops were found to be excellent hosts for the stubby-root nematode. (table 3).

Table 2—Population densities of stunt nematodes in containers with various cover crops 12 weeks after infestation with 1,000 stunt nematodes/container (1600 cc [98 in^3]).

Plant species	Stunt nematodes per 100 cc soil[a]	
Rye grain ('Elbon')	7,393	a
Loblolly pine	6,753	a
Corn ('Roundup Ready')	3,545	ab
Sorghum sudan ('Ultra')	1,905	bc
Oats ('Mora')	1,478	c
Rye grass ('TAM-90')	949	c
Brown top millet ('DW01')	319	d
Pearl millet ('ET-300')	148	d
Fallow	35	e

[a] Means followed by the same letter do not differ significantly (alpha = 0.05) according to Tukey's HSD test. Logarithmic transformation of nematode counts performed before analysis. Data analyzed as a randomized complete block design (100 cc = 6 in^3).

Table 3—Population densities of stubby-root nematodes in containers with various cover crops 18 weeks after infestation with 1000 nematodes/container (1600 cc [98 in^3]).

Plant species	Stubby-root nematodes per 100 cc soil[a]	
Sorghum sudan ('Ultra')	8,498	a
Rye grain ('Elbon')	1,536	b
Loblolly pine	1,111	b
Brown top millet ('DW01')	670	b
Buckwheat ('Mancan')	334	bc
Rye grass ('TAM-90')	68	c
Pearl millet ('Tiff')	4	d
Pearl millet ('ET-300')	0	d
Fallow	1	d

[a]Means followed by the same letter do not differ significantly (alpha = 0.05) according to Tukey's HSD test. Logarithmic transformation of nematode counts performed before analysis. Data analyzed as a randomized complete block design (100 cc = 6 in^3).

Discussion

The stunt nematode, *T. claytoni*, and stubby-root nematode, *P. minor*, are known parasites of loblolly pine (Ruehle 1966), and both of these nematodes have been associated with stunting of loblolly pine when populations are high (Hopper 1958; Ruehle 1969). The level of stubby-root nematodes remained relatively low in our survey. The stunt nematode increased dramatically in some sections of the fields. Hopper (1958) observed severely stunted loblolly pine seedlings in association with *T. claytoni* population densities of 315 to 422/100 cc (52 to 69/1 in^3) of soil, and some sections of the sorghum field reached those levels by December. Our sampling, however, was done systematically over a large area and did not focus on individual spots of damaged seedlings. This type of sampling can be used to follow general populations over time, but will not determine the number of nematode clusters or the cluster populations. The seedling response to specific numbers of nematodes cannot be determined by this survey. Comparing the average seedling measurements between fields is further confounded by variations among fields with respect to seed sources, planting times, and fertilization.

The survey results indicate that plant parasitic nematodes were present at sowing in the sorghum and pine fields, and the potential for damping-off based on the greenhouse assays was similar in all three fields at sowing. The potential for nematodes to interact with fungi and cause damping-off and root disease in loblolly pine is presently unknown. There have been some experiments with other tree species that found a nematode-fungal complex causing damage (Ruehle 1973). Controlled studies are needed to determine if interactions between stunt nematodes and fungi are a threat to production of loblolly pine.

The pattern of nematode occurrence in the sorghum and pine fields indicates that the nematodes occurred at greater frequency in the sample areas adjacent to roads and is probably influenced by the movement of nematodes with nursery equipment. Detection of incipient populations of plant-parasitic nematodes may be increased when sampling in areas where equipment most often enters a field.

The increase in populations of the stunt nematode from August to December could be due to a number of factors. The most obvious factor is the higher volume of root systems during these months. Temperature may also play a role in seasonal fluctuations. Other research on *T. claytoni* populations in eastern North Carolina has also noted that populations are lower in the summer and peak in the winter (Baker and others 1969). The impact of predaceous nematodes on the stunt and stubby-root nematode populations in the present survey is unclear; however, the increased densities of the stunt nematode from August to December indicate that predators present in the fields were incapable of suppressing this species.

The nursery had been alternating sorghum and grain rye with pine crops, all of which are hosts for the stunt and stubby-root nematodes found in this survey. Continuously growing crops that are hosts for a nematode can lead to damaging populations (Dropkin 1989; Cram and others 2003). Although fumigation will depress nematode populations (Dropkin 1989), they can rebound and significantly impact subsequent seedling crops (McKenry and Thomason 1976; Fraedrich and Dwinell 2003). The use of fallow or alternating hosts with non-hosts can help managers control plant-parasitic nematodes (Vargas-Ayala and Rodriguez-Kabana 2001; Fraedrich and others 2005). In the host range tests, population densities of the stubby-root and stunt nematodes declined with fallow treatment or a cover crop of pearl millet. These results are similar to the host range tests for the stunt nematode, *T. ewingi* (Cram and others 2003).

The application of cultural controls, such as fallow and cover crops, requires understanding the survivability of a pest in the absence of a host. The survivability of stunt and stubby-root nematodes in fallow soil and on pearl millet are currently being evaluated over time.

References

Baker KR, Nusbaum CJ, Nelson LA. 1969. Seasonal population dynamics of selected plant-parasitic nematodes as measured by three extraction procedures. Journal of Nematology 1:232-239.

Cram MM, Fraedrich SW. 2005. Management options for control of a stunt and needle nematode in southern forest nurseries. In: Riley

LE, Dumroese RK, Landis TD, technical coordinators. National proceedings: forest and conservation nursery associations—2004. Ogden (UT): USDA Forest Serv ice, Rocky Mountain Research Station. Proceedings RMRS-P-35. p 46-50.

Cram MM, Fraedrich SW, Fields J. 2003. Stunting of southern pine seedlings by a needle nematode (*Longidorus* sp.). In: Riley LE, Dumroese RK, Landis TD, technical coordinators. National proceedings: forest and conservation nursery associations—2002. Ogden (UT): USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-28. p 26-30.

Dropkin VH. 1989. Introduction to plant nematology. 2nd ed. New York (NY): Wiley and Sons Inc. 304 p.

Fraedrich SW, Cram MM, Handoo ZA. 2003. Suitability of southern pines, other selected crops, and nutsedge to a *Longidorus* sp. associated with stunting of loblolly pine seedlings. Plant Disease 87:1129-1132.

Fraedrich SW, Dwinell LD. 2003. Effect of dazomet, metam sodium and oxamyl on *Longidorus* populations and loblolly pine seedling production. Southern Journal of Applied Forestry 29(3):117-122.

Fraedrich SW, Cram MM, Zarnoch SJ. 2005. The effect of fallow on *Longidorus americanus*, a nematode associated with stunting of loblolly pine seedlings in Georgia, USA. Nematology 7:487-493.

Hopper BE. 1958. Plant-parasitic nematodes in soils of southern forest nurseries. Plant Disease Report 42:308-314.

Jenkins WR. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

Johnson AW, Feldmesser J. 1987. Nematicides—a historical review. In: Veech JA, Dickson DW, editors. Vistas on nematology: a commemoration of the twenty-fifth anniversary of the Society of Nematologists. Hyattsville (MD): Society of Nematologists. P 448-454.

Kannwischer ME, Mitchell DJ. 1981. Relationships of numbers of spores of *Phytophthora parasitica* var. *nicotianae* to infections and mortality of tobacco. Phytopathology 71:69-73.

Kannwischer-Mitchell ME, Barnard EL, Mitchell DJ, and Fraedrich SW. 1994. Organic soil amendments as potential alternatives to methyl bromide for control of soilborne pathogens in forest tree nurseries. In: Landis TD, Dumroese RK, technical coordinators. National proceedings: forest and conservation nursery associations. Fort Collins (CO): USDA Forest Service, Rocky Mountain Forest and Range Experiment Station. General Technical Report RM-GTR-257. p 12-15.

Komada H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Review of Plant Protection Research 8:114-25.

McKenry MV, Thomason IJ. 1976. Dosage values obtained following pre-plant fumigation for perennials. II. Using special methods of applying methyl bromide and 1,3-dichloropropene nematicides. Pesticide Science 7:535-544.

Ruehle JL. 1966. Nematodes parasitic on forest trees I. Reproduction of ectoparasites on pine. Nematologica 12:443-447.

Ruehle JL. 1969. Influence of stubby-root nematode on growth of southern pine seedlings. Forest Science 15:130-134.

Ruehle JL. 1973. Nematodes and the forest trees—types of damage to tree roots. Annual Review of Phytopathology 11:99-118.

Shurtleff MC, Averre CW III. 2000. Diagnosing plant diseases caused by nematodes. St Paul (MN): APS Press. 187 p.

Sipes BS, Schmitt DP. 1998. Nematode interactions. In: Barker KR, Pederson GA, Windham GL, editors. Plant nematode interactions. Madison (WI): USA Publishers. p 173-185.

Vargas-Ayala R, Rodriguez-Kabana R. 2001. Bioremediative management of soybean nematode population densities in crop rotations with velvetbean, cowpea, and winter crops. Nematropica 31:37-46.