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PATHOGENICITY AND MANAGEMENT OF *TYLENCHORHYNCHUS CLAYTONI* IN SOUTHERN USA FOREST NURSERIES

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ABSTRACT

The stunt nematode, *Tylenchorhynchus claytoni*, has been a recognized problem in pine nurseries in the southern USA since the 1950's. Pathogenicity tests for *T. claytoni* on loblolly (*Pinus taeda*) and slash pine (*P. elliottii*) seedlings found that populations of 125 nematodes or greater per 100 cc soil caused a reductions in root volume. Host range tests of common cover crops found that sorghum-sudan grass (*Sorghum bicolor* 'SG Ultra'), rye (*Secale cereale* 'Elbon'), corn (*Zea mays* 'Roundup Ready'), ryegrass (*Lolium multiflorum* 'TAM 90'), oats (*Avena sativa* 'Mora'), buckwheat (*Fagopyrum 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. Pearl millet (*Pennisetum americanum* 'ET-300') and brown top millet (*Panicum ramosum* 'DW-01') were found to be poor hosts and had the lowest population densities of the stunt nematode among the cover crops. Only the fallow treatment performed better. A subsequent two 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 reduces the population densities of the stunt nematode in infested fields, and did not differ statistically from the fallow treatment after the first year.

INTRODUCTION

Loblolly (*Pinus taeda*) and slash pine (*P. elliottii*) are known hosts for the stunt nematode *Tylenchorhynchus claytoni* (Ruehle 1966). Although stunting of loblolly pine seedlings has been associated with high population densities of *T. claytoni* (Hopper 1958, Ruehle 1969), pathogenicity testing has only been performed on 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 the impact of this nematode.

In southern forest tree nurseries, cover crops are alternated with tree seedlings for maintaining organic matter and soil stabilization as well as a reduction of pests (Boyer and South 1984). The use of non-host and poor-host cover crops, as well as fallow, has shown promise for controlling a needle nematode (*Longidorus americanus*) and a stunt nematode (*Tylenchorhynchus ewingi*) in pine nurseries (Fraedrich and others 2003, Cram and Fraedrich 2005, Fraedrich and others 2005). Host-range testing may help forest nursery managers identify non- or poor-host cover crops to be used in a pest management program for *Tylenchorhynchus claytoni*. Growth chamber tests provide a practical and efficient means of

screening a wide range of species for host status, but field testing is necessary to determine crop performance and nematode population changes over time under operational conditions.

A southern forest nursery historically had problems with smaller loblolly pine seedlings in some fields during the second year of production following fumigation. A 2005 survey of a loblolly pine field in the first pine production following fumigation revealed *T. claytoni* increased from a field average of 0.17 (April) to 126 (December) per 100 cc soil. By April 2006 the population density of *T. claytoni* averaged 402 nematodes / 100 cc soil and ranged as high as 788 nematodes / 100 cc soil (Fig. 1). A cover crop field test was initiated in this field using pearl millet, a cover crop determined to be a very poor to non-host for *T. claytoni*.

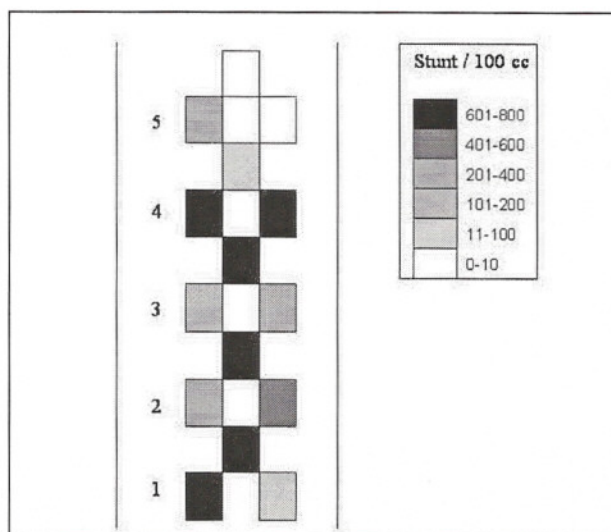


Figure 1. Field diagram of stunt nematode populations by plot in April 2006.

METHODS

Pathogenicity Test

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 cc of a loamy sand soil that was microwaved in 2000 g batches for eight 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 Avere 2000). Nematodes were added to containers at rates of 0, 500, 1000, 2000 and 4000 individuals/container, and there were four replications for each nematode dose. Containers were placed in growth chambers at 25°C 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 15-30 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. Root volume (cm³) was calculated by WinRHIZO Version 2003b scanning system. Nematodes were extracted from

100 cc of soil using the centrifugal-flotation method (Shurtleff and Averre 2000). The relationship between the initial *T. claytoni* dose and root volumes was determined by regression analysis using a nonlinear, negative exponential model. The analysis was conducted using the regression analysis package of SigmaPlot (Version 8.0). 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 Range Tests

Cover crops, typically used in the southern USA forest nurseries, were tested for their suitability as hosts for *T. claytoni*. The first host test included sorghum-sudan (*Sorghum bicolor* 'SG Ultra'), rye (*Secale cereale* '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 second host test included buckwheat (*Fagopyrum esculentum* 'Mancan'), velvetbean (*Mucuna pruriens*), Kobe lespedeza (*Lespedeza striata* 'Kobe'), and bicolor lespedeza (*Lespedeza bicolor*), as well as purple nutsedge (*Cyperus rotundus*); a common southern weed. Loblolly pine and bare fallow treatments were also included as controls in each test.

In each host test, soil with a loamy sand texture was microwaved for 8 minutes in 2000 g batches, and containers were filled with 1600 cc of soil. There were four replications (containers) for each treatment and 5 plants were established in each container (except the fallow containers). Stunt nematodes were extracted from stock cultures using a Baermann funnel method (Shurtleff and Averre 2000). In host test 1 and each treatment container was infested with 1,000 nematodes. In host test 2 containers were infested with 500 nematodes. Containers were placed in a growth chamber at 25°C with a 15 hr photoperiod. Stunt nematode population densities were determined after 12 weeks in both tests. Nematodes closely associated with roots were extracted by placing roots in approximately 1 liter 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 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 and dry weights subsequently determined.

The final population densities of nematodes were compared among treatments in each host range test using an ANOVA and Tukey's HSD test. Data were transformed with the $\log_{10}(x+1)$ transformations prior to analysis, but only nontransformed values are presented in tables.

Field Cover Crop Trial

The soil type for the field trial 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%) at a rate of 350lb/ac (392.9 kg/ha), and then sown with loblolly pine seeds in 2005. In the spring of 2006, a test was established with sorghum-sudangrass hybrid 'Sugar Graze', pearl millet hybrid 'Tifleaf 3', and fallow treatments. The 'Tifleaf 3' cultivar was selected because this cultivar was readily available to the nursery, and the nursery had just begun using this cultivar operationally.

Each treatment had five replications and the study was established as a randomized complete block design. The checkerboard pattern of plots was created by dividing the field into three 3 m widths by ten 15.24 m lengths and leaving sections between the treatment plots as fallow buffers (Fig. 1). Sorghum-sudangrass and pearl millet were sown on April 25, 2006 and again on May 4, 2007. The sowing rate was 33.6 kg/ha for sorghum-sudangrass and 16.8 kg/ha for pearl millet. The study area was watered with approximately 2.5 cm of water per week for 12 weeks. One application of granular ammonium nitrate at a rate of 57.16 kg/ha of N was applied after 6 weeks. Fomesafen sodium (Reflex®) and lactofen (Cobra®) were each applied at 2.3 l/ha in fallow areas at sowing. Glyphosate (Gly 4plus) 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 of each year. The soil was systematically sampled from the center of each treatment plot and consisted of 6 corings taken to a 15 cm depth. Composite soil samples were mixed and nematodes were extracted from a 100 cc sub-sample using the centrifugal-flotation method (Shurtleff and Averre 2000). The percent organic matter for soil samples collected in April 2007 and 2008 was determined using the Dumas combustion elemental analysis at the University of Georgia's Soil Biology Laboratory in the Institute of Ecology.

Nematode population densities were compared among cover crop treatments by an ANOVA using the PROC GLM procedure of SAS (the SAS System for Windows), and mean separation was performed by Tukey's HSD test. Block 5 was removed from the analysis due to low initial nematode population. Data were transformed with the square root ($x + \frac{1}{2}$) 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* (Figure 2). The relative fit of the negative exponential model, based on the R^2 values and MSE's, 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 cc soil (125/100 cc) greatly reduced the root volume of both pine species. The level of damage was similar for all doses of the stunt nematode.

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 1). All other crops and purple nutsedge were hosts for the stunt nematode *T. claytoni* (Tables 1 and 2). The fallow treatment had the lowest number of stunt nematodes in both tests.

Field Cover Crop Trial

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*, and predacious nematodes (*Mylonchulus* sp., *Mononchus* sp.). 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 per 100 cc soil.

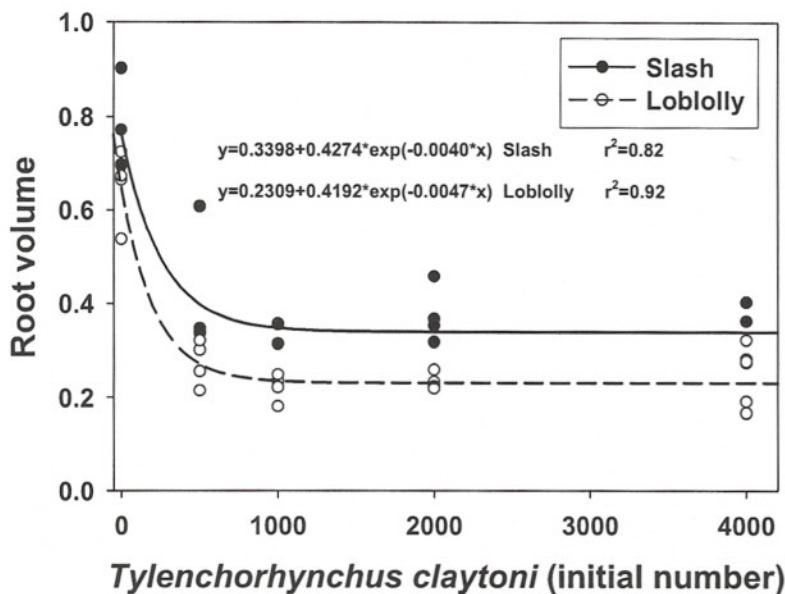


Figure 2. Relationship between initial population of stunt nematode (*T. claytoni*) and root volume (cm^3) of seedlings after 10 weeks.

Table 1. Population densities of stunt nematodes in containers with various cover crops 12 weeks after infestation with 1000 stunt nematodes/container (1600 cc).

Plant species	Stunt nematodes per 100 cc soil [†]
Rye grain ('Elbon')	7393 a
Loblolly Pine	6753 a
Corn ('Roundup ready')	3545 ab
Sorghum sudan ('Ultra')	1905 bc
Oats ('Mora')	1478 c
Rye grass ('TAM-90')	949 c
Brown Top Millet ('DW01')	319 d
Pearl Millet ('ET-300')	148 d
Fallow	35 e

[†]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

Table 2. Population densities of stunt nematodes in containers with various crop and weed species 12 weeks after infestation with 500 stunt nematodes/container (1600 cc soil).

Plant species	Stunt nematodes per 100 cc soil [†]		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

[†]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.

Over a two 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 cc 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 cc soil until April of the second year. An examination of the average population densities of the stunt nematode in the sorghum-sudangrass over the two year study indicated nematode densities decreased during August and September 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 cc soil during the two years (Fig. 4). Population densities of predacious nematodes remained very low (0.5 - 17.5 predators/100 cc) throughout the two year study and did not appear to be affected by season.

Soil organic matter was similar for the sorghum-sudangrass and the pearl millet treatments by April of both years (Table 3). Pearl millet significantly improved the percent organic matter in the soil as compared to the fallow treatment.

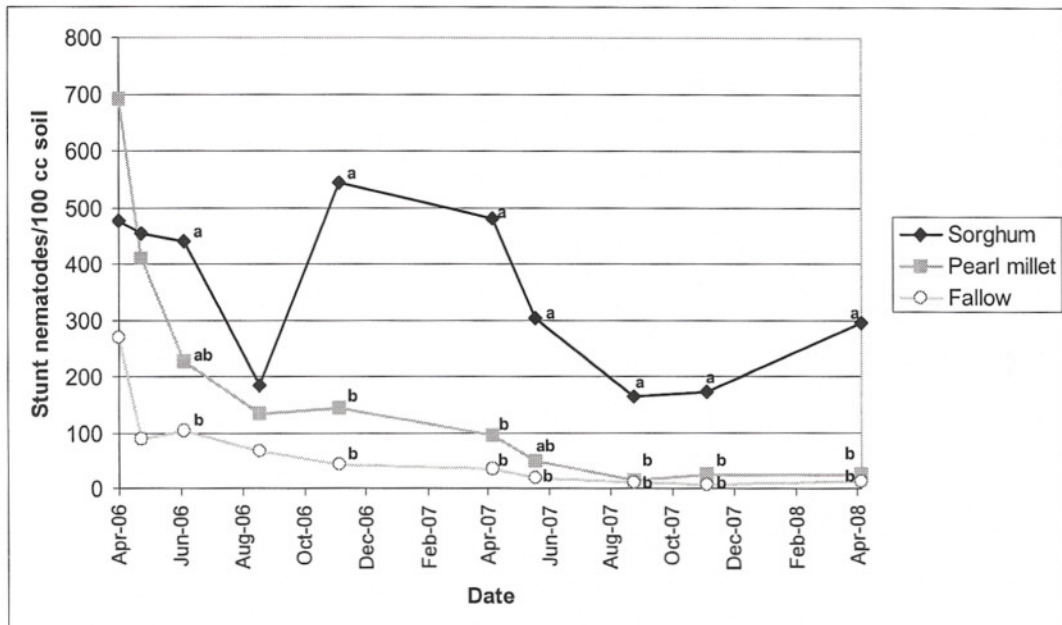


Figure 3. Relationship between stunt nematode (*T. claytoni*) population densities and cover crop treatment over 2 years. Means were transformed by square root of $(x + \frac{1}{2})$; treatments in block 5 were removed from analysis due to low initial nematode pop. Data points followed by a different letters by date are significantly different using Tukey's HSD test (Alpha 0.05).

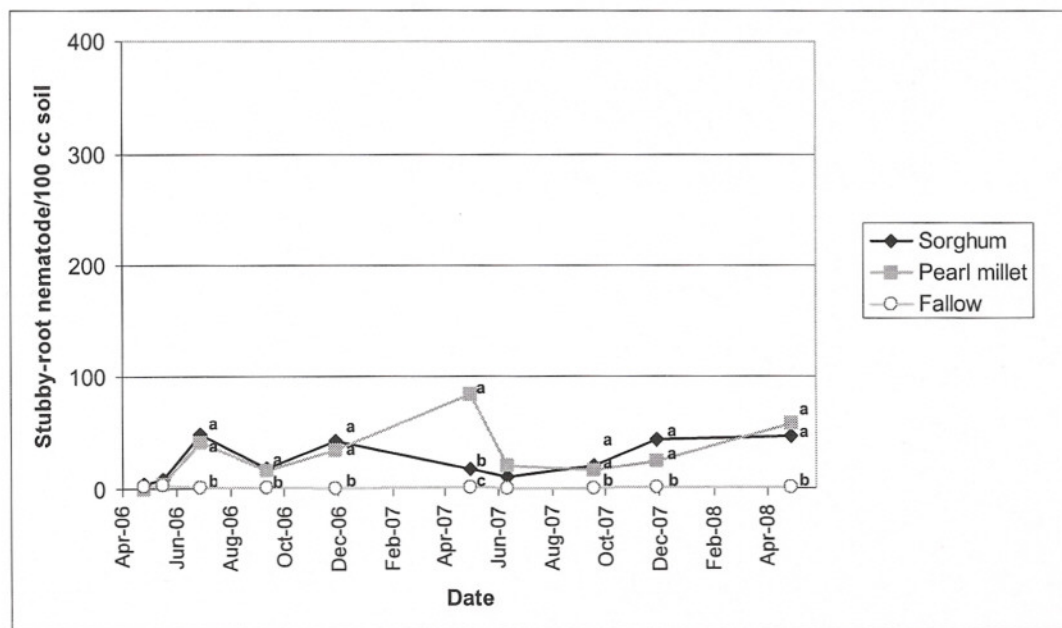


Figure 4. Relationship between stubby-root nematode (*M. minor*) population densities and covercrop treatment over 2 years. Data transformed by square root of $(x + \frac{1}{2})$; treatments in block 5 were removed from analysis due to low initial nematode pop. Data points followed by a different letters by date are significantly different using Tukey's HSD test (Alpha 0.05).

Table 3. Percent carbon as a measure of organic matter in soil by cover crop after the first and second year of treatment.

Cover crops	April 2007	April 2008
	Percent carbon ⁺	Percent carbon ⁺
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

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

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 early 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 suggest that the high densities of the stunt nematodes present in the field study on April 25, 2006 would probably have led to areas of seedling damage and losses had the nursery produced pine seedlings in the field as they would have under their typical crop rotation.

The nursery typically alternated sorghum and grain rye with pine crops, all of which are good hosts for *T. claytoni* according to our results. Continuously growing crops that are hosts for a nematode can lead to damaging populations (Dropkin 1989, Cram and others 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). The host range test results for the population densities of *T. claytoni* with various cover crops were similar to the host range tests for another stunt nematode, *T. ewingi* (Cram and others 2003).

The velvetbean species tested, *M. pruriens*, has 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. More research on velvetbean is warranted before it is entirely ruled out as a control option for nursery fields infested with the stunt nematode.

The field test of the hybrid pearl millet cultivar 'Tifleaf 3' showed that this cultivar is not a host for the stunt nematode *T. claytoni* and appears to be a good alternative to fallow to decrease nematode populations in fields. Other pearl millet cultivars have also been found to be resistant to a variety of plant-parasitic nematodes, including *P. minor*, *Meloidogyne* spp.,

Belonolaimus longicaudatus, and *Pratylenchus brachyurus* (Timper and others 2002, Timper and Hanna 2005).

The low levels of stubby-root nematodes in the sorghum-sudangrass field plots could be the result of many factors including less favorable environmental conditions and competition by stunt nematodes. The densities of stubby-root nematodes in the field may have been too low to expect substantial damage on loblolly or slash pine seedlings (Ruehle 1969); additional work is needed regarding the affect of this plant-parasitic nematode on pines.

The predacious nematodes (*Mylonchulus* sp., *Mononchus* sp.) 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 (MacGuidwin and Layne 1995, Farris and others 1996). Predacious nematodes are only one component of the organisms that control plant parasitic nematodes, and it is possible that they are not efficient control agents or even 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 cc as indicated by the pathogenicity test. Although population levels of nematodes are lowered significantly by pearl millet in one year, it may take two 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. Populations of *T. claytoni* can explode quickly on hosts because of its relatively short lifecycle (31 to 38 day) (Wang 1971) and production of 1-15 eggs per female (Krusberg 1959). Even with the use of soil fumigation to control nematodes (Johnson and Feldmesser 1987, Dropkin 1989), the population densities can rebound and damage the second production crop (McKenry and Thomason 1976, Sipes and Schmitt 1998, Fraedrich and Dwinell 2005). 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 a nurseries 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 is a very poor host (Ruehle 1971). Hardwoods tolerant to stunt nematodes could be used in a rotation with a pine crop (e.g. a pine crop followed by summer fallow with weed control and fall planting of hardwoods). Further investigation into nematode population response to hardwood crops are needed before implementing rotations with pine production systems, as unforeseen problems can occur (Cram and Fraedrich 2005)

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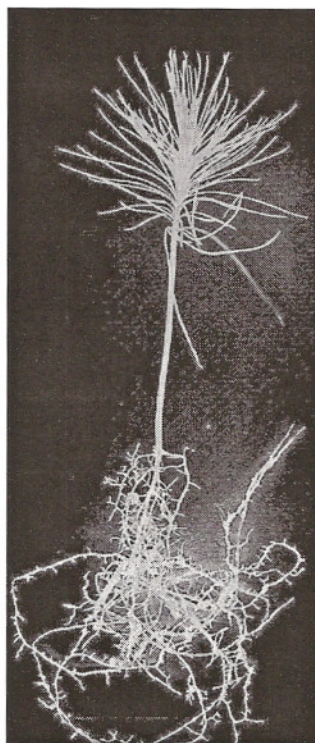
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