Suitability of Southern Pines, Other Selected Crops, and Nutsedge to a *Longidorus* sp. Associated with Stunting of Loblolly Pine Seedlings

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ABSTRACT

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An undescribed needle nematode *(Longidorus* sp.) has been associated with severely stunted loblolly pine seedlings at a south Georgia nursery. Containers with selected crop and weed species were infested with 100 or 200 adults and juveniles of the *Longidorus* individuals to evaluate host suitability. Nematode populations increased in containers with slash, loblolly, and longleaf pine seedlings. The *Longidorus* sp. significantly reduced the dry root weights of slash ($P = 0.008$) and loblolly ($P = 0.047$) but not longleaf ($P = 0.095$) pine compared with controls. Populations of *Longidorus* decreased on nutsedge and small grains, including wheat, rye, oat, sorghum, and millet; and, in most experiments, populations decreased to levels found in fallow containers. Populations decreased on tomato and cabbage but increased slightly on red oak. The periodic removal of fields from pine production to grow cover crops consisting of small grains or to maintain fallow fields may be an important practice for management of this *Longidorus* sp.

Areas of stunted and chlorotic loblolly pine (*Pinus taeda* L.) seedlings have been periodically observed at the Flint River Nursery (Byromville, CA). An undescribed needle nematode *(Longidorus* sp.) has been associated with the problem and, in a controlled study, the nematode was found to damage root systems of loblolly pine (6). Root systems of seedlings affected by the disease arc greatly reduced in size and lack lateral and feeder roots, resulting in seedlings that are severely stunted. Seedling damage usually is confined to small patches when initially observed; however, in 2001, seedlings were noticeably affected by the disease over large areas of one field, rendering much of the production unharvestable.

The Flint River Nursery was established in 1987 on agricultural fields that reportedly were used for the production of cabbage and other crops. The nursery currently produces a large variety of forest tree seedlings to support Georgia's reforestation programs. Loblolly, longleaf (*P. palustris* Mill.). and *slash (I? elliottii* Engelm. var. *elliottii*) pine seedlings constitute a major portion of the yearly production. Hardwood seedlings also are produced and include numerous species of oak (*Quercus* spp.). The nursery uses a variety of small grains as cover crops when fields are not in production. Certain weed species, most notably purple and yellow nutsedge (*Cyperus rotundus* L. and C. *esculentus* L., respectively), can be problems in production fields if not controlled by fumigation or presowing herbicide applications. The field with the nematode problem also has been used in recent years to produce oak and other hardwood seedlings.

Management of plant-parasitic nematode populations can be achieved with the rotation of host and nonhost crops if the species of nematode has a narrow host range (13). The host range of the *Longidorus* sp. associated with damage to loblolly pine seedlings presently is not known. This study was undertaken to determine it slash and longleaf pines arc also suitable hosts for the *Longidorus* sp., and if their root systems are susceptible to damage. We also evaluated the host suitability of small grains that typically are used as cover crops at the nursery, as well as oak, cabbage, tomato, and yellow and purple nutsedge.

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MATERIALS AND METHODS

Pine species. Host suitability and susceptibility to damage by the *Longidorus* sp. was determined for slash, longleaf, and loblolly pine in two experiments. The experiments were conducted in growth chambers. In each experiment, *Longidorus*-infested and noninfested treatments were established for each pine species. There were four containers (replications) for each pine species and treatment combination. Containers were 7 cm high by 10 cm wide, and the soil used in containers was a loamy sand (86% sand, 9% silt, 5% clay; pH 5.6, 1.8% organic matter) from the Flint River nursery. Soil was microwaved in 2,000-g batches for 8 min and approximately 650 g was added to each container. Seeds were placed in 30% hydrogen peroxide for up to 60 min (I) and rinsed three times with sterile distilled water. Loblolly pine seeds then were stratified for 30 to 60 days prior to germination: slash and longleaf pine seeds were not stratified. Seeds were germinated under sterile conditions at 25°C on germination paper in clear containers (17.5 by 12.5 by 6 cm). Five germinated seeds were transplanted to containers with microwave-treated soil. The *Longidorus* sp. was produced on loblolly pine seedlings grown in containers at 22°C for 14 to 26 weeks, and extracted from soil using the procedure of Flegg (4) with modifications by Fraedrich and Cram (6). Nematodes were hand picked, and 100 individuals in experiment l or 200 individuals in experiment 2 were added to each container in the *Longidorus*-infested treatment. Containers were placed in a growth chamber at 22°C with a 14-h photoperiod for 19 weeks in experiment l and 26 weeks in experiment *2*. At the end of the experiments, nematodes were extracted from soil as previously described and populations of the *Longidorus* sp. were determined for each container. Roots of seedlings were removed at the root collar and dried for 4X h at 80°C, after which root dry weights were determined. The nematode reproduction factor (Rf = final population of all juveniles and adults divided by the initial population) was calculated as a measure of host suitability among the different plant treatments tested (1 l). Plant species with Rf values less than l were considered to be nonhosts or poor hosts for the *Longidorus* sp., and those species with Rf values greater than l were considered to be hosts.

Statistical comparisons of the final *Longidorus* populations among pine species in infested containers were conducted by an analysis of variance (ANOVA) using PROC GLM in SAS (The SAS System for Windows, version 8.01 ; SAS Institute, Inc., Cary, NC), and Tukey's honest significant difference (HSD) test was used

for mean separation. Seedling root dry weights were compared between infested and noninfested treatments within pine species by a *t* test (PROC TTEST; The SAS System for Windows).

Nutsedge, small grains, and other selected crops. The suitability of other selected crops and weeds to the *Longidorus* sp. was determined in a series of four experiments. The number of hosts that could he tested within each experiment was limited by our abilities to produce, extract, and handle the *Longidorus* sp. In experiment 1, host suitability was evaluated for yellow nutsedge and cultivars of wheat (*Triticum aestivum* L. 'Saluda'), rye (*Secale cereale* L. 'Wrens Albruzzi'), and grain sorghum (*Sorghum bicolor* L. 'Richardson 9300'). Experiment 2 was a repeat of experiment 1, with the exception that purple nutsedge was tested in place of yellow nutsedge. Northern red oak (*Quercus rubra* L.), tomato (*Lycopersicon esculentum* Mill. 'Rutgers'), cabbage (*Brassica oleracea* L. 'Copenhagen Market Early'), and oat (*Avena sativa* L. 'FLA 501') were evaluated in experiment 3. In experiment 4, brown top millet (*Panicum ramosum* L. 'DW-01') and Sorghum-sudan (S. *bicolor* 'SG Ultra') were evaluated. A *Longidorus*-infested loblolly pine treatment and a *Longidorus*-infested fallow treatment were included in all experiments with the exception of experiment 3, for which a *Longidorus*-infested fallow treatment was not included. A noninfested loblolly pine treatment also was included in all experiments to monitor possible cross-contamination among treatments. There were four containers (replications) for each treatment in each experiment. Containers, soil, and production and extraction of the *Longidorus* sp. were as previously described. In all experiments, 100 adults and juveniles of the *Longidorus* sp. were added to containers in the infested treatments. Seeds of all species except northern red oak were germinated under sterile conditions and transplanted to containers. Loblolly pine seeds were treated, stratified, and germinated as previously described. Oak acorns were germinated in a potting medium consisting of sphagnum peat moss, vermiculite, and perlite (MIX #2; Fafard, Agawam, MA) before transplanting to containers with nursery soil. Nutsedge tubers were obtained from the Flint Rivet Nursery, washed to remove soil, and sprouted in microwave-treated soil. Plants in the three- to four-leaf stage were transplanted to containers. Five seedlings or plants were established in containers for most species, but only three seedlings were established in containers for oak because of their rapid growth and greater size. Containers were placed in a growth chamber at 22°C with a 14-h photoperiod for 12 to 14 weeks. At the end of the experiments, *Longidorus* populations were determined in containers of each treatment. The determination of Rf values and basic assessments of host suitability were determined as discussed previously for pine species. Additional conclusions regarding the suitability of individual plant species were based on statistical comparisons of final *Longidorus* populations among treatments within experiments.

Statistical comparisons were conducted on the final *Longidorus* populations for all treatments (except noninfested loblolly pine) within each experiment 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. Nematode counts were transformed with the log,& + 1) transformation (IS) prior to analysis, but only nontransformed values are presented in text and tables.

Table I. Initial and final *Longidorus* populations, reproductive factor (Rf) values, and root dry weights of southern pine species 19 weeks (experiment 1) or 26 weeks (experiment 2) after infestation with *Longidorus* sp.[y]

Experiment, pine species	Nematodes/container		Rf[z]	Root dry weight (g)
	Initial	Final		
1				
Loblolly	0	2		0.166 ns
	100	164	1.64	0.166
Slash	0	0		0.246 ns
	100	343	3.43	0.271
Longleaf	0	2		0.325 ns
	100	652	6.52	0.378
2				
Loblolly	0	0		0.295 *
	200	1,257	6.28	0.159
Slash	0	0		0.556 *
	200	1,683	8.41	0.334
Longleaf	0	3		0.825 ns
	200	820	4.10	0.681

[y] Means for root dry weight within experiment and pine species followed by ns were not significantly different ($P > 0.05$) between *Longidorus*-infested and uninfested treatments according to a *t* test; those followed by an * were significantly different ($P \leq 0.05$). Means are based on four replications.

[z] Rf = final population/initial population.

RESULTS

Pine species. Populations of the *Longidorus* sp. increased in infested containers for all pine species during each experiment (Table 1), and the final *Longidorus* populations did not differ among pine species in infested containers in experiment 1 or 2 ($P = 0.2345$ and 0.2310, respectively). In experiment I, root dry weights did not differ between control containers and those infested with 100 nematodes for any of the pine species. In experiment 2, root dry weights were significantly reduced in containers infested with 200 nematodes compared with controls for slash ($P = 0.008$) and loblolly pine ($P = 0.047$) but not longleaf pine ($P = 0.095$).

Nutsedge, small grains and other selected crops. Results of ANOVAs indicated that the final populations of *Longidorus* differed significantly among plant species within each of the four experiments ($P < 0.0001$ for each experiment). *Longidorus* populations increased in containers with loblolly pine in all experiments, and populations were significantly greater in containers with loblolly pine than for all other plant species (Table 2). Nutsedge and all small grains appeared to

Table 2. Final *Longidorus* populations and reproductive factor (Rf) values in fallow containers and in containers with selected small grain and vegetable crops. nutsedge, red oak, and loblolly pine 12 to 14 weeks after infestation with 100 individuals of a *Longidorus* sp.

Experiment, plant	Final[y]	Rf[z]
1		
Loblolly pine	1,089 a	10.89
Grain sorghum	7 b	0.07
Fallow	6 b	0.06
Wheat	3 b	0.03
Yellow nutsedge	3 b	0.03
Rye	1 b	0.01
2		
Loblolly pine	251 a	2.51
Purple nutsedge	14 b	0.14
Fallow	8 b	0.08
Rye	7 bc	0.07
Grain sorghum	5 bc	0.05
Wheat	0 c	0
Loblolly pine	887 a	8.87
Northern red oak	112 b	1.12
Tomato	73 b	0.73
Cabbage	16 c	0.16
oat	1 d	0.01
Loblolly pine	1,405 a	14.05
Fallow	36 b	0.36
Sorghum sudan hybrid	20 b	0.20
Brown top millet	12 b	0.12

[y] Final population, nematodes/container. Duration of experiment 1 = 12 weeks; 2 = 14 weeks. 3 = 14 weeks, and 4 = 12 weeks. Means within an experiment followed by the came letter arc not significantly different ($P > 0.05$) according to Tukey's HSD test. Means for each plant species are based on four replications.

[z] Rf = final population/initial population.

be nonhosts for the *Longidorus* sp. In experiments 1, 2, and 4, *Longidorus* populations decreased in containers with small grains and nutsedge to the levels found in fallow containers. In experiment 3. where there was no fallow treatment, the Rf value for oat was only 0.01 and the final population of *Longidorus* in this treatment was significantly less than populations for all other plant species evaluated in this experiment. Final *Longidorus* populations in containers with cabbage and tomato were greater than those in containers with oat, but Rf values for cabbage and tomato were below 1 .0, indicating that these species were also nonhosts or poor hosts. The Rf value of I. 12 for northern red oak indicated that this species is a host. although not as suitable as loblolly pine. The *Longidorus* sp. was not found in noninfested containers with loblolly pine at the end of experiments 1 and 2, but minor contamination was noted in experiments 3 and 4. One uninfested container had 6 *Longidorus* nematodes in experiment 3 and, in experiment 4, one container was contaminated with 22 nematodes and another container with 1 nematode of the *Longidorus* sp.

DISCUSSION

Slash, loblolly, and longleaf pines should he regarded as hosts for the *Longidorus* sp., and the possibility of seedling damage by this nematode exists for any of these pine species. Populations of *Longidorus* developed similarly **on** all pine species, with concomitant root damage on loblolly and slash pine seedlings in experiment 2. The lack of discernable differences for the root dry weight of longleaf pine seedlings in infested and noninfested treatments may be related to the growth and development characteristics of this species. Longleaf pines are unique among pine species in that their seedlings remain in a stemless condition known as a 'grass stage' during the early years of their development. Seedlings develop extensive root systems during this stage (2). This unique feature may have prevented the expression of differences between treatments at the initial *Longidorus* populations used in these experiments.

In a previous study, 100 nematodes of the *Longidorus* sp. per container were sufficient to cause a reduction in the dry weigh1 of loblolly pine root systems (6). Although a reduction in root weight for loblolly pine was observed at the rate of 200 nematodes per container in the present study, no reduction was observed at 100 nematodes per container. However. slight methodological changes in the present study may have affected results. In the previous study, less soil was used in each container and, therefore, there were more nematodes per unit volume of soil than in the present study. Furthermore. nematodes in the previous study were extracted from field soil, and most nematodes were adults or large juveniles (>5.0 mm in length). In the present study, the *Longidorus* sp. was reared in growth chambers, and a 50/50 ratio of adults and larger juveniles to smaller juveniles was used to infest containers.

Longidorus Rf values for loblolly pine ranged from 1 .64 to 14.05 among the six experiments in this study. This variation likely was due to a number of factors. including subtle variation among the experiments in nematode production, elapse time between extraction of nematodes and handpicking them, and nematode storage prior to infesting containers. In the first experiment, nematodes used to infest many containers were stored in water at 6°C for up to 24 h; however, in later experiments, nematodes were placed immediately into containers after they were handpicked. The Rf value of 1.64 for loblolly pine in the first experiment was the lowest among the six experiments in this study. This low Rf value may have been the result of nematode mortality or loss of vigor during the brief storage prior to infesting containers.

Sorghum, wheat, rye, oat, millet, and yellow and purple nutsedge appeared to be nonhosts for the *Longidorus* sp. In all of the experiments, the *Longidorus* population decreased in containers with small grains and nulsedge similarly to populations found in **fallow** containers, or declined to exceptionally low population levels (i.e., oat in experiment 3). Many of the *Longidorus* individuals that survived in containers with small grains and nutsedge were larger juveniles and adults, and generally there was no evidence of reproduction in these containers. According to Nusbaum and Barker (IO), there will be no reproduction at all with nonhosts, and populations will decline at about the same rate as those under bare fallow. The use of small grains as cover crops appears to be an acceptable and desirable practice in pine seedling nurseries infested with this *Longidorus* sp. Additional research is necessary to better clarify the suitability of oak and tomato as hosts for this *Longidorus* sp. Although the Rf value was above 1 .0 for oak and below 1 .0 for tomato in experiment 3, the final population of *Longidorus* did not differ significantly between these plant species.

The *Longidorus* sp. at the Flint River Nursery probably was not carried over from cultivation of cabbage because this species appears to be either a nonhost or a poor host. Presently, the origin of this nematode is not clear. We have been unable to find the *Longidorus* sp. in natural areas around the nursery that contain pine, oak. and bald cypress (*Taxodium distichum* (L.) Rich.), although we have found a morphologically identical *Longidorus* sp. in pine seed orchards that border the nursery (7). These seed orchards have been established since the founding of the nursery in 1987, and their importance as a source of inoculum for nursery beds is unknown. Surveys for the *Longidorus* sp. are continuing, and possible associations of the nematode with various herbaceous and woody plants that occur within and around the nursery are being investigated.

The nursery normally produces two pine seedling crops in fields following soil fumigation. Cover crops that consist of various small grains then are grown in fields for 2 years before refumigation. The field at the Flint River Nursery where stunted pine seedlings have occurred has not been part of this standard regime. The field has been in continuous seedling production since 1990 and was used to produce pine and hardwood seedlings. Patches of stunted loblolly pine seedlings were observed in the southernmost sections of the field in most years from 1996 through 1999, although the problem was undiagnosed. Some sections of this field were fumigated in 1998 and again in 2000; however, in each instance, the disease recurred within these sections in the second year of pine production. A complete history of the fumigation and crop production in this field has been documented (3).

According to Ruehle (13), nematode damage in forest tree nurseries typically is restricted to spots in nursery beds when first observed. This normally has been our experience with the damage caused by the needle nematode at the Flint River nursery, although the disease has appeared to spread over time with the yearly development of new infestation foci and enlargement of older foci. When seedling damage first appears, patches typically are small, often ranging in length from 3 lo 9 m of nursery bed (6). In 2001, seedling damage was exceptional in some sections during the second year of production following fumigation (7). Affected areas were considerably larger and exceeded 90 m in length in some beds.

Longidorus spp. are known to occur at soil depths of at least a meter (8,14), and some *Longidorus* spp. are most abundant at soil depths greater than 60 cm (5.12). Nursery fields typically are fumigated with methyl bromide injected at a depth of approximately 15 to 20 cm. Although these relatively shallow injections can provide high concentrations of methyl bromide in the upper 30 cm of soil, methyl bromide concentrations decrease rapidly with soil depth, und may occur only at insignificant quantities deeper in the soil (9). Therefore, nematodes found in the deeper soil strata most likely are below the zone where standard fumigation practices used in forest tree nurseries are most effective. Although the elimination of the *Longidorus* sp. from the upper soil strata by fumigation permits pine seedlings to develop normal root systems early in the growing season, abundant roots are available later in the

growing season for needle nematodes that survive fumigation. These factors could favor increased population development that could greatly impact subsequent pine crops grown in an infested field. Practices such as maintaining fallow fields or growing cover crops for one or more years following production of pine seedling crops may be important components of an integrated management program for this *Longidorus* sp.

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