The Association of a *Longidorus* Species with Stunting and Root Damage of Loblolly Pine (*Pinus taeda* L.) Seedlings

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ABSTRACT

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A *Longidorus* species was consistently associated with patches of stunted and chlorotic loblolly pine seedlings at a forest-tree nursery in Georgia. Seedlings from affected areas had poorly developed root systems that lacked lateral and feeder roots. *Longidorus* population densities in composite soil samples from the margins of patches ranged from 9 to 67 nematodes per 100 cm^3 of soil. In a growth chamber experiment, seedling root dry weight decreased with respect to the initial *Lungidorus* dose as well as the final *Lungidorus* populations in containers. The dry root weight of seedlings were 0.117, 0.090, 0.066, and 0.065 g *in* containers initially infested with 0, ***50, 100,*** and ***200 Longidorus,*** respectively. Lateral and fine roots were lacking on seedlings at the highest doses. Populations of *Longidorus* increased in all containers during the experiment. Damage to loblolly pine seedlings caused by *Longidorus* is a previously undescribed problem in southern pine nurseries. Proper diagnosis of the problem by nematode testing laboratories may require the use of extraction techniques specific for larger nematodes such as *Longidorus.*

Many species of plant parasitic nematodes are associated with roots of conifers (10), and some can cause significant damage (9,11,12,16). Nursery seedlings are particularly vulnerable to damage by nematodes because of the continuous culture of single species, use of irrigation to maintain soil moisture, and maintenance of high soil fertility for optimal plant growth (12). Fumigation has been used routinely for more than 40 years in many southern forest-tree nurseries to control weeds, insects, fungal pathogens, and nematodes. However, the most widely used fumigant, methyl bromide (6), is scheduled to be phased out by 2005. Many aspects of nursery operations have changed since the 1950s, and there is presently a lack of information about soilbome diseases that may affect pine production in southern nurseries.

Areas of stunted pine seedlings have been periodically observed in fields at the Flint River Nursery (Byromville, GA) since its establishment in 1987. During 1998 and 1999, patches of stunted and chlorotic loblolly pine (*Pinus taeda* L.) seedlings occurred in one field at this nursery. Soil samples from the affected areas were forwarded to a nematode testing laboratory for evaluation of plant parasitic nematodes, and seedlings were sampled for fungal pathogens, but a definitive cause of the problem could not be determined. Sections of the field affected by the disease were fumigated in the spring of 2000, and the disease was not observed in the pine seedling crop produced in these sections during 2000.

In July 2000, we again observed patches of stunted loblolly pine seedlings but in different sections of the field. These sections contained a fumigation study (3) that was in its third year of continuous pine seedling production. Seedling damage was observed in nonfumigated control plots, and soil in these plots had not been fumigated since October 1993. Many seedlings were chlorotic and severely stunted in affected areas (Fig. 1A). Compared to healthy seedlings, stunted seedlings had poorly developed root systems that lacked lateral, feeder, and mycorrhizal roots (Figs. 1B and 1C). Soil samples were forwarded to a nematode-testing laboratory where lesion (*Pratylenchus* sp.) and ring (*Criconemella* sp.) nematodes were found at low levels in areas with diseased seedlings as well as areas with healthy seedlings. Roots were plated on various agar media, and *Fusarium* spp. were routinely isolated from both healthy and diseased seedlings. A Rhizoctonia-like fungus was also occasionally isolated from roots of stunted seedlings in some patches. Upon closer inspection of the unwashed roots in water under a dissecting microscope, we routinely observed needle nematodes (*Longidorus* sp.) associated with diseased seedlings. This nematode had not been reported by the nematode testing laboratory. We summarize in this paper our findings of a survey for a ***Longidorus*** sp. in affected areas of seedbeds at the Flint River Nursery and results of a growth chamber experiment to determine the effect of *Longidorus* sp. on loblolly pine seedlings.

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MATERIALS AND METHODS

Field survey. Seedlings and soil were sampled in four areas that contained patches of stunted loblolly pine seedlings. Each patch occurred in a separate bed within a 12-seedbed area of one field. Patches ranged from 3 to 9 m long and were one seedbed (1.2 m) wide. Samples of seedlings with soil attached were collected from each of the four patches in August and October 2000. In August, diseased seedlings were collected at patch centers and margins, and healthy-appearing seedlings were collected at distances of 1.5 and 3.0 m outside the margins of patches (seven sample locations per patch). In October, seedlings were collected at patch margins and at distances of 1.5 m outside the patch margins (four sample locations per patch). At each sample location and sample time, 10 to 25 seedlings were lifted. Seedlings with attached soil were placed in plastic bags. A 25 g sample of soil from the root zone of seedlings at each sample location was used for nematode extractions. At patch centers, only 8 to 15 g of soil was used because of the sparseness of seedling roots and lack of associated soil. The population density of ***Longidorus*** in these samples was expressed on a 25 g weight basis. Nematodes were extracted using the technique of Flegg (5) but the technique was modified such that 90 pm aperture sieves were used in place of 150 pm sieves. In addition, nematodes and debris from sieves were placed in water under a dissecting scope, and nematodes were counted directly without the use of the Baermann funnel apparatus.

Composite soil samples were collected with a soil sampler in September and again in October 2000 from the centers, margins, and 1.5 m outside the margins of each of the four patches (five sample locations per patch). Soil samples from each location consisted of 5 to 6 soil cores (2.5 cm diameter to a 1.5 cm depth). Nematodes were extracted from 200 cm^3 of soil using the technique of Flegg (5) with 90 μm aperture sieves as previously noted. In addition, Kimwipes (Kimberly-Clark Corp., Roswell, GA) were used instead of 90 pm aperture nylon screen in the Baermann funnel apparatus. After 48 h, about 2.5 ml of water with nematodes was removed from the Baermann funnels, and *Longi-*

dorus were counted under a dissecting microscope. Debris with nematodes was washed from the Kimwipes into a petri dish, and remaining *Longidorus* were counted under a dissecting microscope by systematically sorting through the debris.

Growth chamber experiment. The objective of the experiment was to examine the effect of the *Longidorus* sp. on growth of loblolly pine seedlings. *Longidorus* were extracted from soil using the technique of Flegg (5) with the 90 µm aperture sieves and without additional modifications. The nematodes were hand picked, placed in water in groups of 50, and stored at 5°C for up to 48 h before introduction into containers. Soil was obtained from areas of the nursery not affected by disease and was microwaved in 2 kg batches for 8 min. Approximately 330 g of soil was placed in each container (7 cm high by 10 cm wide). Loblolly pine seeds were surfaced sterilized with hydrogen peroxide (30%) for 60 min (1), rinsed with sterile distilled water, and stratified for 30 to 60 days. Seeds were germinated under sterile conditions, and five germinated seeds were transplanted to containers with microwaved soil.

Needle nematodes were added to containers at rates of 0, 50, 100, or 200 individuals per container, and there were four replications of each nematode dose. The seedlings were placed in a growth chamber at 22°C with a 14-h photoperiod and were watered every 1 to 3 days as needed. At the end of 22 weeks, seedlings were removed from containers and dried at 80°C for 48 h. The dry root and shoot weights of each seedling was determined. The final population of *Longidorus* in containers was determined using the extraction technique of Flegg (5) with the 90 µm aperture sieve and without additional modification.

The experiment was established as a completely randomized design with four treatments (*Longidorus* doses) and four replications (containers) per treatment. The effect of the initial *Longidorus* dose and final *Longidorus* populations on seedling root and shoot dry weights was determined by regression analysis (4). A nonlinear, negative exponential model was used to characterize the relationship between the initial *Longidorus* dose and seedling root dry weight. Parameter estimates were determined using PROC NLIN (The SAS System for Windows, Version 8.01, SAS Institute, Inc., Cary, NC). The criteria for fit of the model were based on the mean square error (MSE), the significance of the overall regression, and a lack of fit analysis. Linear regression using PROC REG (The SAS System for Windows, Version 8.01) was used to analyze the relationship between initial *Longidorus* dose and seedling shoot dry weight, and relationships between final population of *Longidorus* per container and seedling root and shoot dry weights. *Longidorus* populations in containers at the end of the experiment were analyzed among treatments using an analysis of variance and Tukey's honest significant difference (HSD) procedure (α = 0.05) for mean separation (8).

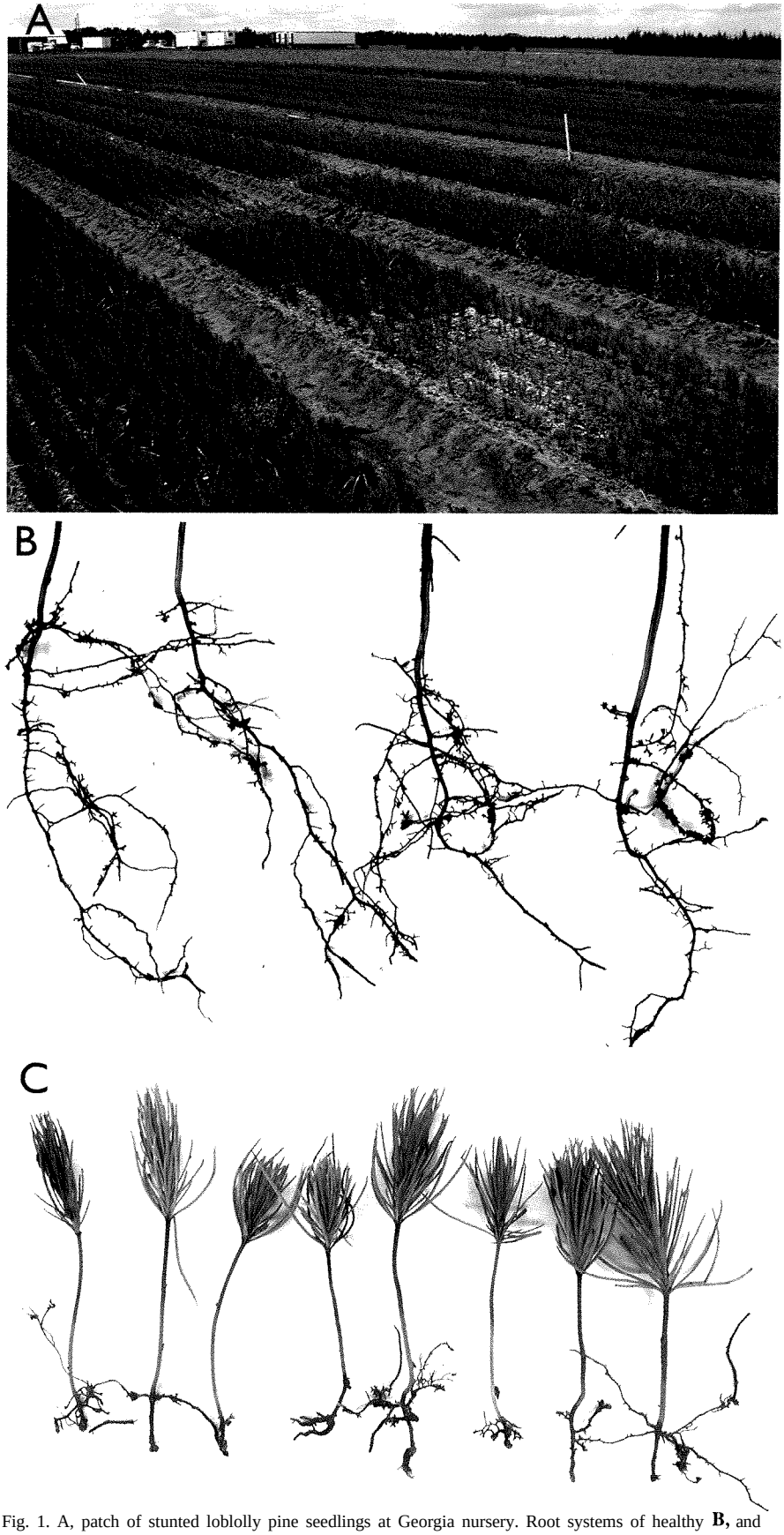

Fig. 1. A, patch of stunted loblolly pine seedlings at Georgia nursery. Root systems of healthy **B,** and diseased C, IO-week old seedlings.

RESULTS

Longidorus survey. In August 2000, *Longidorus* population densities were

greater in soil samples from the root zone of seedlings at the centers and margins of patches than areas with healthy seedlings at distances of 1.5 and 3.0 m from the patch margins (Fig. 2A). The October sampling of soil from the root zone provided similar results. An average of 25.8 Longidorus per 25 g soil (range: 10 to 52) were extracted at the margins of affected areas and an average of 1.5 *Longidorus* (range: 0 to 6) were extracted 1.5 m outside the margins.

In the composite soil samples obtained in September, *Longidorus* were most frequently extracted at the margins of affected areas (Fig. 2B). Fewer nematodes were extracted at the centers of affected areas or 1.5 m outside the margins of the affected areas. The findings were similar for the composite soil samples collected in October. The mean number of *Longidorus* extracted at the margins of patches was 17.5/100 cm^3 soil (range: 9 to 30), and at 1.5 m outside the margins only 2.5/100 cm^3 (range: 1 to 7).

Growth chamber experiment. Damage to root systems similar to that found in seedbeds occurred in containers infested with the *Longidorus* sp. Seedlings typically lacked lateral and feeder roots in containers infested with 100 and 200 individuals. Seedling root dry weight decreased exponentially as the initial *Longidorus* dose increased (Fig. 3A, MSE = 0.00026, $P = 0.0006$), and there was no evidence of lack of fit for the relationship ($P = 0.369$). Root dry weight was also inversely related to the final number of *Longidorus* per container (Fig. 3B, $R^2 = 0.60$, $P = 0.0004$). Shoot dry weight did not vary with respect to initial dosage levels of *Longidorus* ($R^2 = 0.078$, P = ,296) or final counts of *Longidorus* ($R^2 = 0.0005$, $P = 0.933$) in containers. Mean shoot dry weight ranged from 0.14 to 0.17 g among treatments.

Longidorus increased in all containers during the experiment, but final populations differed among treatments ($P = 0.01$). Containers initially infested with 50, 100, or 200 *Longidorus* had a average of 555, 582, and 672 *Longidorus*, respectively, at the end of the experiment, and there were no differences among these treatments. Containers initially free of nematodes at the beginning of the experiment had been contaminated and had an average of 74 *Longidorus* per container at the end of the experiment. Nonetheless, control containers had significantly fewer *Longidorus* than containers of the other treatments.

DISCUSSION

Longidorus spp. have been reported to damage pine seedlings in Germany (15), and have been previously found in soil from areas where southern pines were grown (7,13). In a survey of soils of southern nurseries, Hopper (7) found the needle nematode at only 1 of 16 nurseries. However, we have not found published accounts of *Longidorus* spp. parasitizing and damaging roots of loblolly pine or other southern pine species. Based on the results of our survey and growth chamber experiment, we believe that the *Longidorus* sp. is responsible for the damage that we observed on loblolly pine seedlings at the Flint River Nursery. The possible involvement of fungal pathogens in the seedling losses at this nursery requires additional study.

The threat of this *Longidorus* sp. to pine seedling production is presently not known. During 2000, the disease was primarily restricted to areas of 12 seedbeds that had not been fumigated in recent years and approximately 4% of the nonfumigated area was affected. However, during the summer of 2001, the problem recurred in areas of the field fumigated in 2000. Thus far, the problem has been localized to sections of this one field and has not spread to other fields. In fact, six consecutive crops of pine seedlings were produced in nonfumigated study plots in an adjacent field between 1995 and 2000, and the disease did not develop.

Specimens of the *Longidorus* sp. were forwarded to the USDA, ARS, Nematology Laboratory in Beltsville, MD for identification. The nematode is not believed to conform to *any* known *Longidorus* sp. and is presently regarded as undescribed (Z. Handoo, *personal communication*). The nematode is extremely large (7 to 8 mm long), and is found at low population levels compared to many nematodes that damage plants. These factors may partially account for the failure to detect *Longidorus* by nematode testing laboratories that processed soil from affected areas. The techniques for extraction of large nematodes such as *Longidorus* spp. are quite specific (5,14), and methods routinely used for the extraction of plant parasitic nematodes from soil may not be suitable for extraction of larger nematodes. In our laboratory, we initially substituted Kimwipes for the 90 µm aperture nylon screen used by Flegg (5) in the Baermann funnel apparatus. Kimwipes were not a suitable support for extraction of this *Longidorus sp.* because 70% of the *Longidorus* in samples did not move through this material. In a small experiment that we conducted, three times as many *Longidorus* could be obtained from soil samples using a 90 µm aperture nylon screen in the Baermann funnel apparatus compared to Kimwipes (*unpublished data*).

In patch centers, fewer nematodes were extracted in the composite soil samples (range: 2 to 8 nematodes per 100 cm^3) than from soil obtained from the root zone of seedlings (range: 7 to 48 nematodes per 25 g). The difference is most likely due to the very limited root distribution of seedlings

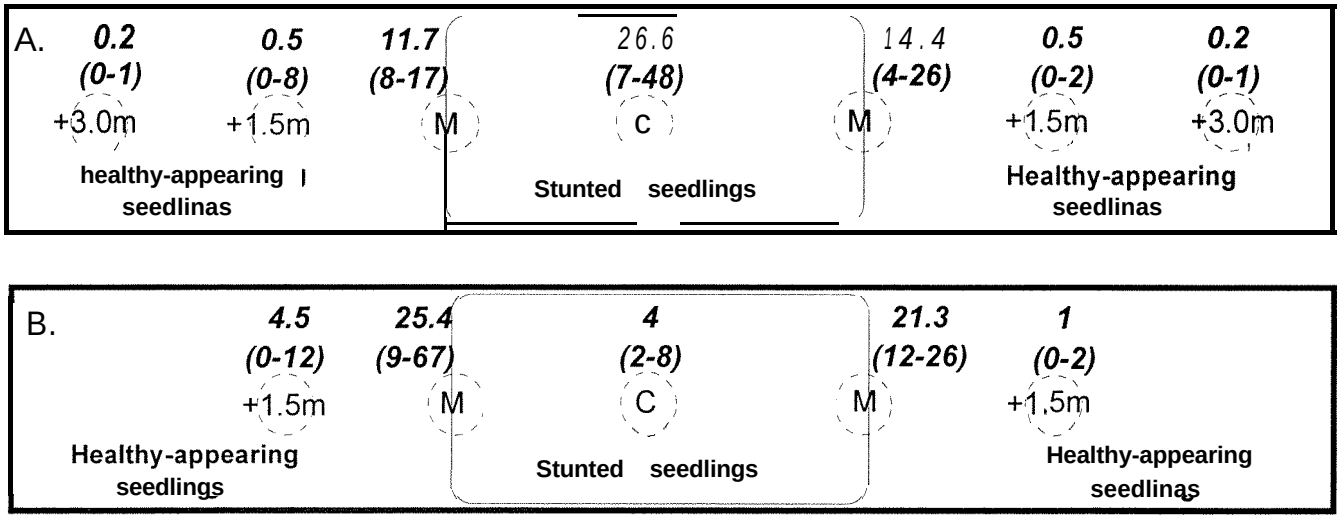

Fig. 2. Mean number (and range) of *Longidorus* per 25 g soil collected from the root zone of loblolly pine seedlings A, and mean number (and range) of *Longidorus* per 100 cm^3 soil extracted from composite soil samples B. Mean values are from four areas with patches of stunted seedlings, and includes sample locations at the centers (C), margins (M), and 1.5 m (and 3.0 m [A]) from the margins of patches.

in the patch centers and the close association of ***Lmgidorus*** with the pine roots. The composite soil samples probably included many cores void of roots, which greatly reduced the presence of *Longidorus* in these soil samples.

The presence of ***Longidorus*** in control containers at the end of the growth chamber experiment was most likely due to contamination from infested containers. Containers were randomized in the growth chamber in close proximity to one another. The contamination of noninfested containers probably resulted from splash during watering. Although reductions in the size of root systems were found in our growth chamber experiments, we did not observe the stunting of seedling shoots that was prevalent under field conditions in affected areas. However, stresses due to high summer temperatures and daily water deficits that would occur normally under field conditions were not imposed in the growth chamber experiments.

Considerable changes have occurred in many aspects of forest-tree nursery operations since nursery managers began fumigating more than 40 years ago. From a pest management standpoint, one important change is the placement of newer nurseries on sites with sandier soils than in the past (2). The soil type at the Flint River Nursery is a loamy sand, and classified in the Eustis soil series. Although sandier soils provide better drainage that may prevent the development of some root diseases, ***Longidorus*** spp. are usually confined to sandy and sandy loam soils (14).

Damage to pine seedlings by the needle nematode is a previously undescribed problem in southern nurseries. Nursery managers and pest management specialists who suspect nematode damage to pine crops should alert nematode testing laboratories to examine soil samples for *Longidorus* spp. Where patches of stunted seedlings occur, soil samples should be taken at the margins of the patches, and stunted seedlings with rhizosphere soil should be lifted for evaluation. Nematode testing laboratories should consider using the procedure of Flegg (5) or other techniques (14) specific for extraction of larger nematodes such as *Longidorus* spp. when nematode damage is suspected on seedlings of loblolly pine or other southern pines.

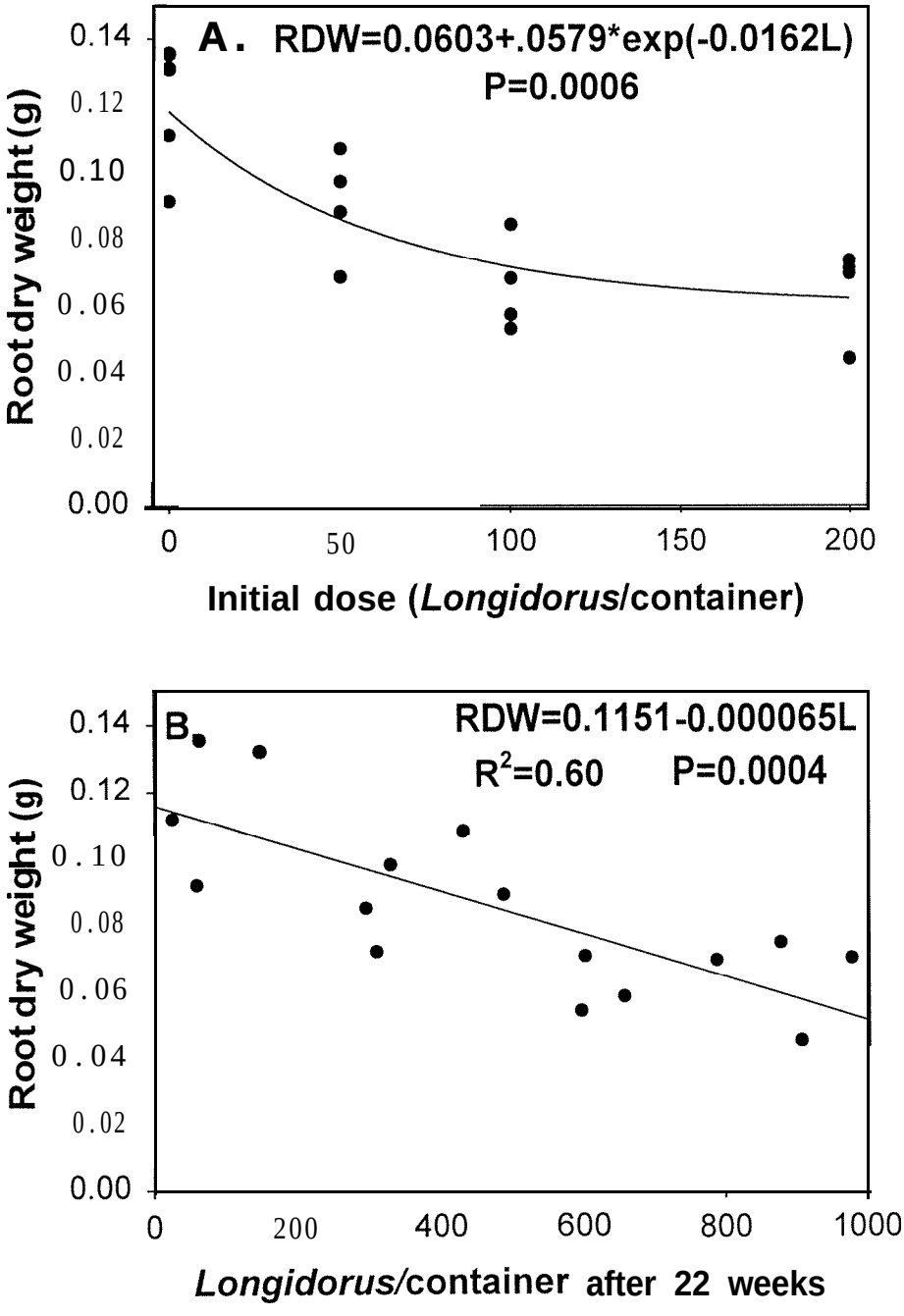

Fig. 3. Relationships between the initial dose of Longidorus (L) and root dry weight (RDW) of loblolly pine seedlings **A,** and final population of *Longidorus* per container and root dry weight **B.** Data was collected 22 weeks after infestation. Each data point represents the mean root dry weight of five seedlings per container.

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