Journal of Nematology 24(3):432–437. 1992.

Dynamics of *Belonolaimus longicaudatus* Parasitism on a Susceptible St. Augustinegrass Host[1]

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Abstract: St. Augustinegrass (*Stenotaphrum secundatum*) cv FX-313 was used as a model laboratory host for monitoring population growth of the sting nematode, *Belonolaimus longicaudatus,* and for quantifying the effects of sting nematode parasitism on host performance in two samples of autoclaved native Margate fine sand with contrasting amounts of organic matter (OM = 7.9% and 3.8%). Following inoculation with 50 *Belonolaimus longicaudatus* per pot, nematodes peaked at a mean of 2,139 nematodes per pot 84 days after inoculation, remained stable through 168 days at 2,064 nematodes per pot, and declined at 210 days. The relative numbers of juveniles and adults demonstrated senescence after 84 days. Root dry weight of nematode-inoculated plants increased briefly to an apparent equilibrium 84 days after inoculation, whereas root weights of uninoculated controls continued to increase, exceeding those of inoculated plants from 84 to 210 days ($P < 0.01$). At 210 days, uninoculated plants had 227% the root dry weight of inoculated plants. Transpiration of FX-313 was reduced by nematodes ($P < 0.0001$) at 84 and 126 days after inoculation; reduction was first observed at 42 days and last observed 168 days after inoculation ($P < 0.05$). OM content affected all plant performance variables at multiple dates, and generally there were no inoculation × OM content interactions. OM content had no effect on nematode numbers per pot, although there was a slight ($P < 0.05$) increase in the number of nematodes per gram root dry weight in the low-OM soil compared with the high-OM soil.

Key words: Belonolaimus longicaudatus, nematode, population dynamics, resistance screening, soil organic matter, St. Augustinegrass, *Stenotaphrum secundatum,* sting nematode, turfgrass.

St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze, is well adapted to warm humid and subtropic regions and is the most commonly planted turfgrass species in Florida (1). The sting nematode, *Belonolaimus longicaudatus* Rau, is highly pathogenic to St. Augustinegrass (2) and most other warm-season turfgrass species (8). Ten *B. longicaudatus*/100-cm^3 soil is the reported damage threshold for turfgrasses (3). Recently, an ultradwarf, diploid St. Augustinegrass (FX-313) was shown to be a highly suitable and susceptible host for *B. longicaudatus* relative to three other diploid St. Augustinegrass genotypes (2). FX-313 supported a large population (12,300 nematodes per g of root dry weight) of *B. longicaudatus* within 128 days of inoculation, under laboratory conditions with artificially low light levels and moderate air temperatures (27–33 C). FX-313 is therefore an excellent candidate laboratory host for studying *B. longicaudatus* parasitism on a susceptible turfgrass.

Soil type and composition have been identified as major limiting factors for *B. longicaudatus* reproduction (e.g., soils with less than 80% sand are not suitable) (7,9). *Belonolaimus longicaudatus* counts in field plots of FX-313 at the Fort Lauderdale Research and Education Center, Davie, Broward County, Florida, are inversely correlated with the organic matter (OM) content of native Margate fine sand (siliceous, hyperthermic, Mollic Psammaquent), and plant performance is less affected by nematode parasitism in soil with a higher OM content (unpubl. data).

The purpose of this study was to use FX-313 St. Augustinegrass as a model laboratory host for monitoring *B. longicaudatus* population growth over time and for quantifying the effects of sting nematode parasitism on host performance in two samples of autoclaved native Margate fine sand with contrasting amounts of OM.

Received for publication 10 February 1992.

[1] Contribution of the Florida Agricultural Experiment Station, Journal Series R-02189.

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We thank Frank G. Bilz and Edna I. Zaenker for technical assistance, Harlan L. Rhoades for a culture of *B. longicaudatus,* and John L. Cisar and Tom Weissling for critical review of the manuscript. This project was partially funded by a grant from the Florida Turfgrass Research Foundation to P.B. and R.M.G.-D.

Quantification of host performance was assessed by destructive root and shoot harvests and a nondestructive measurement of transpiration.

Materials and Methods

Treatments involved a harvest factor (harvested 42, 84, 126, 168, and 210 days after inoculation) and two potential plant establishment and performance factors: nematode inoculum (inoculated vs. uninoculated) and OM content (low OM vs. high OM). The resulting 20 combinations were arranged in a randomized complete block design with six replications. In addition to the 120 experimental units, a set of 12 uninoculated plants were also harvested on the day of inoculation.

Washed aerial stolons of FX-313 were harvested from a 6-year-old field plot and planted in autoclaved (90 minutes at 121 C at 103 kPa), 60-mesh sand in 26- × 52-mm plastic trays for root development. Sprigs (6–8 cm long) were terminal cuttings with two or three nodes and weighed 1.03 ± 0.04 (SD) g per sprig. After 14 days, sprigs were transplanted to square tapered pots (80 mm wide at the top, 60 mm wide at the bottom, and 75 mm deep). At the time of transplanting, sprigs averaged 1.8 g fresh weight and had about five nodes, of which three had newly initiated roots. Drainage holes at the bottom of the pots were covered with an 85-mesh synthetic fabric, which was mounted by means of Super 77 Spray Adhesive (3M Brand, St. Paul, MN). Pots were filled with moist soil to within 15 mm of the top of each pot; the total compacted soil volume in the pot at the end of the experiment was 250 cm^3. Sprigs were planted with their roots distributed throughout the soil and with the proximal stolon node buried slightly. Soil type was Margate fine sand (siliceous, hyperthermic, Mollic Psammaquent) from two different field areas, which differed primarily in organic matter content, determined by dichromate digestion (6). The high-OM Margate fine soil sample was pH 6.5 and contained 7.9% OM. The low-OM soil sample was pH 6.5 and contained 3.8% OM. Soil was sieved (2 mm), thoroughly mixed, and autoclaved (90 minutes at 121 C at 103 kPa).

On 7 June 1991, after 23 days of transplant rooting, pots were inoculated with *B. longicaudatus* from a stock culture maintained on St. Augustinegrass. Nematodes were extracted by centrifugal flotation (4) and hand picked under a dissecting microscope. Fifty *B. longicaudatus*, mostly adults, in 2 ml water were pipetted into a single 10-mm-deep soil depression near the most proximal rooted node. The mean saturated pot weight was 421 g. Pots were placed on a laboratory bench. Daily maximum and minimum temperatures averaged 30.4 C ± 0.9 SD and 25.6 C ± 0.8 SD, respectively. Soil temperatures were generally between 22 and 23 C. Photosynthetic photon flux density was 138 μmole/m^2 per second (ca. 7% of maximum sunshine at latitude 26° N) for 16 hours/day. Plants did not cover the soil; thus, in order to minimize evaporation from the soil, 0.4 g polystyrene nuggets were placed on the soil surface of each pot. Pots were watered every 3 days to within 400 ± 5 g, and after every harvest they were watered to excess, to leach away possible salt buildup. With few exceptions, plants were not allowed to wilt. Pots were fertilized with 56 mg N per pot, 3 days after inoculation, and with 19 mg N per pot after each harvest. Fertilizer analysis was 0.2 N, 0.2 P_2O_5, 0.2 K_2O, with micronutrients, and was dissolved in 15 ml water per pot. Plants were sprayed once to runoff with fluvalinate at 157 mg a.i./liter to control mites. Plants were trimmed periodically to remove stolons, which surpassed the edges of the pots. All trimmings were weighed and added to the eventual shoot harvest (below).

Transpiration was used as a noninvasive method for quantifying plant performance of inoculated versus uninoculated FX-313. Quantification of evapotranspiration (ET) began during the week before each harvest. Individual pot ET was determined by weighing the pots daily for 3 days, followed by uniform rewatering and

reweighing to 400 ± 1 g. This procedure was performed twice for each of the five harvests, 42 to 210 days after inoculation. Six pots with the plant cut off at the soil level were used as a control to estimate soil evaporation; their mean value, 3.7 g/pot/day, was subtracted from all other pot values to provide an estimate of plant transpiration per pot, which was calculated on a daily basis. Because transpiration measurements were similar in the two determinations for each harvest, and to summarize overall response, they were combined for statistical analysis as repeated measures within harvests.

For harvest, the soil was washed from the root ball of each pot, and nematodes were extracted from the entire soil volume by centrifugation-flotation (4). Number of nematodes (juveniles + adults) were counted in an aliquant representing one-half, one-eighth, or all of the sample, depending on the harvest. For estimating the total population, the number of nematodes counted was divided by the aliquant fraction. For a random subsample of 100 nematodes, the number of juveniles (J2–J3 and J4) and of adults (males and females) were counted separately, and their proportion was used to estimate the total number of juveniles and adults in the total sample. Following nematode extraction, roots were cut from the plant stolons. Roots and shoots (leaves + stolons) were dried at about 60 C for 72 hours and weighed.

Data for each of six harvests (0 to 210 days after inoculation) were analyzed separately by analysis of variance (10), pooling the mean squares for blocks and interactions with block. Variables analyzed were number of nematodes per pot, root and shoot weights (destructive harvest of 24 pots per harvest), and trimmings yield and transpiration (diminishing from 120 pots at 42 days to 24 pots at 210 days).

Results and Discussion

Belonolaimus longicaudatus reproduced rapidly, reaching a maximum combined mean of 2,139 nematodes (adults + juveniles) per pot for both high and low OM content at 84 days after inoculation. The nematode population thereafter remained stable at 2,064 per pot through 168 days, and then declined (Fig. 1A). There was no effect of soil OM content on total number of nematodes per pot. This suggests that inverse correlations of *B. longicaudatus* numbers with OM content in sandy soils (with >80% sand) probably involved interactions between OM and biological antagonists or other biotic factors destroyed during autoclaving. Similarly, Rhoades (unpubl.) observed that native muck soil was repressive to *B. longicaudatus* growth and reproduction, but was a suitable medium when autoclaved.

Adult *B. longicaudatus*, which were in a 1:1.1 female:male sex ratio, increased continuously in number until 168 days and then dropped in the low-OM soil, but leveled off after 126 days in the high-OM soil (Fig. 1B). In both soils, the proportion of juveniles peaked at about 80% of the total population at 84 days after inoculation and declined gradually to less than 40% at 210 days. This decline was slightly faster in the low-OM soil than in the high-OM soil. In the low-OM soil, J2–J3 and J4 densities increased rapidly up to 84 days after inoculation and declined thereafter (Fig. 1B). In the high-OM soil (Fig. 1C), the density versus time plot for the J2–J3 stages of *B. longicaudatus* was similar to that observed for the low-OM soil (Fig. 1B). However, the J4 density in the high-OM soil increased continuously until 168 days. This population data suggests that *B. longicaudatus* was in a growth and reproduction (expansion) phase from 0 to about 84 days, followed by senescence.

St. Augustinegrass root dry weight was progressively reduced in nematode-inoculated plants relative to uninoculated controls, starting 84 days after inoculation and continuing through 210 days. Absolute values for root dry weight remained constant or declined slightly from 84 to 210 days for inoculated plants (Fig. 2A), whereas the root dry weight continued to increase in uninoculated plants between 84

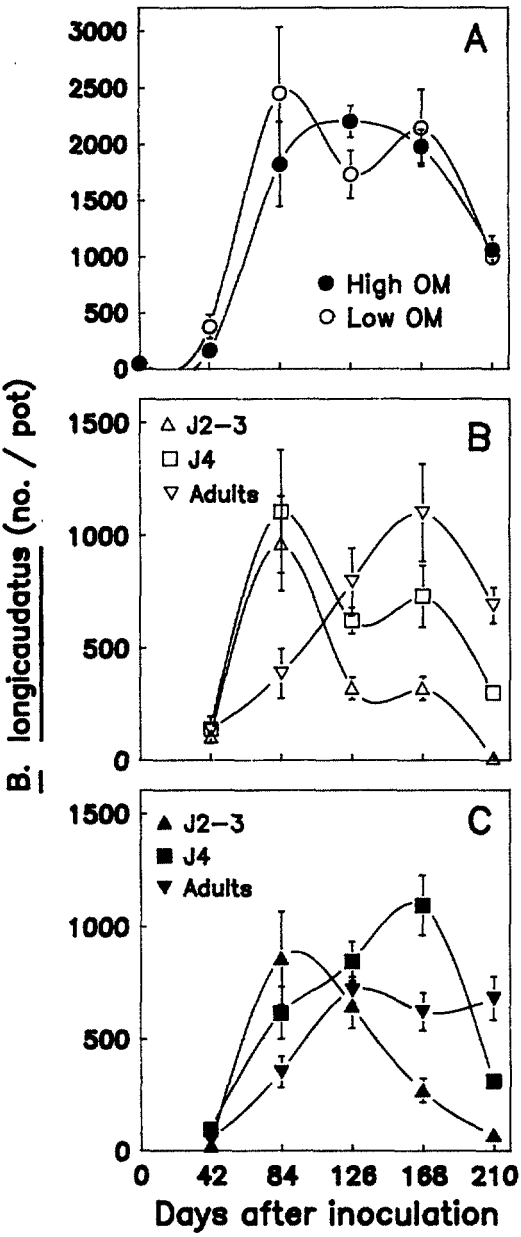

FIG. 1. Response over time of *Belonolaimus longicaudatus* on FX-313 St. Augustinegrass. A) Nematode numbers per pot (250 cm^3 soil volume) for successive harvests in autoclaved Margate fine sand soil with high OM (7.9% organic matter) and low OM (3.8% organic matter). B) Population changes in different life stages in low-OM soil. C) Population changes in different life stages in high-OM soil. Curves connect means of six observations ± standard error for each treatment combination for each harvest interval.

and 168 days. The plateau in root dry weight of inoculated plants corresponded to a stable phase in nematode population (Fig. 1A), suggesting that density-dependent equilibrium existed between 84 and 168 days after inoculation. In the field, where other stresses can occur, *B. longicaudatus* can cause stand thinning and death of FX-313 about 2 years after establishment in soil fumigated with methyl bromide (2). Density-dependent population growth has been reported for *B. longicaudatus* in field-grown soybean in Florida (5) and for *Belonolaimus* sp. in corn in southeastern Kansas (11), but not for *B. longicaudatus* in corn in Florida (5).

Shoot weight was increased ($P < 0.05$) in inoculated plants compared with controls at 84 and 168 days after inoculation (Fig. 2B). Cumulative trimming weight was unaffected except for a slight ($P < 0.05$) increase at 126 days (data not shown). Plant transpiration was reduced ($P < 0.05$) by nematodes 42 days after inoculation, compared with uninoculated plants (Fig. 2C). Thus, reduced transpiration, which can be observed nondestructively, was an early indicator of other plant damage responses that had to be measured destructively. The reduction of transpiration by nematodes was strongest ($P < 0.0001$) at 84 and 126 days after inoculation, and was last observed 168 days after inoculation ($P < 0.05$). The tendency for reduced transpiration in *B. longicaudatus*-parasitized plants, despite the slight increase in shoot biomass, indicates a possible resistance in vascular conductance.

OM content affected all plant performance variables at multiple dates of observation in both inoculated an uninoculated pots. Shoot weight and transpiration were greater ($P < 0.01$) in the high-OM soil than in low-OM soil from 42 to 210 days after inoculation. Root weight was greater ($P < 0.01$) in the high-OM soil 126 and 210 days after inoculation and was slightly greater ($P < 0.05$) on the day of inoculation. Trimmings were increased in the high-OM soil compared with the low-OM soil from 42 to 126 days after inoculation.

Several performance ratios are interesting. Nematode number per g root dry weight was consistently greater in the low-

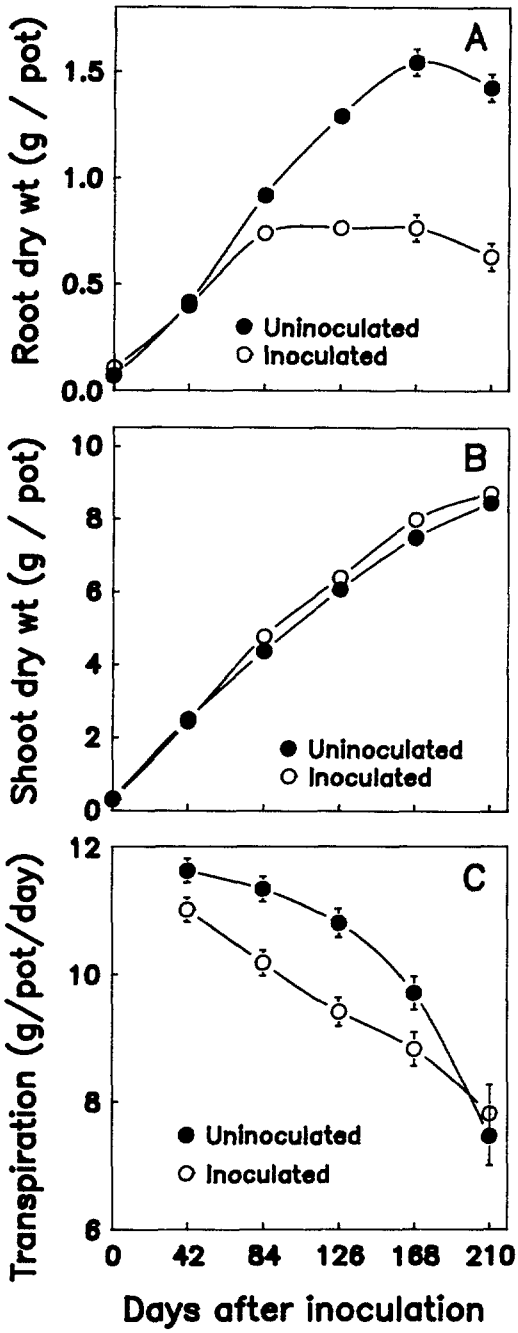

FIG. 2. FX-313 St. Augustinegrass performance indicators over time in response to *Belonolaimus longicaudatus* inoculum. A) Root dry weight B) Shoot dry weight. C) Transpiration. Curves connect means of 12 observations ± standard error for each treatment combination for each harvest interval, except for transpiration data where the n for means declined in increments of 12 for each harvest interval (n = 60 at 42 days to n = 12 at 210 days). High- and low-OM content treatments were pooled.

OM soil (Fig. 3A). When analyzed over time, this effect was significant ($P < 0.05$). The maximum value, 3,733 nematodes per gram root dry weight, was less than the 12,300 previously observed in FX-313 (2). This difference may be attributed to the higher temperature or other factors that prevailed in the previous study (2). The relative effect of inoculum on root dry weight [100% × (inoculated − uninoculated)/uninoculated] showed a progressively more severe divergence after 42 days (Fig. 3B). By 210 days, uninoculated root dry weight was 227% of inoculated root dry weight. At 126 days, roots in the

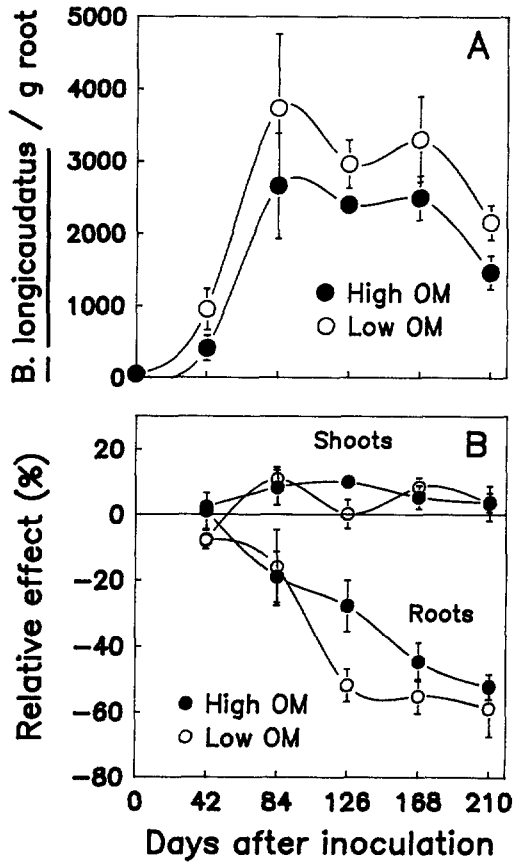

FIG. 3. Performance ratios derived from *Belonolaimus longicaudatus* and FX-313 St. Augustinegrass responses over time. A) Nematode density relative to root dry weight. B) Relative effect of nematode inoculum on root and shoot dry weight [100% × (inoculated − uninoculated)/uninoculated]. Curves connect means of six observations ± standard error for each treatment combination for each harvest interval.

low-OM soil were more reduced ($P < 0.05$) by nematodes, relative to controls, than those in the high-OM soil (Fig. 3B). There was no other inoculation × OM content interaction for any other variable throughout the study.

Physical differences between the two soils might explain plant performance differences. At the rewatering weight of 400 g, the high-OM soil had 94 g plant available water holding capacity (difference from wilted pot weight), compared with only 69 g for the low-OM soil. Soil porosity would also be expected to differ. Despite the obvious soil physical differences, and their direct effect on plant performance, their interaction with nematode stress was slight compared with the differences observed in unautoclaved soil in the field, where other biotic factors may have been present.

St. Augustinegrass grows vegetatively by stolons and is a perennial crop managed for many years. In an annual crop there are discrete starting and stopping points (benchmarks) to associate plant performance with the dynamics of a nematode species. This allows for the development of damage threshold models. Typically, a count of nematodes from soil is taken at the time of planting and is used for predictions of nematode numbers or yield reductions at harvest, which can feed back into management decisions (5,11). Unfortunately, there are no easy benchmarks for the perennial turfgrass ecosystem. A perennial lawn is composed of many plants of different ages juxtaposed over soil with slightly different composition and patches of different below-ground parasites (e.g., *B. longicaudatus*). Turfgrass is slow to show the damage from root defoilation unless stressed, and by the time above-ground symptoms are distinctive, nematode counts can often be used only as a retrospective sign. Much more research is needed to elucidate how different biotic and abiotic variables affect the population dynamics of *B. longicaudatus* in turfgrass.

FX-313 was an excellent model laboratory host for research on the population dynamics of *B. longicaudatus* and should be useful for future expanded studies on host-plant resistance, biological antagonists, and pesticide evaluations for the management of the sting nematode.

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