Stunt Nematode (*Tylenchorhynchus claytoni*) Impact on Southern Pine Seedlings and Response to a Field Test of Cover Crops

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Abstract: The stunt nematode, *Tylenchorhynchus claytoni*, was found to cause a reduction in root volume (cm^3) of loblolly pine at population densities equivalent of 125 nematodes/100 cm^3 (6 in^3) soil and greater. The results of a host range test conducted in containers under controlled conditions determined that buckwheat cultivar (*Fagopryum esculentum* 'Mancan'), velvetbean (*Mucuna pruriens*), Kobe lespedeza (*Lespedeza striata* 'Kobe'), bicolor lespedeza (*Lespedeza bicolor*), and purple nutsedge (*Cyperus rotundus*) are suitable hosts for the stunt nematode. Previous container studies concluded that pearl millet (*Pennisetum glaucum*) hybrid 'ET-300' was a nonhost for the stunt nematode. A 2-year field test of sorghum-sudangrass hybrid 'Sugar Graze,' pearl millet hybrid 'Tifleaf 3,' and fallow found that the use of pearl millet as a cover crop greatly restricts population development of the stunt nematode in infested fields, and its use would be a good alternative to fallow.

Keywords: nematode, stunt, stubby-root, *Tylenchorhynchus claytoni*, *Paratrichodorus minor*, *Pinus taeda*, pine, cover crops, sorghum-sudangrass, pearl millet, fallow, *Fagopyrum esculentum*, *Mucuna pruriens*, *Lespedeza striata*, *Lespedeza bicolor*, *Cyperus rotundus*

Introduction

Loblolly (*Pinus taeda*) and slash pine (*P. elliottii*) are known hosts for the stunt nematode, *Tylenchorhynchus claytoni* (Ruehle 1966), and stunting of loblolly pine seedlings has been associated with high population densities of this nematode (Hopper 1958; Ruehle 1969). However, the only southern pine species that has been shown to be stunted by this nematode is longleaf pine (*P. palustris*) (Ruehle 1973). The population densities at which *T. claytoni* can damage loblolly and slash pine seedlings remains a basic gap in our understanding of this nematode and the impact it has on pine seedling production.

Cover crops are alternated with tree seedlings in southern forest tree nurseries for maintaining organic matter and soil stabilization, as well as other benefits (Boyer and South 1984). In the southern United States, sorghum-sudangrass, corn, rye, and oats have been common cover crops in forest nurseries (Boyer and South 1984). Unfortunately, these cover crops are hosts for the stunt nematode *T. claytoni* (Cram and Fraedrich 2007). Based on host range tests conducted under controlled conditions, hybrid pearl millet (*Pennisetum glaucum*) 'ET-300' cultivar from the East Texas Seed Company (Tyler, TX) appeared to be a nonhost for *T. claytoni* (Cram and Fraedrich 2007). The search for other nonhosts and the testing of common cover crops used by southern nursery managers, as well as a noxious weed (purple nutsedge [*Cyperus rotundus*]), is continued in this paper. The growth chamber tests provide a practical way of screening species for host status, but field testing is necessary to determine crop performance and nematode population changes over time under field conditions.

Materials and Methods

Pathogenicity

The effect of *T. claytoni* population density on loblolly and slash pine seedlings was evaluated in a growth chamber experiment. Containers were filled with approximately 400 cm^3 (24 in^3) of a loamy sand soil that was microwaved in 2,000-g

(70.5-oz) batches for 8 minutes. Containers were planted with five germinating loblolly pine seeds. Nematodes were reared on roots of loblolly pine seedlings and subsequently extracted with Baermann funnels (Shurtleff and Averre 2000). Nematodes were added to containers at rates of 0, 500, 1,000, 2,000, and 4,000 individuals/container, and there were four replications of each nematode dose. Containers were placed in growth chambers at 25 °C (77 °F) with a 14-hour photoperiod and watered every 1 to 3 days, as needed. After 10 weeks, plants were removed from the containers and placed in tap water for 15 to 30 minutes to remove soil and nematodes from plant roots. These nematodes were washed back into the soil sample using a 325-mesh screen, and soil samples were mixed thoroughly. Roots were placed in plastic bags and stored at 6 °C (43 °F). Root volume (cm^3) was calculated by WinRHIZO Version 2003b scanning system (Regent Instruments, Quebec, Canada). Nematodes were extracted from 100 cm^3 (6 in^3) of soil using the centrifugal-flotation method (Shurtleff and Averre 2000). The relationship between the initial *T. claytoni* dose and root volumes were determined by regression analysis using a nonlinear, negative exponential model. The analysis was conducted using the regression analysis package of SigmaPlot®, Version 8.0 (SYSTAT, San Jose, CA). The criteria for fit of the model were based on the mean square error (MSE), r-square values, and the significance of the overall regression.

Host Suitability

Host suitability was evaluated for loblolly pine, buckwheat (*Fagopryum esculentum* 'Mancan'), velvetbean (*Mucuna pruriens*), Kobe lespedeza (*Lespedeza striata* 'Kobe'), bicolor lespedeza (*Lespedeza bicolor*), and purple nutsedge (*Cyperus rotundus*). A fallow treatment was also included. Soil was treated as previously described and each container held about 1,600 cm^3 (98 in^3) of the loamy sand soil. In each experiment, four containers for each plant species were sown. Stunt nematodes were produced as previously described, extracted with Baermann funnels, and 500 nematodes were added to each container. Containers were placed in a growth chamber at 25 °C (77 °F) with a 14-hour photoperiod for 12 weeks. Plants were removed from the containers and placed in water for 15 to 30 minutes to separate the soil and nematodes from the roots. Nematodes were extracted from the water using a 325-mesh screen, washed back into the soil sample, and soil samples were thoroughly mixed. Nematodes were extracted from 100 cm^3 (6 in^3) of soil using the centrifugal-flotation method (Shurtleff and Averre 2000). Statistical comparisons were conducted among treatments on the final population densities of nematodes by an ANOVA and Tukey's HSD test. Data were transformed with the log10$^{(x+1)}$ transformations prior to analysis, but only non-transformed values are presented in tables.

Field Cover Crop Trial

The site selected for a field trial was surveyed in 2005 and found to be infested with the stunt nematode *T. claytoni* (Cram and Fraedrich 2007). The soil type of the field was a sandy loam soil in the Wagram Sand soil series. The field had been fumigated in the fall of 2004 with methyl bromide (67%) and chloropicrin (33%), then sown with loblolly pine seeds in 2005. In spring 2006, a test was established of sorghum-sudangrass hybrid 'Sugar Graze,' pearl millet hybrid 'Tifleaf 3,' and fallow treatments. Each treatment had five blocks. 'Tifleaf 3' was selected because this cultivar was readily available to the nursery, and the nursery was switching from sorghum-sudangrass to the pearl millet and was using this cultivar operationally. A previous test found this cultivar was not a host of the stubby-root nematode *Paratrichodorus minor*, and we hypothesized that 'Tifleaf 3' would also prove to be a nonhost for the stunt nematode *T. claytoni*.

The checkerboard pattern of plots was created by dividing the field into three 3-m (10-ft) widths by ten 15.3-m (50-ft) lengths and leaving sections between the treatment plots as fallow buffers (fig. 1). Sorghum-sudangrass and pearl millet were sown on 25 April 2006 and again on 4 May 2007. The sowing rate was 33.6 kg/ha (30 lb/ac) for sorghum-sudangrass and 16.8 kg/ha (15 lb/ac) for pearl millet. The study area was watered with approximately 2.5 cm (1 in) of water per week for 12 weeks. One application of granular ammonium nitrate at a rate of 57.2 kg/ha (51 lb/ac) of N was applied after 6 weeks. Fomesafen sodium (Reflex®) and lactofen (Cobra®) were each applied at 2.3 L/ha (1 qt/ac) in fallow areas at sowing. Glyphosate (Gly-4 Plus) was added as a 5% solution as needed during the growing season.

Soil samples were obtained in April (prior to sowing), May, June, September, and November each year. The soil was systematically sampled from the center of each treatment
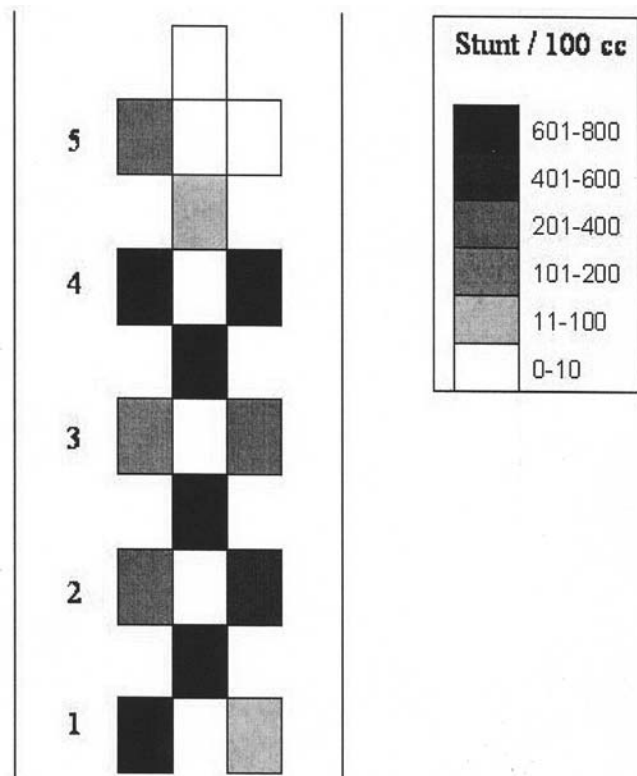

Figure 1. Field diagram of stunt nematode populations by plot in April 2006.

plot and consisted of six cores taken to a 15 cm (6 in) depth. Soil samples from each plot were composited and nematodes were extracted from a 100 cm^3 (6 in^3) subsample using the centrifugal-flotation method (Shurtleff and Averre 2000). The percentage of organic matter for soil samples collected in April 2007 and 2008 was determined using the Dumas combustion elemental analysis at the University of Georgia Institute of Ecology, Soil Biology Laboratory (Athens, GA).

Statistical comparisons were conducted on the nematode population densities between cover crop treatments by an ANOVA using the PROC GLM procedure of SAS® software (SAS Institute Incorporated, Cary, NC), and mean separation was performed by Tukey's HSD test. Prior to analysis, block 5 was removed due to low initial nematode population. Data were transformed with the square root (x + ½) transformations prior to analysis, but only nontransformed values are presented in graphs.

Results

Loblolly and slash pine root volume decreased with respect to the initial populations of the stunt nematode *T. claytoni* (fig. 2). The relative fit of the negative exponential model, based on the R^2 values and MSEs, was slightly better for loblolly pine (MSE = 0.0027; R^2 = 0.92) than slash pine (MSE = 0.0073; R^2 = 0.82). Initial population densities as low as 500 nematodes per 400 cm^3 (25 in^3) soil (125/100 cm^3 [6 in^3]) reduced the root volume of both pine species. The level of damage was similar for all doses of the stunt nematode.

Host Range Tests

All crops and purple nutsedge were hosts for the stunt nematode *T. claytoni* (table 1). Only the fallow treatment had a lower population of stunt nematodes than the original inoculum of 500 nematodes. In all crop and weed treatments,
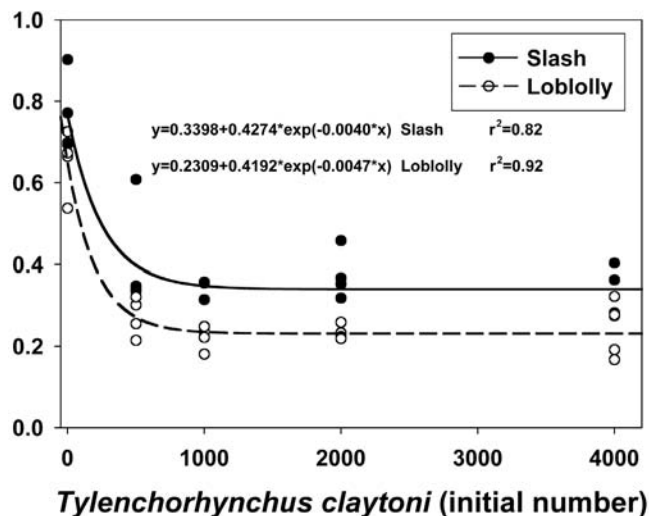
Table 1. Population densities of stunt nematodes in containers with various crop and weed species 12 weeks after infestation with 500 stunt nematodes/container (1600 cm^3 [98 in^3] soil).

Plant species	Stunt nematodes per 100 cm^3 (6 in^3) soil[a]		Stunt nematodes per container[+]	
Buckwheat 'Mancan'	798	a	12760	a
Velvetbean	615	a	9840	a
Loblolly pine	318	a	5080	a
Kobe lespedeza	243	a	3880	a
Bicolor lespedeza	159	a	2540	a
Purple nutsedge	135	a	2160	a
Fallow	5	b	80	b

[a]Non-tranformed means followed by the same letter do not differ significantly (α = 0.05) according to Tukey's HSD test. Logarithmic transformation of nematode counts performed before analysis.

the number of nematodes increased. No significant differences in stunt nematode population densities were observed among hosts.

Field Test

The stunt nematode *T. claytoni* was the predominant nematode species isolated from treatment plots in the field study. Some plots also had stubby-root nematodes, *Paratrichodorus minor*, at less than 10 individuals/100 cm^3 (6 in^3), and predacious nematodes (*Mylonchulus* spp., *Mononchus* spp.) at less than 15 individuals/100 cm^3 (6 in^3) soil. One other plant parasitic nematode, *Paratrichodorus porosus*, was found during the second year of the field test in the sorghum-sudangrass treatments only. Population densities of *P. porosus* were usually less than 50 nematodes/100 cm^3 (6 in^3) soil.

The population densities of the stunt nematode within plots at the time of sowing in April 2006 ranged from 0 to 788 individuals/100 cm^3 (6 in^3) soil. The stunt nematodes were not uniformly distributed among plots, and few to no nematodes were found in some treatment plots of block 5 (fig. 1).

Over a 2-year period, the average population densities of the stunt nematode decreased significantly in the fallow and pearl millet cover crop treatments (fig. 3). The population of stunt nematodes in the fallow treatment fell below 100 individuals/100 cm^3 (6 in^3) soil by the end of the first year. The number of stunt nematodes within the pearl millet treatment did not fall below 100 individuals/100 cm^3 (6 in^3) soil until the second year. An examination of the average population densities of the stunt nematode in the sorghum-sudangrass over the 2-year study indicated nematode densities decreased during August and September sampling dates and increased greatly in the winter and spring.

The population densities of the stubby-root nematode *P. minor* were greater in the sorghum-sudangrass and pearl millet plots than in the fallow, although the densities remained under 100 nematodes/100 cm^3 (6 in^3) soil during the 2 years (fig. 4). Population densities of predacious nematodes remained low (0.5 to 17.5 predators/100 cm^3 [6 in^3]) throughout the 2-year study and did not appear to be affected by season.

Figure 2. Relationship between initial population of stunt nematode (*T. claytoni*) and root volume (cm^3) of seedlings after 10 weeks.

y=0.3398+0.4274*exp(-0.0040*x) Slash r^2=0.82

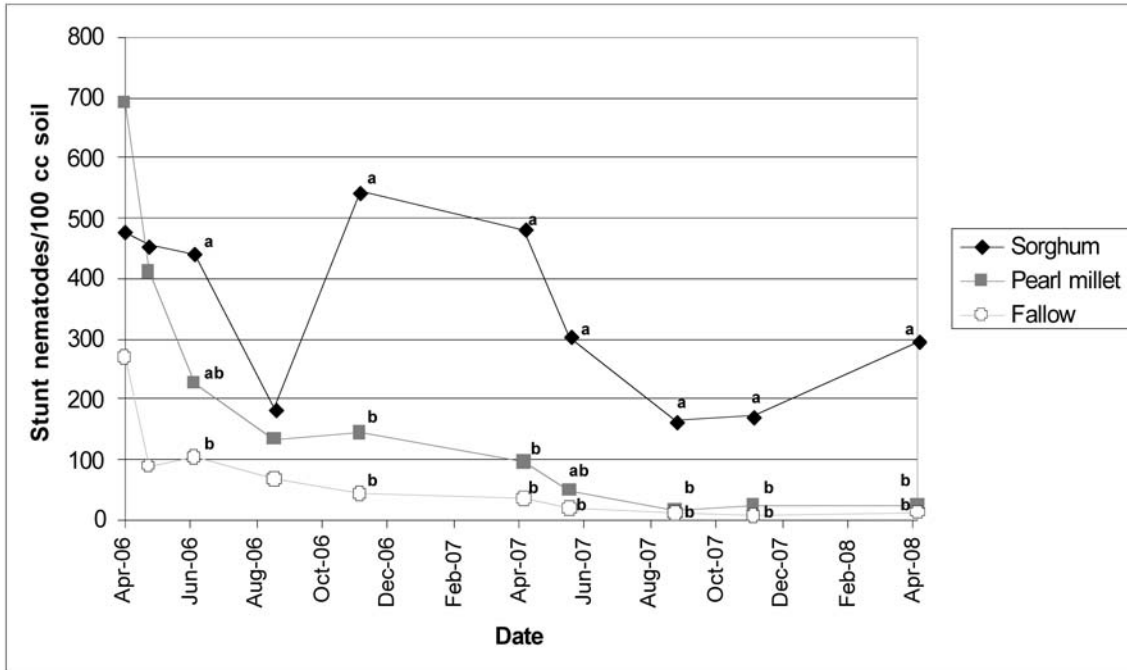y=0.2309+0.4192*exp(-0.0047*x) Loblolly r^2=0.92

Figure 3. Relationship between stunt nematode (*T. claytoni*) population densities and cover crop treatment over 2 years. Data was transformed by square root of (x + ½); treatments in block 5 were removed from analysis due to low initial nematode population. Data points followed by different letters by date are significantly different using Tukey's HSD test (α = 0.05).

Figure 4. Relationship between stubby-root nematode (*M. minor*) population densities and covercrop treatment over 2 years. Data transformed by square root of (x + ½); treatments in block 5 were removed from analysis due to low initial nematode population. Data points followed by different letters by date are significantly different using Tukey's HSD test (α = 0.05).
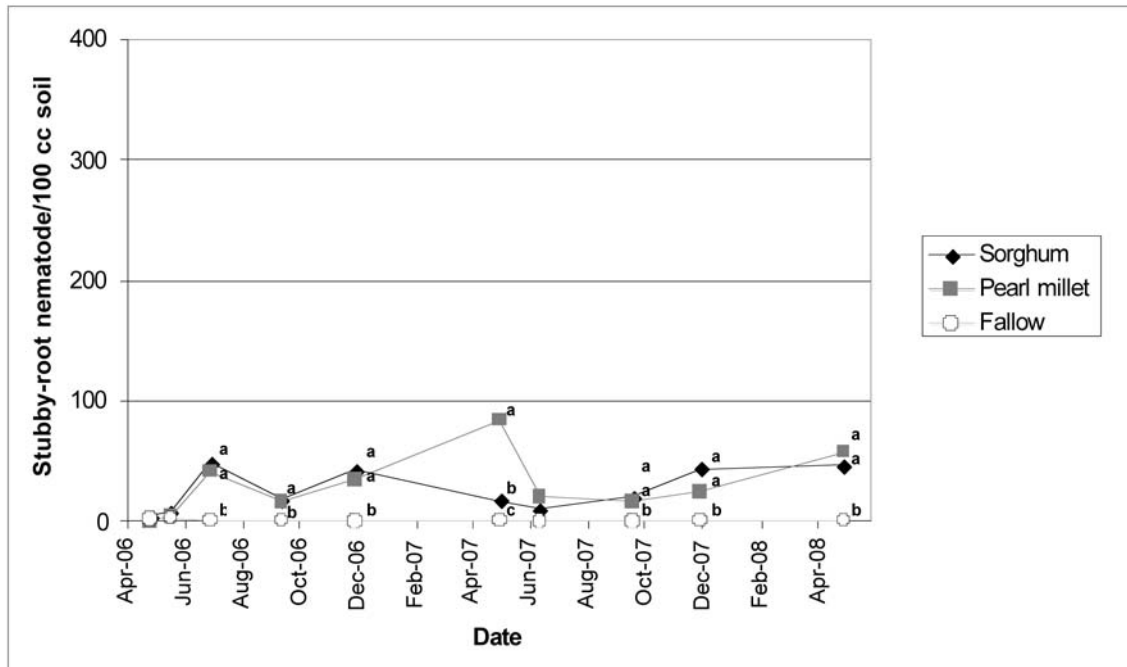
Table 2. Percentage of carbon as a measure of organic matter in soil by cover crop after the first and second year of treatment.

Cover crops	April 2007 carbon (%)[a]		April 2008 carbon (%)[a]	
Sorghum-sudangrass	0.79	ab	0.89	a
Pearl millet 'Tifleaf 3'	0.89	a	0.86	a
Fallow	0.57	b	0.51	b

[a]Means within columns followed by the same letter to not differ significantly ($\alpha = 0.05$) according to Tukey's HSD test.

Soil organic matter was similar for the sorghum-sudangrass and the pearl millet treatments by April of both years (table 2). Pearl millet significantly improved the percentage of organic matter in the soil as compared to the fallow treatment.

Discussion

The results of the dosage response trials indicate that the stunt nematode, *T. claytoni*, can directly cause stunting of loblolly and slash pine root systems when the nematode is present during seed germination and growth of young seedlings. The impact of the stunt nematode on loblolly and slash pine seedlings during the first 10 weeks in this study is similar to what occurs on these pine species in nursery beds (Hopper 1958; Ruehle 1973). The results of our pathogenicity test also suggest that the high densities of the stunt nematodes present in the field study on 25 April 2006 would probably have led to areas of seedling damage and losses had the nursery produced pine seedlings in the field.

Our host range test failed to identify a new nonhost of the stunt nematode, although some of the crops may be less favorable hosts than others. The buckwheat cultivar 'Mancan,' Kobe lespedeza, and bicolor lespedeza have been grown in some southern forest tree nurseries, and are now confirmed hosts for the stunt nematode. Our finding that purple nutsedge is a host for the stunt nematode adds to the list of common weeds that are hosts of *T. claytoni*. Other host weeds include: crabgrass (*Digitaria ischaemum* and *D. sanguinalis*), redwort pigweed (*Amaranthus retroflexus*), witchgrass (*Panicum capillare*), stinkgrass (*Eragrostis cilianensis*), carpet weed (*Molugo verticillata*), and dandelion (*Taraxacum officinale*) (Miller and Ahrens 1969).

The velvetbean species, *M. pruriens*, had shown some promise as a less favored host than sorghum-sudangrass for the stunt nematode in Florida (Crow and others 2001). Extracts from velvetbean stems and roots have also been shown to have nematicidal effects on a root-knot nematode when tested under laboratory conditions (Zasada and others 2006). Our results indicate that this species of velvetbean is a good host for the stunt nematode *T. claytoni*; however, we did not test the potential toxic effects of this species on nematode populations after plant parts are incorporated into the soil. Perhaps more research on velvetbean is warranted before it is entirely ruled out as a control option for fields infested with the stunt nematode.

The hybrid pearl millet, 'Tifleaf 3,' is not a host for the stunt nematode *T. claytoni* and appears to be a good alternative to fallow as a means to decrease nematode populations in fields. These field results mirror the container study with pearl millet 'ET-300' and fallow treatment (Cram and Fraedrich 2007). Other pearl millet cultivars have also been found to be resistant to plant-parasitic nematodes, including *P. minor*, *Meloidogyne* spp., *Belonolaimus longicaudatus*, and *Pratylenchus brachyurus* (Timper and others 2002; Timper and Hanna 2005).

The host test conducted under controlled conditions in a previous paper found that pearl millet 'ET-300' and 'Tiff' (abbreviated for 'Tifleaf 3') were nonhosts for the stubby-root nematode *P. minor*, and that sorghum-sudangrass was a preferred host (Cram and Fraedrich 2007). Our field test results for the stubby-root nematode in this study did not mirror the results of previous container studies. The low levels of stubby-root nematodes in the sorghum-sudangrass plots could be the result of many factors including less favorable environmental conditions and competition by stunt nematodes. In general, the densities of stubby-root nematodes in the field were too low to have expected substantial damage on loblolly or slash pine seedlings (Ruehle 1969).

The predacious nematodes (*Mylonchulus* spp., *Mononchus* spp.) appeared to have no impact on the stunt nematode population, as their densities did not change over time. The low population of predators and lack of effect on other nematodes has been noted in other studies that have monitored these nematodes (Ferris and others 1996; MacGuidwin and Layne 1995). Predacious nematodes are only one component of the organisms that control plant parasitic nematodes, and it is possible that they are neither effective, nor even suited, as predators of the stunt nematode.

The threshold population density of stunt nematodes that young loblolly and slash pine seedlings can tolerate without stunting remains unknown, but seedling size can be significantly reduced at 125 stunt nematodes/100 cm^3 (6 in^3), as indicated by the pathogenicity test. Although population levels of nematodes are lowered significantly by pearl millet in 1 year, it may take 2 years to get population densities sufficiently low that they will not damage pine seedlings. Nurseries that use a 2:1 rotation of seedling production to cover crops may be better off using fallow (or a combination of organic matter treatments and fallow). Unfortunately, stunt nematode populations in the fallow treatments were not reduced to zero in all plots over the 2 years of this study. Managers need to be aware that populations of the stunt nematode *T. claytoni* can explode quickly because its lifecycle is relatively short (31 to 38 days) (Wang 1971), and each female can produce 1 to 15 eggs (Krusberg 1959).

Fields that have stunt nematodes may not be able to have successive pine crops without the use of a fumigant before each crop. Other options depend on nursery land base and access to organic amendments. Managers may consider alternative cropping strategies, such as a 1:1 rotation of pine with fallow (including organic amendments if needed). Crop rotations with hardwoods, pine, and cover crops may also be possible. Associations between the stunt nematode and stunting of hardwood tree seedlings have not been well documented (Ruehle 1968). The only known nonhost hardwood species identified to date is sweetgum (*Liquidambar styraciflua*), while yellow poplar (*Liriodendron tulipifera*) is a very poor host (Ruehle 1971). Hardwoods tolerant to stunt nematodes could be used in a rotation with a pine crop (for example, a pine crop followed by summer fallow with weed control and fall planting of hardwoods). Further investigation

into nematode population response to hardwood crops over time may be required before implementing a hardwood/pine/cover crop rotation.

Acknowledgments

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