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Influence of *Belonolaimus longicaudatus* on Nitrate Leaching in Turf

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Abstract: Experiments were conducted to quantify the effects of the sting nematode (*Belonolaimus longicaudatus*) on root reductions and quantity of nitrate (NO_3^-) leached from 'Tifdwarf' bermudagrass in lysimeters. Forty lysimeters were planted with 'Tifdwarf' bermudagrass, of which 20 were inoculated with *B. longicaudatus* and 20 were noninoculated. Root length was compared between treatments at six, 12, and 18 weeks after initiation of the experiments. Turf was fertilized every three weeks, and leaching events were simulated at 21 and 42-day intervals in trial one and trial two, respectively. Leachate was collected, and the quantity of NO_3^- leached was compared between treatments. Root reductions were observed in lysimeters inoculated with *B. longicaudatus* at all evaluation dates. Quantity of NO_3^- leached was greater in inoculated lysimeters at the 18-week evaluation during both trials. This study indicates that nematode damage to turf roots limits root vigor and N uptake, thereby increasing nitrate leaching, adding to water quality concerns.

Key words: Belonolaimus longicaudatus, bermudagrass, *Cynodon dactylon*, nitrate leaching, sting nematode, turfgrass, management, water quality.

Belonolaimus longicaudatus, the sting nematode, was initially found on several crops including bermudagrass (*Cynodon dactylon*) (Rau, 1958). *Belonolaimus longicaudatus* has an extensive host range and has been recognized as a parasite of many agronomic, horticultural, and ornamental crops (Abu-Gharbieh and Perry, 1970). While it can be devastating to a wide range of crops, *B. longicaudatus* is found predominately in sandy coastal areas of the southeastern United States where soils consist of > 80% sand and < 10% clay with minimal organic matter (Robbins and Barker, 1974; Rhoades, 1980).

Feeding by *B. longicaudatus* usually causes root tips to stop growing, which is particularly devastating to young plants with a developing root system (Perry and Rhoades, 1982; Crow et al., 1997; Huang and Becker, 1997; Crow et al., 2003). Damage caused by *B. longicaudatus* to bermudagrass root systems can decrease water and nutrient uptake and reduce plant growth (Johnson, 1970).

In recent years, heightened environmental awareness has brought water quality and consumption to the forefront of public concern and focused attention on heavy users of water, fertilizers, and pesticides (Haydu and Hodges, 2002). Nitrogen (N) is normally the limiting nutrient in the turfgrass system. Generally, bermudagrass greens are fertilized at 49 kg/ha per month during the growth season. However, grass cultivar, age, pests, pathogens, cultural practices, and management skill can all alter fertility level. Similarly, N source and method of release can alter N availability (Unruh et al., 1999). The intensive use of N fertilizers on golf courses coupled with a 12.7% increase in maintained turf area over the past five years has added to water quality concerns and spurred questions to the fate of N following application. Because plant-parasitic nematodes cause reductions to turf root systems, NO_3^- leaching may be elevated in turfgrass systems. The objective of this study was to determine if damage caused by *B. longicaudatus* to turfgrass roots increases nitrate leaching in 'Tifdwarf' bermudagrass.

Materials and Methods

Two trials were conducted in a glass house at the University of Florida Turfgrass Envirotron in Gainesville, FL, from 29 January 2002 to 13 September 2002 and from 1 November 2002 to 16 April 2003. Data were collected over a 126-d period for each trial.

Trial one: Establishment of experimental units: Forty lysimeters (15-cm-diam.; 45.75-cm high; 8,339-cm^3 volume) were used to simulate a golf course putting green soil profile. In the bottom of the lysimeters was placed 15 cm of gravel (2-mm-diam.) covered with an additional 30 cm of nematode-free U.S. Golf Association (USGA) specification root-zone sand (Anonymous, 1993). The lysimeters were brought to field capacity and weighed. Aerial sprigs of 'Tifdwarf' bermudagrass were planted at 218 kg/ha (0.4 g/lysimeter), after which approximately 0.3 cm of nematode-free sand was added to the surface of each lysimeter. During establishment, the turf was watered 6 times/d starting at 0700 hr at 2-hr intervals with 8 ml of water from a mister irrigation system. Turf was fertilized once, 5 d after planting, with 20-20-20 (N-P_2O_5-K_2O) soluble fertilizer (United Industries Corp., St. Louis, MO). Nutrient inputs were 91.96 kg/ha N, 40.46 kg/ha P, 76.33 kg/ha K, and trace amounts of essential micronutrients. The turf was allowed to establish a root system for 6 wk before being inoculated with nematodes.

Trial one: Nematode inoculation: Following turf establishment, 20 lysimeters were inoculated with *B. longicaudatus* and 20 remained noninoculated, using a com-

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pletely randomized design. Nematode inoculum was obtained from soil collected from a golf course putting green in Palatka, FL. Nematodes were extracted from the soil using a modified Baermann method (McSorley and Frederick, 1991). Inoculum consisted of mixed life stages of *B. longicaudatus* that were hand picked into 10 ml of water. Nematode inoculum was poured into four holes (1-cm-diam. × 2.5-cm-deep) in the soil, adding 138 nematodes/lysimeter, which were then allowed to reproduce for a period of 8 wk.

Trial one: Turf maintenance: Following nematode establishment, turf was watered 3 times/wk. The first and second watering were 150 ml of water per application, followed by a third where measured amounts of water were added to achieve field capacity. Turf was fertilized every 3 wk with potassium nitrate 14-0-46 ($N\text{-}P_2O_5\text{-}K_2O$) soluble fertilizer. Nutrient inputs were 91.96 kg/ha N and 255.35 kg/ha K.

Trial one: Evaluation and sampling: Nematode population counts and root lengths were assessed 6, 12, and 18 wk after nematode establishment. Nematode population counts and root lengths were measured from four, five and 11 replications of each treatment at the 6, 12, and 18 wk evaluations, respectively. Nematode and root samples were obtained by removing the entire 30 cm soil profile above the gravel layer (15-cm-diam.) from each lysimeter. The sample was cut in 7.5 ± 0.1 cm lengths to determine nematode counts and root lengths at four soil depths (0–7.5cm, 7.5–15.0 cm, 15.0–22.5 cm, and 22.5–30.0 cm). Each subsample was placed onto a 135-µm sieve. The roots were rinsed with water, and the sand and nematodes were collected. Rinsates were agitated with water and poured into a 25-µm sieve to catch any *B. longicaudatus* present (Cobb, 1918). Nematodes were collected and counted using an inverted light microscope. Roots were collected, stained with methlyene blue, and refrigerated for at least 24 hr. The stained roots were placed into a glass-bottom tray and scanned with an HP Scanjet 2cx desktop scanner (Hewlett Packard, Boise, ID) to obtain bitmap images of the root system (Pan and Bolton, 1991; Kaspar and Ewing, 1997). The bitmap images were imported into the GSRoot (Louisiana State University, Baton Rouge, LA) software program for analysis. This program is designed to determine root length in millimeters.

Leaching events were simulated using three soil pore volumes of water at 21 ± 1-d intervals. The leaching technique requires the lysimeters be brought to field capacity and then water added that is equal to three times the pore space of the soil (3,750 ml). The leachate from each lysimeter was measured, and a 20 ml subsample was taken for analysis. Samples were analyzed for NO_3^- per liter of water using an air segmented continuous flow auto spectrometer (Flow Solution IV, O.I. Analytical, College Station, TX). The equation (mg NO_3^-/liter × volume of leachate) was used to determine the mg of NO_3^- leached from each lysimeter during leaching events.

Trial two: Establishment of experimental units: Soil profiles, turf, and irrigation were established as previously stated for trial one. Turf was fertilized once, 5 d after planting, with 20-20-20 ($N\text{-}P_2O_5\text{-}K_2O$) soluble fertilizer. Nutrient inputs were 109.3 kg/ha N, 48.09 kg/ha P, 90.72 kg/ha K, and trace amounts of essential micronutrients. The turf was allowed to establish a root system for 3 wk prior to nematode inoculation.

Trial two: Nematode inoculation: Belonolaimus longicaudatus cultures were established from inoculum obtained from Sanford, FL (Giblin-Davis et al., 1992). The cultures were maintained on 'Tifdwarf' bermudagrass grown on nematode-free USGA specification putting green sand mix for several months. Following turf establishment, four lysimeters within each block of eight were inoculated with *B. longicaudatus* using a random complete block design with five replications. *Belonolaimus longicaudatus* were extracted by the decant and sieve method, collected into a beaker, and the volume

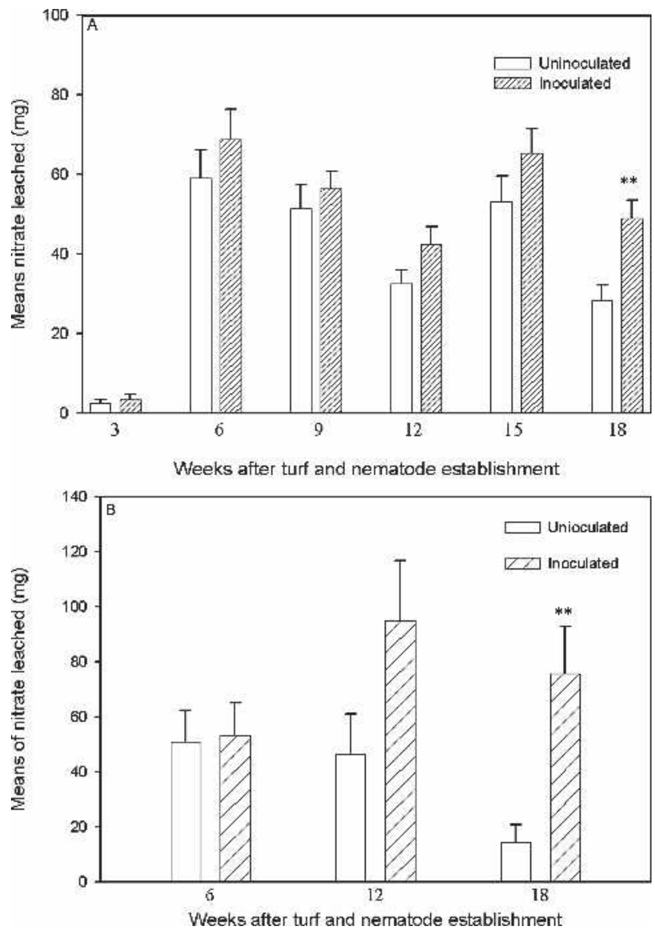

FIG. 1. Effects of inoculating with *Belonolaimus longicaudatus* on mg nitrate leached from lysimeters at 3, 6, 9, 12, 15, and 18, or 6, 12, and 18 wk after turf and nematode establishment during trial one (A) and trial two (B), respectively. Inoculated plants received 138 (trial one) and 300 ± 40 *B. longicaudatus* (trial two), whereas noninoculated plants received no nematodes. Error bars indicate standard error of individual population means.

brought up to 500 ml (Cobb, 1918). One milliliter of water and nematodes was placed onto a counting slide (Hawksley and Sons Limited, Lancing, Sussex, UK) to determine the number of nematodes per ml. Nematode counts were replicated five times with 15 ± 2 nematodes/ml. Nematode inoculum was pipetted into four holes (1-cm-diam. × 2.5-cm-deep) in the soil at 300 ± 40 nematodes/lysimeter and allowed to reproduce for a period of 3 wk.

Trial two: Turf maintenance: Following turf and nematode establishment, the turf was watered twice daily with 25 ± 3 ml of water from an overhead mister irrigation system. Turf was fertilized every 3 wk following leaching events with potassium nitrate 14-0-46 (N-P_2O_5-K_2O) soluble fertilizer. Nutrient inputs were 109.3 kg/ha N and 303.5 kg/ha K.

Trial two: Evaluation and sampling: The sampling and evaluation processes were the same as in trial one, with the following exceptions. Nematode population counts and root lengths were measured from one inoculated and one noninoculated lysimeter from each block at the 6 wk and 12 wk evaluation, leaving two inoculated and two noninoculated lysimeters from each block at the end of the study. Nematode and root samples were obtained from a single core sample (5-cm-diam.) from the middle of each lysimeter, whereas the entire lysimeter volume was used in trial one. Leaching events were simulated at 42 ± 1-d intervals instead of 21-d intervals as in trial one.

Data analysis: Nitrate leached data collected at 6 wk after nematode establishment was transformed by $\sqrt{(x+1)}$. All other data were normally distributed. *T*-tests were performed using SAS (SAS Institute, Cary, NC) to compare noninoculated and inoculated lysimeters for root length and NO_3^- leached at individual sampling dates.

Results

Lysimeters inoculated with *B. longicaudatus* leached more ($P \leq 0.05$) NO_3^- than noninoculated lysimeters at 18 wk after turf and nematode establishment during both trials (Fig. 1). Compared with noninoculated controls, nitrates leached from the inoculated lysimeters were increased from 9% to 72% and 4% to 428% during trial one and trial two, respectively. Differences ($P \leq 0.05$) in root length were observed between noninoculated and inoculated turf at 6, 12, and 18 wk after turf and nematode establishment for both trials (Table 1). Root lengths of inoculated root systems were reduced 30%, 42%, and 43% at 6, 12, and 18 wk after turf and nematodes establishment during trial one and 50%, 66%, and 94% during trial two. *Belonolaimus* populations increased in both trials (Table 2).

Discussion

Differences in NO_3^- leached and root length were observed in both trials. However, differences in root growth, N assimilation, and feeding by *B. longicaudatus* influenced the way these outcomes were expressed in each trial. In both trials, nematode populations reproduced to levels considered damaging (10 nematodes/100 cm^3 of soil) in the root zone (Crow, 2005). In trial one, actively growing root systems were relatively well established prior to inoculation, and *B. longicaudatus*

Table 1. Effects of *Belonolaimus longicaudatus* on 'Tifdwarf' bermudagrass root length at different soil profile depths, 6, 12, and 18 wk after turf and nematode establishment in lysimeters.

	Profile depth (cm)				
Weeks[a]	0 to 7.5	7.5 to 15.0	15.0 to 22.5	22.5 to 30.0	All depths
	Trial one Total Root Length (mm)[b]				
6 (N)	7,104 ± 210[c]	4,335 ± 597	3,043 ± 61	2,528 ± 110	17,011 ± 528
6 (I)	4,570 ± 608**	3,564 ± 268	1,832 ± 132***	1,991 ± 173*	11,957 ± 359***
12 (N)	6,028 ± 151	4,385 ± 335	3,195 ± 78	3,083 ± 157	16,691 ± 297
12 (I)	3,994 ± 566**	2,908 ± 408*	1,445 ± 306*	1,399 ± 120***	9,746 ± 1,221**
18 (N)	6,809 ± 522	3,299 ± 48	2,565 ± 59	2,476 ± 76	15,151 ± 591
18 (I)	3,170 ± 329***	2,459 ± 132***	1,759 ± 157***	1,209 ± 123***	8,597 ± 472***
	Trial two Total Root Length (mm)[b]				
6 (N)[d]	475 ± 73[e]	164 ± 44	89 ± 30	77 ± 32	807 ± 92
6 (I)	216 ± 84*	66 ± 19	74 ± 29	47 ± 26	404 ± 107*
12 (N)	479 ± 128	223 ± 47	144 ± 41	331 ± 118	1,176 ± 232
12 (I)	138 ± 48*	111 ± 30	86 ± 36	71 ± 31	406 ± 85*
18 (N)	1,801 ± 441	1,228 ± 266	777 ± 197	443 ± 115	4,249 ± 958
18 (I)	99 ± 10**	77 ± 9**	48 ± 12**	26 ± 11**	250 ± 25**

*, **, ***Inoculated different from noninoculated at a specified depth.

[a] Weeks after turf and nematode establishment. (N) = noninoculated, (I) = inoculated plants receiving 138 *B. longicaudatus*/lysimeter.

[b] In trial one, root lengths are from the entire volume of the lysimeter for each depth, in trial two root lengths are from a 5-cm-diam. core through the middle of each lysimeter.

[c] Means and mean standard error for 4, 5, and 11 replications at 6, 12, and 18 wk, respectively.

[d] (N) = noninoculated, (I) = inoculated plants receiving 300 ± 40 *B. longicaudatus*/lysimeter.

[e] Means and mean standard error for 5, 5, and 10 replications at 6, 12, and 18 wk, respectively.

Table 2. Counts of *Belonolaimus longicaudatus* in inoculated lysimeters with 'Tifdwarf' bermudagrass at 6, 12, and 18 wk after turf and nematode establishment during trials one and two.

Weeks[a]	Nematodes/lysimeter	Nematodes/100cc of soil
	Trial one[b]	
6	1002 ± 30[c]	12
12	996 ± 186	12
18	392 ± 80	5
	Trial two[d]	
6	1651 ± 374[e]	20
12	3451 ± 161	41
18	4362 ± 458	52

[a] Weeks after turf and nematode establishment.
[b] Inoculated plants received 138 *B. longicaudatus*/lysimeter and were allowed to reproduce for 8 wk.
[c] Means and mean standard error for 4, 5, and 11 replications at 6, 12, and 18 wk, respectively.
[d] Inoculated plants received 300 ± 40 *B. longicaudatus*/lysimeter and were allowed to reproduce for 3 wk.
[e] Means and mean standard error for 5, 5, and 10 replications at 6, 12, and 18 wk, respectively.

reduced the root systems over time. Initially, the root systems may have been able to compensate for root reductions with increased N assimilation throughout the remaining root system. However, later in the experiment, root reductions by *B. longicaudatus* inhibited the plants' ability to assimilate N, which led to the differences observed in NO_3^- leached. Conversely, during trial two, the root systems were slow to establish and develop due to limited light intensity and duration during winter months. Likewise, the period of nematode establishment was reduced from eight weeks, during trial one, to three weeks, during trial two, which reduced the amount of root growth observed. As the experiment progressed, light intensity and duration improved, which led to increased root growth and N assimilation in noninoculated root systems, but feeding by *B. longicaudatus* retarded root development in the inoculated lysimeters. Differences in nitrate leached were not observed until 18 weeks after turf and nematode establishment, when reductions in root length were observed throughout the soil profile. This directly relates to the ability of the entire root system of actively growing turf to assimilate N (Clarkson and Hanson, 1980). Differences in nitrate leached may occur sooner in mature stands of turf with increased lignin and suberin of large diameter roots which can shift the burden of N assimilation to root tips, root hairs, and finer lateral roots where nematode feeding typically occurs.

These trials indicate that *B. longicaudatus* can increase nitrate leaching in turfgrass systems. However, glass house trials are often not good predictors of what happens in the field. Therefore, field studies need to be conducted before the impact that sting nematode has on nitrate leaching in the environment can be estimated. Several factors indicate that nitrate leaching resulting from sting nematode damage in the field may be even greater than shown here. Bermudagrass grown in lysimeters was able to produce some roots despite sizeable populations of sting nematode. In the field, bermudagrass infested with sting nematode often has no roots growing in the soil. In these trials, water and nitrogen rates were kept constant between inoculated and noninoculated treatments. However, most golf course superintendents managing sting nematode infestations increase irrigation and nitrogen application frequency and quantity in an attempt to compensate for the nematode damage, which could further increase the likelihood of leaching.

In conclusion, whether turfgrass root systems were well established or newly forming, damage caused by *B. longicaudatus* to the entire turfgrass root system can increase nitrate leaching. However, the rates of root growth, N assimilation, and feeding by *B. longicaudatus* can all influence the amount of time needed to observe differences in NO_3^- leached.

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