

Susceptibility of Diploid St. Augustinegrasses to *Belonolaimus longicaudatus*¹

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Abstract: A fine-textured, dwarf St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze) genotype, FX-313, was severely damaged in plots in the third year of evaluation in sandy soil in southern Florida. Damage was associated with numerous (> 40/100-cm³ soil) sting nematodes, *Belonolaimus longicaudatus* Rau. Damage was ameliorated ($P < 0.05$) by fenamiphos applied broadcast at 2.2 g a.i./m², and *B. longicaudatus* numbers were reduced ($P < 0.01$), compared with untreated plots. Root dry weights of four diploid ($2n = 18$) St. Augustinegrasses—FX-261, FX-299, FX-313, and Seville—were reduced ($P < 0.001$) by *B. longicaudatus* in a temperature- and light-controlled experiment. Estimated daily transpiration, an indicator of plant health, was reduced ($P < 0.001$) after 112 days to 3.32 g/pot for inoculated plants, compared with 5.10 g/pot for uninoculated plants. Genotypes did not differ in nematode number per pot (mean 551/215 cm³ soil) 128 days after inoculation, but differed ($P < 0.05$) in nematode numbers on a root dry weight basis, with FX-313 and Seville representing the extremes, 12,300 and 4,000 *B. longicaudatus*/g root dry weight, respectively. The diploid St. Augustinegrasses evaluated were good hosts for *B. longicaudatus*, but field data and controlled inoculation demonstrate genetic variation in susceptibility.

Key words: *Belonolaimus longicaudatus*, breeding, fenamiphos, nematode, resistance, St. Augustinegrass, *Stenotaphrum secundatum*, sting nematode, turfgrass.

St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze, is the most important turfgrass species in Florida (1). The sting nematode, *Belonolaimus longicaudatus* Rau, is pathogenic to St. Augustinegrass (7) and other turfgrasses (6). The damage threshold to turfgrasses has been reported as 10 *B. longicaudatus*/100 cm³ soil (3). Turfgrasses in Florida are often grown in coastal developments on irrigated fine sands, an optimum environment for the sting nematode (9). Sting nematode pathogenicity is probably enhanced in southern Florida by warm soil temperature. The optimum temperature for *B. longicaudatus* reproduction is 25–30 C (9), and the most sting nematodes on limpgrass, *Hemarthria altissima* (Poir.) Stapf & C. E. Hubbard, occurred at soil temperatures from 26 to 34

C (2). Sting nematode damage may also be enhanced by the perennial, shallow-rooted characteristics of most turfgrasses. Depleted oxygen concentrations, as might be expected in deep soil levels, reduce the activity of ectoparasitic nematodes (11), and aeration is critical for the survival of *B. longicaudatus* (9). Multiple annual applications of the nematicide fenamiphos are required to maintain bermudagrass golf greens at many locations in southern Florida. However, fenamiphos cannot legally be applied to home lawns, which are planted with primarily St. Augustinegrass.

The comparative susceptibility of St. Augustinegrass cultivars to phytoparasitic nematodes is not known, except that seven diploid and polyploid genotypes are similar in host suitability to the lance nematode, *Hoplolaimus galeatus* (Cobb) Thorne (4). In contrast to the polyploid ($2n = 32$) cultivar Floratam, which has very coarse texture (broad leaf blade width), diploid ($2n = 18$), St. Augustinegrass cultivars such as Seville (8) are attractive alternatives for use in home lawns because of their fine texture, low growth habit, and improved adaptation to shade (Busey, unpubl.). Additional fine-textured genotypes of St. Augustinegrass have been developed at the Univer-

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sity of Florida, Fort Lauderdale Research and Education Center, with potential applications for home lawns. Unfortunately, during long-term field studies of an ultradwarf, diploid St. Augustinegrass genotype, FX-313, there was rapid decline in plots, probably due to *B. longicaudatus*. The basis for this supposition was that observed numbers of *B. longicaudatus* exceeded the reported damage threshold (3). Damage was delayed or absent in other genotypes. The objective of this study was to determine if FX-313 has greater susceptibility to *B. longicaudatus* than do other diploid St. Augustinegrasses. In addition to field experiments, controlled inoculation was performed on diploids with similar growth habits.

MATERIALS AND METHODS

Field experiments: Twenty St. Augustinegrass genotypes were planted 18 March 1986 in field plots in eight blocks (replicates) at Fort Lauderdale Research and Education Center (Davie, Broward County, FL). Soil was Margate fine sand (siliceous, hyperthermic, Mollic Psammaquent; 94% sand, 5% silt, 1% clay; pH 7.2, 41 mg P/kg, 13 mg K/kg, 5.5% OM), which was fumigated 14 August 1985 with 5.1 g methyl bromide/m². Each plot (4.6 m × 6.7 m) was planted with 24 prerooted sprigs. After transplanting sprigs to the field, plots were irrigated up to 3 times per day during the first 6 months to prevent wind erosion. Thereafter, plots were irrigated in response to wilt. Plots were fertilized with 37 g N/m² during the first 4.5 months. When plots were fully covered 7 months after planting, they were fertilized at 31 g N/m² per year through 1988, and 15 g N/m² in 1989 and in 1990. Fertilizer analysis was 0.16 N, 0.04 P₂O₅, 0.08 K₂O, with micronutrients. Nitrogen source was primarily ammonium nitrate, with 22% of N from isobutylidene diurea. Plots were treated individually with 0.2 g chlorpyrifos/m² or 0.9 g diazinon/m², in response to damage by the southern chinch bug (*Blissus insularis* Barber) and were spot-treated several times per year with 30 g hydramethylnon/

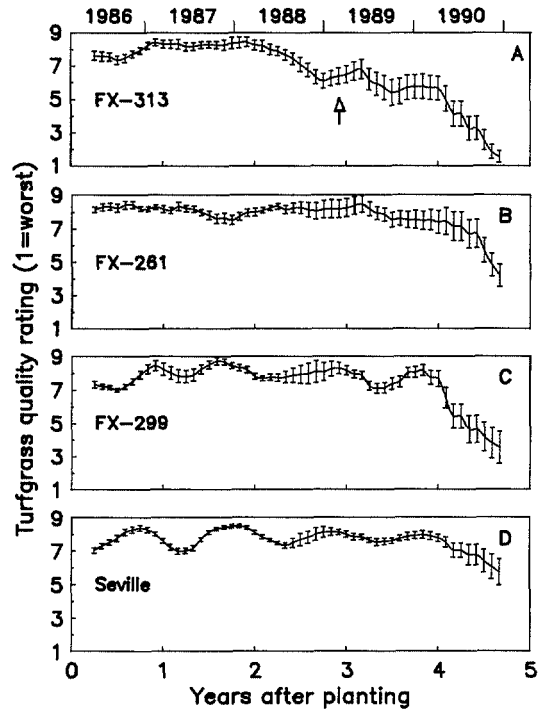


FIG. 1. Monthly turfgrass quality ratings of four diploid St. Augustinegrass genotypes, 1986–90. Curves connect 5-month moving means \pm standard error for each genotype for each month. Arrow signifies the date of first nematode sampling.

mount to control the fire ant (*Solenopsis invicta* Buren). Encroachment by different St. Augustinegrasses across alleyways was prevented by selective application of 0.4 g glyphosate/m². No broadcast pesticides were applied. All plots were mowed at ca. 65 mm with a riding rotary mower at an interval of ca. 2 weeks.

Plots were rated for turfgrass quality, usually once each month, beginning 3 months after planting, on a 1–10 scale: 1 = poorest (grass completely dead) and 10 = best (high cover, dark green, fine texture, and smooth growth habit). Some plots of FX-313 displayed loss of turfgrass quality associated with high numbers of *B. longicaudatus*. For purposes of presenting this trend, 5-month moving means of quality ratings were obtained for FX-313 and three other diploid genotypes, FX-261, FX-299, and Seville (Fig. 1).

Nematode samples were taken 9 Feb-

ruary 1989 from the eight replicated plots of FX-313. For each plot there were two subsamples containing 14–20 random cores taken to a 10–15 cm depth with a cone-shaped sampling tube (2.5-cm-d orifice). Cores were mixed thoroughly within subsamples, and a 100-cm³ aliquant was taken from each subsample for extraction of plant-parasitic nematodes by centrifugation-flotation (5). Subsamples within replications were averaged for analysis and presentation. Each of five replicate plots with high numbers of *B. longicaudatus* received treatments with or without fenamiphos 10G applied broadcast at 2.2 g a.i./m² on 21 February 1989. There were two subsamples for each treatment, and these were pooled in the subsequent analysis. Fenamiphos was reapplied at 2.2 g a.i./m² after 4 weeks because 116 mm of rain fell 10 days after the first treatment. Nematode counts were made 20 April 1989. Root dry weights of turfgrass were determined from cores 10-cm-d by 10-cm deep, with three sub-subsamples within each subsample, all of which were pooled. Visual ratings of turfgrass quality were made before treatment, 13 February 1989, and after treatment, 31 March 1989 and 21 April 1989. Nematicide effect was evaluated within replicates (blocks), and nematicide \times replicate mean square (Error a) was the denominator for determining the significance of nematicide effect mean square.

Controlled inoculation: Nematode-free stolons of FX-299, FX-261, FX-313, and Seville were grown in trays on raised benches. On 6 February 1990, rootless, aerial sprigs (two-node or three-node terminal cuttings ca. 6 cm long) were removed and planted in heat-treated (4 hours at 71 C) Margate fine sand. Following a 4-week rooting period, sprigs were harvested, washed, and weighed. Sprigs were assigned to pairs within genotypes according to similarity of individual sprig weights. For each of eight pairs for each genotype, one sprig would later be assigned randomly to inoculation with *B. longicaudatus*, and the other would serve as an uninoculated control. Sprigs were planted in autoclaved (1

hour at 121 C at 103 kPa) Margate fine sand in square pots (80 mm wide at the top, 60 mm wide at the bottom, 75 mm deep). Moist soil was compacted to within 20 mm of the top of each pot. Sprigs were planted with their roots distributed throughout the soil and with the distal stolon node buried slightly.

Pots were inoculated 13 March 1990 with *B. longicaudatus* from a stock culture maintained on bermudagrass (*Cynodon* sp.). Nematodes were extracted by centrifugal-flotation and hand picked under a dissecting microscope. Fifty *B. longicaudatus*, mostly adults, in 2 ml water were pipetted into a 10-mm-deep soil depression. Pots were distributed randomly between two shelves of a growth chamber. Daily maximum and minimum temperatures averaged 28.8 C \pm 1.6 SD and 22.2 C \pm 1.0 SD, respectively. Fluorescent tubes provided photosynthetic photon flux at turf height of 65 μ mole/m² per second, for 16 hours/day. Pots were watered daily with 20–30 ml water, and pot locations were rearranged once or twice each week. All pots were rewatered to constant weight or flooded to saturation at weekly intervals. Because of a lapse in irrigation during one weekend, several plants died and had to be eliminated from the experiment. Some resulting unpaired plants were reassigned with other plants of similar initial sprig weight, which left six pairs for each genotype (except seven for FX-313). On 6 April 1990, soil from uninoculated pots was added to bring all pots to a uniform soil weight, which resulted in mean saturated pot weight of 399.3 g \pm 2.0 SD (ca. 215 ml).

Pots were moved 3 July 1990 from the initial growth chamber to a laboratory bench to reduce air flow. Daily maximum and minimum temperatures averaged 30.3 C \pm 0.5 SD and 25.2 C \pm 2.0 SD, respectively. Light was 79 μ mole/m² per second (ca. 4% of maximum sunshine at latitude 26°N) for 14 hours/day. Plants did not cover the soil; thus, in order to minimize evaporation from the soil, polystyrene nuggets were placed on the surface of each pot. In-

TABLE 1. Nematode numbers per 100 cm³ soil in field plots of FX-313 St. Augustinegrass.

Nematode	Pretreatment† (February 1989)	Posttreatment (April 1989)		Economic threshold‡
		Control	Fenamiphos	
<i>Belonolaimus longicaudatus</i>	66 ± 23	132 ± 19	9 ± 4**	10
<i>Criconemella ornata</i>	45 ± 43	58 ± 19	25 ± 13	500
<i>Hoplolaimus galeatus</i>	28 ± 32	36 ± 14	24 ± 12	40
<i>Helicotylenchus microlobus</i>	2 ± 3	9 ± 6	1 ± 1	300
<i>Hemicriconemoides annulatus</i>	19 ± 16	47 ± 13	10 ± 4	—
<i>Meloidogyne</i> spp.	14 ± 15	33 ± 10	18 ± 7*	80

All data are means ± standard deviations of 10 observations in five replicates.

*,** Plots treated with fenamiphos differed from controls at $P < 0.05$ and 0.01 , respectively.

† Nematode densities for three untreated plots are not included.

‡ According to Dunn (3).

dividual pot evapotranspiration was determined by weighing the pots daily for 3 days, followed by uniform rewatering to 390, 380, or 370 g, respectively, for each of three successive evapotranspiration trials. Evapotranspiration trials began 112 days after inoculation and lasted for 9 days. Pots were rearranged randomly at least once a day. A pot with a plant cut off at the soil level was used as a control for soil evaporation; its value was subtracted from all other pot values to provide an estimate of transpiration per pot. Because transpiration was similar among trials, and to summarize overall response, separate trials were combined as repeated measures for statistical analysis.

Grasses were harvested after 128 days on 19 July 1990. Leaves, stolons, and other shoot portions were removed from each pot at the soil line, and fresh and dry weights were determined. Soil was washed from the roots, and nematodes were extracted from the entire soil volume as described above. The GLM procedure, a type of general linear model, was used to partition effects and interactions (10). Treatments (inoculated vs. uninoculated) were split plots within pairs within genotypes (main plots), all in a completely randomized design.

RESULTS

Field experiments: Severe decline in turfgrass quality ratings of FX-313 was noticed by July 1988, 28 months after planting (Fig. 1). The symptoms that appeared to be re-

lated to reduced quality ratings were reduced response to fertilization, premature wilting within 2 days after soil moisture saturation, and loss of cover. At the time of nematode sampling, February 1989, one plot had high damage and high numbers of *B. longicaudatus* (92/100-cm³ soil). Four plots in other blocks of the field had moderate damage and numerous *B. longicaudatus* (40 to 90/100-cm³ soil). Those five highly infested plots were used in subsequent fenamiphos treatments. Finally, three plots of FX-313 with low numbers of *B. longicaudatus* (1.3/100-cm³ soil) in 1989 did not show any apparent damage until 1990.

Although other plant-parasitic nematodes were present (Table 1), only *B. longicaudatus* number was correlated ($P < 0.01$) with low quality ratings (Fig. 2). All plots of Seville remained healthy and intact until 1990, when an infestation of southern chinch bug was untreated. Although Seville showed seasonal reduction in quality ratings associated with seedhead production, plots returned to high ratings within each year (Fig. 1). Compared with other genotypes, Seville showed stable quality ratings, evidenced by small standard errors (Fig. 1).

Fenamiphos ameliorated nematode damage in FX-313, evidenced by improved ($P < 0.05$) turfgrass quality ratings compared with untreated controls 31 March 1989, 22 days after the second treatment, and 21 April 1989. High-quality (rating ≥ 7) turf was generally associated (Fig. 2) with

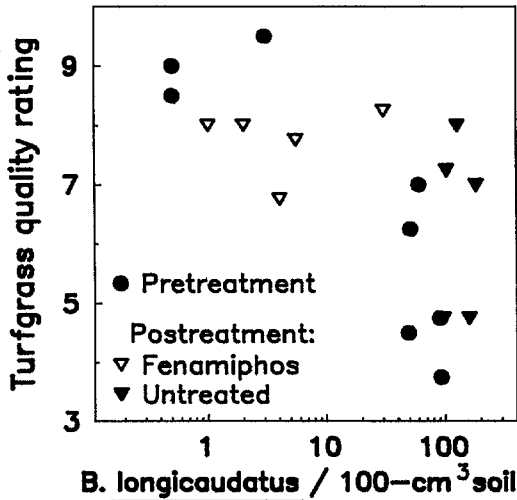


FIG. 2. Turfgrass quality ratings (1 = poorest and 10 = best [high cover, dark green, fine texture, and smooth growth habit]) and densities of *Belonolaimus longicaudatus* for FX-313 field plots. Pretreatment observations include three plots with low densities of *B. longicaudatus* that received no fenamiphos.

low *B. longicaudatus* numbers (< 10 nematodes/100 cm³ soil), in agreement with published thresholds (3). Root mass increased 36% ($P < 0.10$) in nematicide-treated plots compared with controls. There was an 86% reduction in *B. longicaudatus* numbers in treated plots compared with pretreatment numbers, whereas untreated plots showed a 100% increase in *B. longicaudatus* numbers (Table 1). Numbers of one other nematode group, *Meloidogyne* spp., were reduced ($P < 0.05$) after fenamiphos treatment compared with controls, but *Meloidogyne* numbers increased after treatment compared with pretreatment, and were always below economic thresholds (3).

Controlled inoculation: On 5 May 1990, 53 days after inoculation, severely chlorotic leaves were noticed on eight plants inoculated with *B. longicaudatus*. Chlorosis was greatest on young leaves and was only slightly more noticeable in the interveinal regions than intraveinal regions. By 18 July 1990, 127 days after inoculation, most inoculated plants were symptomatic, whereas uninoculated plants were not symptomatic. The frequency of symptoms differed

($P < 0.01$) among genotypes, with six of six FX-299, five of seven FX-313, three of six Seville, and one of six FX-261 plants symptomatic.

Transpiration was reduced 35% in inoculated plants compared with uninoculated plants from 112 days to 121 days post-inoculation (Table 2). Transpiration was estimated for each pot by subtracting the evaporation component of 3.95 g/pot per day for a control pot from which the plant had been cut off. Although the overall genotype \times inoculum interaction was not statistically significant for transpiration, individual *t*-test comparisons were made for each genotype. FX-299 and Seville had no transpiration effect from inoculation with *B. longicaudatus* (Table 2), whereas FX-261 and FX-313 were seriously affected ($P < 0.01$). St. Augustinegrasses grew over the edge of pots but did not fully cover any pot; thus comparison of transpiration estimates is useful only as a bioassay of nematode activity, not as a predictor of field performance.

Nematode inoculation reduced ($P < 0.05$) shoot dry weights 13% and reduced ($P < 0.001$) root dry weights 72% across St. Augustinegrass genotypes (Table 2). Primary roots of inoculated plants were very dark brown and appeared "stubby," with very closely spaced, small (< 2 mm) secondary roots at right angles to the primary roots. Primary roots of control plants were buff to light brown and had dense, white, fine secondary roots, which were elongate and similar in branching pattern to the primary roots. There was no difference in *B. longicaudatus* numbers per pot among St. Augustinegrass genotypes. Nematode numbers among inoculated pots were positively correlated ($P < 0.05$) with root dry weights. This relationship led us to speculate that the extremely high nematode numbers had caused such severe damage to St. Augustinegrass roots that nematode populations might be declining in response to a lack of available roots. This feedback response would predict a compensatory nematode reduction in highly susceptible genotypes, explaining the lack

TABLE 2. Means and statistical analysis of initial sprig fresh weight, transpiration, dry weight, and *Belonolaimus longicaudatus* number and density per gram root dry weight of four St. Augustinegrass genotypes inoculated (I) with 50 *B. longicaudatus* per pot or uninoculated (U).

Genotype	Initial sprig fresh weight (g/pot)		Estimated daily transpiration (g/pot)		Posttreatment dry weight (g/pot)				Final nematode numbers	
	U	I	U	I	Shoot		Root		Per pot	Per g root dry weight
					U	I	U	I	I	I
FX-261	2.09	2.10	6.04	3.98**	1.54	1.32	0.375	0.088**	598	6,700 b
FX-299	1.48	1.48	4.01	2.64	1.40	1.14	0.287	0.062**	539	8,900 ab
FX-313	1.63	1.65	5.00	2.40***	1.56	1.22	0.213	0.059**	610	12,300 a
Seville	2.88	2.88	5.36	4.38	1.77	1.84	0.282	0.119**	446	4,000 b
Mean	2.01	2.01	5.10	3.32***	1.57	1.37*	0.286	0.081***	551	8,200

Analysis of variance:		Mean squares							
Source	df	Initial sprig fresh weight		Estimated daily transpiration		Posttreatment dry weight		Final nematode numbers	
Genotype	3	4.819**	8.671**	0.656*	0.021	34,900	798 × 10 ⁵ *		
Error (a)	21	0.819***	1.153	0.177	0.011	59,700	180 × 10 ⁵		
Inoculum	1	0.000	38.084***	0.434*	0.534***				
Gen × I	3	0.000	1.688	0.100	0.012				
Error (b)	21	0.004	0.718	0.083	0.007				

Means of six replications, except seven replications for FX-313.

*, **, *** U vs. I differ or mean squares significant at $P < 0.05$, 0.01, or 0.001, respectively.

† Values in column with a letter in common are not different by the Waller-Duncan k-ratio *t*-test ($k = 100$).

of significant difference in *B. longicaudatus* numbers among genotypes. An analytic remedy for this was to calculate nematode densities on a root dry weight basis (Table 2). By this interpretation, FX-313 had the highest density of *B. longicaudatus*, 12,300/g root dry weight, which was greater ($P < 0.05$) than FX-261 and Seville (Table 2). Seville had the lowest density of *B. longicaudatus*, 4,000/g root dry weight. These levels were greater than the highest number of *B. longicaudatus*, 128 nematodes/g root dry weight, reported by Rhoades (7).

DISCUSSION

The field evidence for severe pathogenicity of *B. longicaudatus* on FX-313 St. Augustinegrasses was circumstantial but cumulative: 1) declining quality ratings associated with population densities of *B. longicaudatus* ($\geq 40/100\text{-cm}^3$ soil), above accepted thresholds and 2) amelioration of turfgrass quality and increase in root density by fenamiphos, associated with highly significant reduction only in *B. longicaudatus* numbers. Additionally, circumstantial evidence for differential susceptibility

is based on the relatively stable performance of other diploid St. Augustinegrasses in the same sandy field. It is presumed that nematodes were randomly distributed in the fumigated field area and were not brought in with the sprigs during planting.

Controlled inoculation showed that *B. longicaudatus* could colonize and damage all of the tested diploid St. Augustinegrass genotypes. However, host damage was most severe in FX-313 and least severe in Seville. FX-313 and Seville represented the extremes in root dry weight, transpiration, and population density of *B. longicaudatus* per pot and per gram root dry weight. Although only differences in nematode numbers per gram root dry weight were significant ($P < 0.05$), all parameters agreed with differences in decline in the field. FX-313 was the most susceptible, and Seville was the least susceptible. In other areas where FX-313 has been grown (unpubl.), it has provided high turfgrass quality ratings, but in the second year of maintenance in sandy soil with high numbers of *B. longicaudatus*, FX-313 has shown premature wilt, lack of response to fertilization, and

death. In contrast, the polyploid Floratam St. Augustinegrass is grown extensively in fine, irrigated sands throughout Florida, with only occasional decline attributable to *B. longicaudatus*. Additional research is needed to determine whether the severity of *B. longicaudatus* in diploid St. Augustinegrasses would restrict their suitability as lawn grasses.

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