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Parasitism of *Hoplolaimus galeatus* on Diploid and Polyploid St. Augustinegrasses[1]

R. M. Giblin-Davis, P. Busey, and B. J. Center[2]

Abstract: 'Floratam' and 'FX-313' St. Augustinegrasses (*Stenotaphrum secundatum*) were compared in a time-course experiment for host suitability and susceptibility to the lance nematode, *Hoplolaimus galeatus*. Nematode densities were determined in the soil and acid-fuchsin stained roots 42, 84, 126, 168, and 210 days after pots containing 230 cm^3 of autoclaved native Margate fine sand/pot were infested with 104 ± 9 nematodes and maintained at 25 ± 2 C in the laboratory. 'FX-313' was a more suitable host for *H. galeatus*. Numbers of *H. galeatus* reached a maximum at 210 days after inoculation, with 5,550 and 4,120 nematodes (adults plus juveniles)/pot for 'FX-313' and 'Floratam,' respectively. Root and shoot dry weights of both grasses were not affected by *H. galeatus* throughout the experiment. Three polyploid, $2n = 30$ to 32 ('Floratam,' 'FX-10,' and 'Bitterblue') and three diploid, $2n = 18$ ('FX-313,' 'Florida Common,' and 'Seville') *S. secundatum* genotypes were inoculated with *H. galeatus* (99 ± 9/pot) and compared with uninoculated controls 210 days after inoculation. St. Augustinegrass genotypes differed as hosts of *H. galeatus*. 'FX-313' and 'Florida Common' represented the high and low extremes, respectively, for nematode reproduction (9,750 and 5,490 nematodes/pot or 4,239 and 2,387 nematodes/100 cm^3 of soil). However, differences in root and shoot growth were not detected 210 days after inoculation with *H. galeatus*.

Key words: Hoplolaimus galeatus, lance nematode, nematode, population dynamics, resistance, St. Augustinegrass, *Stenotaphrum secundatum,* turfgrass breeding.

St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze, is the most commonly cultivated turfgrass species in Florida (1). The lance nematode, *Hoplolaimus galeatus* (Cobb) Thorne has been associated with poor growth of St. Augustinegrass and is thought to be a moderately pathogenic migratory endoparasite (9,11,13). Forty *H. galeatus*/100 cm^3 soil is the damage threshold for turfgrasses in Florida (5). Seven commonly available cultivars of St. Augustinegrass were shown to have similar host suitability for *H. galeatus* in the greenhouse and in microplots, but nematode population dynamics and nematode-induced pathology were not studied (9). An ultradwarf, diploid St. Augustinegrass ('FX-313') was shown to be a highly suitable and susceptible host for *Belonalaimus longicaudatus* Rau relative to other diploid (3) and polyploid St. Augustinegrass genotypes (2,7). 'FX-313' St. Augustinegrass has been used in controlled laboratory studies to evaluate postplant nematicides (8), natural nematicidal products (12), and host resistance (2). As a susceptible host, 'FX-313' is a good candidate for laboratory studies with *H. galeatus*.

The purpose of this study was to compare diploid and polyploid *S. secundatum* genotypes as hosts for *H. galeatus* and to assess nematode population dynamics inside roots and in soil from the rhizosphere of potted grasses grown in the laboratory. Growth of root and shoots relative to uninoculated controls was assessed.

Materials and Methods

Time-course experiment: Treatments included a nematode inoculum factor (inoculated with *H. galeatus* vs. uninoculated), a St. Augustinegrass ploidy factor ('FX-313' [$2n = 18$] vs. 'Floratam' [$2n \approx 32$]), and a harvest factor (harvested 42, 84, 126, 168, and 210 days after inoculation). The resulting 20 combinations were arranged in a completely randomized design with six replications. In addition to the 120 experimental units, six uninoculated plants of each genotype were harvested on the day of inoculation.

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[2] Professor of Entomology and Nematology, Associate Professor of Environmental Horticulture, and Laboratory Technician, Fort Lauderdale Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 3205 College Ave., Fort Lauderdale, FL 33314–7799.

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Aerial stolons harvested from raised-bed cultures of 'FX-313' and from a field plot of 'Floratam' were washed and planted in autoclaved soil in 26 × 52 cm plastic trays to develop roots. Stolons (6–8 cm long) were terminal cuttings with two or three nodes and weighed 0.74 ± 0.11 g ($\bar{x}$ ± SD) and 4.39 ± 0.50 g/sprig for 'FX-313' and 'Floratam,' respectively. After 14 days, sprigs usually had five nodes, of which three had newly initiated roots. These were transplanted to square tapered pots (80 mm wide at the top, 60 mm wide at the bottom, and 75 mm deep). 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 230 cm^3. Soil type was Margate fine sand (siliceous, hyperthermic, Mollic Psammaquent) at pH 6.5 with 3.8% OM. Soil was sieved (2-mm mesh), thoroughly mixed, and autoclaved. Sprigs were planted with roots distributed throughout the soil, with the proximal stolon node buried slightly.

After 26 days of transplant rooting outside on raised benches, pots were inoculated with *H. galeatus* from a stock culture maintained on St. Augustinegrass. Nematodes were extracted by centrifugal flotation (10). *Hoplolaimus galeatus* (104 ± 9; 4% eggs, 19% J2s, 43% J3-J4s, 17% males, and 17% females) in 1 ml water were pipetted into a single 10-mm-deep soil depression near the most proximal rooted node of each inoculated pot. An additional milliliter of water was added to each inoculation site. Uninoculated controls received 2 ml of deionized water. Pots were placed on a laboratory bench. Soil temperatures ranged between 22 C and 25 C. Photosynthetic photon flux was approximately 170 μmole/m^2/second for 16 hours/day. Pots were fertilized 2 days after inoculation and every 42 days thereafter with 18 mg N, 8 mg P, 15 mg K, 18 μg B, 45 μg Cu, 90 μg Fe, 45 μg Mn, 0.45 μg Mo, and 45 μg Zn/pot, dissolved in 15 ml water/pot. Nitrogen was 40% ammoniacal, 30% nitrate, and 30% urea. Every third day, pots were watered to run-through. Plants were not allowed to wilt. Plants were sprayed once to run-off with the 2FP formulation of fluvalinate applied at the rate of 157 mg a.i./liter to control mites. Plants were trimmed periodically to remove stolons which grew past the edges of the pots. All trimmings were weighed and added to the cumulative shoot harvest (below).

For harvest, the soil was washed from the root ball of each pot, and nematodes were extracted from the entire soil volume by centrifugal flotation (10). Following nematode extraction, roots were cut from the plant stolons. Roots and shoots (leaves plus stolons) were dried at 60 C for 72 hours and weighed. Depending upon the harvest, nematodes were counted and staged in an aliquot representing all, one-half, or one-fifth of the sample. Nematodes in roots were staged and counted in dry-weighed aliquots using a modified acid-fuchsin staining-destaining procedure (4).

Log-transformed nematode numbers and root and shoot dry-weight data were analyzed as a combined experiment in time using a general linear model (14). Root weight proportion was analyzed similarly, without transformation. Class variables were genotype and treatment (inoculated vs. uninoculated); days after inoculation was a linear variable.

St. Augustinegrass genotype evaluation: Six *S. secundatum* genotypes, representing three diploids ($2n$ = 18; 'Florida Common,' 'Seville,' and 'FX-313') and three polypoids ($2n$ = 30–32; 'Bitterblue,' 'Floratam,' and 'FX-10') were evaluated. The 12 treatment combinations (six genotypes inoculated and six uninoculated) were arranged in a randomized complete block design with six replications. All plants were harvested at 210 days after inoculation. Environmental conditions and procedures were the same as for the time-course experiment, except that transplant rooting lasted 23 days and pots were inoculated

with 99 ± 9 *H. galeatus*. Nematode counts were square-root transformed for ANOVA and means comparisons (Waller-Duncan *k*-ratio *t* test). Ploidy levels and ploidy levels × inoculum interactions were tested with single-degree-of-freedom contrasts.

Results and Discussion

Time-course experiment: Nematode numbers were higher on 'FX-313' than 'Floratam' in both soil ($P < 0.01$) and roots ($P < 0.0001$) (Fig. 1A,B). Nematode numbers increased linearly over days after inoculation ($P < 0.0001$). There was no genotype × day interaction between 'FX-313' and 'Floratam' in nematode numbers over time ($P = 0.10$ and 0.91 for soil and roots, respectively). The prediction equation for total nematode number (soil + roots) was $y = 10.3 * 1.028^{days}$ for 'Floratam,' and $y = 17.9 * 1.028^{days}$ for 'FX-313'. The different intercepts and equal slopes are consistent with different effective inoculation rates for the two grasses, followed by equal reproduction rates.

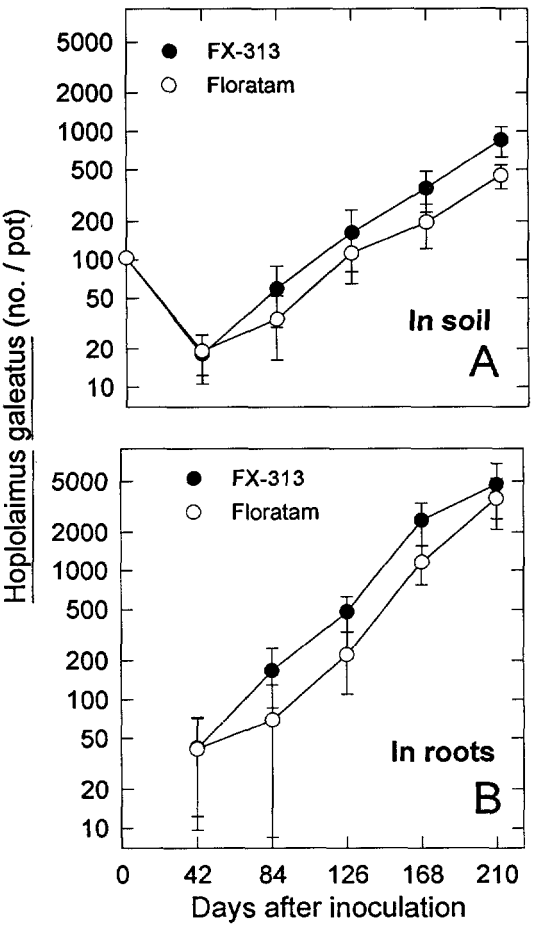

FIG. 1. *Hoplolaimus galeatus* population responses over time on 'FX-313' and 'Floratam' St. Augustinegrass. A) Nematode numbers per pot for harvests from soil. B) Nematode numbers per pot for observations from roots. Lines connect means of six observations ± standard deviation.

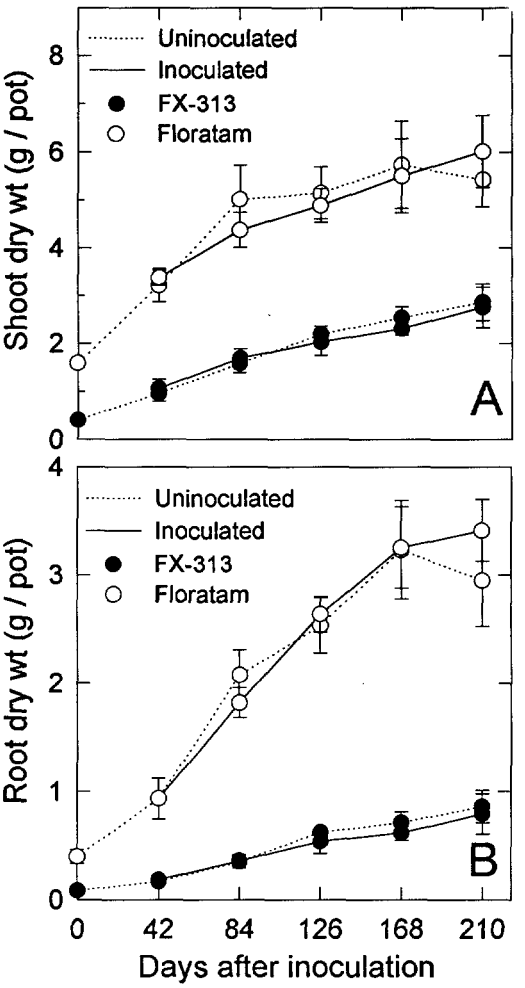

FIG. 2. Effects of inoculation with *Hoplolaimus galeatus* on growth of 'FX-313' and 'Floratam' St. Augustinegrass. A) Shoot dry weight. B) Root dry weight. Lines connect means of six observations ± standard deviation for each treatment for each harvest interval.

Numbers of *H. galeatus* reached a maxi-

TABLE 1. Means and analysis of variance of shoot and root dry weight, root weight ratio, and numbers of *Hoplolaimus galeatus* harvested from six St. Augustinegrass genotypes inoculated (I) with 99 ± 9 nematodes/pot or uninoculated (U).

	Dry weight						Nematodes[a]	
	Shoot total g/pot		Roots g/pot		Ratio		Absolute no./pot	Relative no./g roots
Genotype	U	I	U	I	U	I	I	I
				Diploids				
'FX-313'	4.49	4.35	0.92	0.97	0.23	0.24	9,750 a	10,400 a
'Seville'	5.04	4.81	1.61	1.60	0.29	0.30	9,040 a	5,680 b
'Florida Common'	5.33	5.55	2.12	2.14	0.32	0.32	5,490 b	2,590 d
Mean (diploids)	4.95	4.90	1.55	1.57	0.28	0.29	8,090	6,224
				Polyploids				
'Bitterblue'	5.95	6.25	2.44	2.71	0.33	0.33	9,270 a	3,460 cd
'Floratam'	6.74	6.77	2.81	2.93	0.33	0.34	9,580 a	3,370 cd
'FX-10'	5.95	6.47	1.77	1.72	0.24	0.23	7,490 ab	4,530 bc
Mean (polyploids)	6.21	6.50	2.34	2.45	0.30	0.30	8,780	3,790
Mean (overall)	5.58	5.70	1.95	2.01	0.29	0.29	8,440	5,010

Source	df	Mean squares				
Ploidy	1	36.79***	12.56***	0.0048**	0.08	1.32**
Genotype nested in ploidy	4	2.32***	4.15***	0.0294***	0.41*	1.53***
Inoculum	1	0.25	0.08	0.0000	—	—
Inoculum × ploidy	1	0.51	0.04	0.0001	—	—
Inoculum × genotype nested in ploidy	4	0.18	0.04	0.0002	—	—
Blocks	5	0.06	0.04	0.0005	0.07	0.09
Error	55	0.25	0.07	0.0005	0.13	0.16
CV (%)		8.9	13.1	7.5	33.0	44.1

Means of six replicates.
*,**,*** Mean squares significant, at $P < 0.05$, 0.01, or 0.001, respectively.
[a] Within nematode columns, means followed by the same letter are not different by the Waller-Duncan k-ratio t-test, $k = 100$, $P \approx 0.05$. Nematode numbers (absolute and relative) were square-root transformed for analysis and means comparison, but original values are reported for genotype means and CV%; error degrees of freedom for nematode numbers were 25, not 55.

mum at 210 days after inoculation with 5,550 and 4,120 nematodes (adults + juveniles)/pot for 'FX-313' and 'Floratam,' respectively. About 85% of the nematodes were in the roots of both grasses throughout the experiment. Numbers of eggs (data not shown) showed similar trends. The average frequency of *H. galeatus* juveniles (relative to the total number of adults + juveniles) was 74%. The number of *H. galeatus* reached 7,070 nematodes/g root dry weight at 210 days after inoculation on 'FX-313,' compared with 1,220 nematodes/g root dry weight on 'Floratam' (data not shown).

Shoot and root dry weights, and root weight proportion (root dry weight / root + shoot dry weights), were greater ($P < 0.0001$) for 'Floratam' than for 'FX-313,' which was expected based on different initial sprig sizes (Fig. 2A,B). There was no effect of nematode inoculum on shoot and root dry weights or root weight proportion ($P > 0.66$). There was no interaction of genotype × inoculum or day × inoculum for root and shoot dry weights or root weight proportions ($P > 0.38$). There was a large linear effect of days for all three growth parameters ($P < 0.0001$), indicating uninterrupted growth of both grasses. These data show that under the conditions of this experiment *H. galeatus* was not pathogenic to 'FX-313' and 'Floratam' St. Augustinegrass, despite considerable reproduction.

St. Augustinegrass genotype evaluation: Stenotaphrum secundatum genotypes differed as hosts for *H. galeatus*. Differences

between ploidy levels were not detected, but genotypes differed within ploidy levels ($P < 0.05$). 'FX-313' and 'Florida Common' represented the high and low extremes, respectively, in nematode numbers per pot (Table 1). Nematode numbers were larger for both 'FX-313' and 'Floratam' than in the time course experiment at 210 days after inoculation, though they did not differ from one another.

Nematode inoculum had no overall effect on root or shoot dry weights, or root weight ratio (Table 1). Likewise, there was no ploidy × inoculum interaction and no genotype-nested-in-ploidy × inoculum interaction. These were powerful tests, based on 55 error degrees of freedom, and coefficients of variation of 9% and 13% for shoots and roots, respectively. Nematode numbers per g root dry weight differed ($P < 0.01$) among genotypes, reflecting the genotypic differences in root weights.

Stenotaphrum secundatum genotypes were all suitable hosts for *H. galeatus*, but nematodes did not affect St. Augustinegrass growth for the duration and conditions of the experiments.

The lack of pathogenicity for *H. galeatus* is in marked contrast to experiments on *B. longicaudatus* in which pathogenicity to several *S. secundatum* genotypes has been demonstrated consistently under experimental conditions similar to those used in the present study (2,3,7). Following inoculations of 50 *B. longicaudatus* onto 'FX-313' St. Augustinegrass, nematode densities increased rapidly to >2,000 nematodes/pot (250 cm^3) 84 days after inoculation, leveled, and declined through 210 days (2,7). Root dry weight began to show significant reductions 42 days after inoculation with *B. longicaudatus*, and by 210 days, uninoculated 'FX-313' had >200% the root dry weight of inoculated plants (2,7).

Threshold information is published concerning the numbers of *B. longicaudatus* and *H. galeatus* in soil that justify a post-plant nematicide treatment in turfgrass in Florida (10 and 40/100 cm^3, respectively) (5). Soil counts of *H. galeatus* continuously exceeded 40 nematodes/100 cm^3 for 84 days (betweeen 126 and 210 days after inoculation) on 'FX-313' and 'Floratam' (Fig. 1A), with no effect on plant growth. Soil counts represented only about 10–20% of the total counts of *H. galeatus* per pot.

Future work should examine the possibility of interactions of *H. galeatus* with other species of nematodes (i.e., *B. longicaudatus*) and with other microorganisms in the root rhizosphere (6). Future work should also examine *H. galeatus* pathogenicity to other turfgrass species such as the hybrid bermudagrasses used in golf courses, athletic fields, and cemeteries.

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