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Interaction Between *Belonolaimus longicaudatus* and *Helicotylenchus pseudorobustus* on Bermudagrass and Seashore Paspalum Hosts

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Abstract: *Belonolaimus longicaudatus* and *Helicotylenchus pseudorobustus* are among the most common nematode parasites of turfgrasses in Florida. Bermudagrass (*Cynodon dactylon* × *C. transvaalensis*) and seashore paspalum (*Paspalum vaginatum*) are the two turf species most commonly used on Florida golf courses. This paper explores the interactions between *B. longicaudatus* and *H. pseudorobustus* on bermudagrass and seashore paspalum hosts. Data collected from thousands of nematode samples submitted to the Florida Nematode Assay Lab over a 8-yr period revealed a negative relationship between *B. longicaudatus* and *H. pseudorobustus* on bermudagrass, but not seashore paspalum. In a multi-year field plot experiment using multiple cultivars of bermudagrass, and seashore paspalum *B. longicaudatus* and *H. pseudorobustus* were negatively related on both turf species. Greenhouse trials where multiple cultivars of both turf species were inoculated with different combinations of *B. longicaudatus* and *H. pseudorobustus* found that each nematode species was inhibitory to the other on both host species. *Belonolaimus longicaudatus* and *H. pseudorobustus* clearly impact each other on turfgrass hosts, although the mechanism of the nematode-nematode interactions is unknown.

Key words: *Belonolaimus longicaudatus*, bermudagrass, *Cynodon dactylon*, *Helicotylenchus pseudorobustus*, interaction, *Paspalum vaginatum*, seashore paspalum, spiral nematode, sting nematode, turfgrass.

Bermudagrass (*Cynodon dactylon* × *C. transvaalensis*) and seashore paspalum (*Paspalum vaginatum*) are common warm season turfgrasses used on golf courses, athletic fields, and lawns in the southeastern United States. Sting nematode (*Belonolaimus longicaudatus*) and spiral nematode (*Helicotylenchus pseudorobustus*) are two of the most commonly encountered ectoparasites associated with bermudagrass and seashore paspalum in this region. A survey of seashore paspalum golf courses and lawns in Florida found that 50% of the golf courses and 40% of the lawns were infested with *B. longicaudatus*, while 88% of the golf courses and 85% of the lawns were infested with *Helicotylenchus* spp. (Hixson and Crow, 2004). High numbers of *Helicotylenchus* spp. (> 500 nematodes/100-cm^3 soil) were often found associated with seashore paspalum in Florida (Hixson and Crow, 2004). A survey of bermudagrass golf courses in Florida found *B. longicaudatus* was the most common nematode present at numbers considered potentially damaging according to the thresholds used by the Florida Agricultural Extension Service (Crow, 2005). *Belonolaimus longicaudatus* was present on 84% of golf courses, 60% of fairways, and 52% of greens from bermudagrass golf courses surveyed and at potentially damaging numbers on 60%, 25%, and 21% of those sites, respectively (Crow, 2005). Both bermudagrass and seashore paspalum are damaged by *B. longicaudatus* and *H. pseudorobustus* (Hixson et al., 2004; Pang et al., 2011a, 2011b, 2011c, 2011d), although *B. longicaudatus* is generally considered the most virulent of the two. Populations of these two nematodes often occur concomitantly on turfgrasses in the field.

Previous studies have observed that when multiple plant-parasitic nematode species are present, the numbers of an individual species are often suppressed as compared with when only a single species is present. On turf, inoculation with *B. longicaudatus*, *Mesocriconema ornatum*, and *Tylenchorhynchus annulatus* singly, and in combination on different bermudagrass cultivars, revealed that nematode numbers for individual species were generally lower in the combination treatments than in the single species treatments (Johnson, 1970). Similarly, presence of *Tylenchorhynchus agri* inhibited reproduction of *Meloidogyne naasi* on *Agrostis palustris* (creeping bentgrass) (Sikora et al., 1972). The mode-of-action for these types of nematode-nematode interactions may be variable with the nematode species and hosts involved. These effects have been shown to be physiologically related through an induced resistance mechanism with some sedentary endoparasitic species on cotton (Aryal et al., 2011a, 2011b). However, effects of ectoparasites on other nematode species might be because of competition for feeding sites or structural changes in plant roots resulting from nematode activity. The more virulent species often has the greater effect. Johnson (1970) noted that effects of *B. longicaudatus*, the more virulent species, on *M. ornatum* and *T. annulatus* were greater than the effects of *M. ornatum* or *T. annulatus* on *B. longicaudatus*. Similarly, other research noted that *B. longicaudatus* caused more root damage and plant stunting of *Zea mays* than *Dolichodorus heterocephalus*, and had greater impact on Pf of *D. heterocephalus* than *D. heterocephalus* did on Pf of *B. longicaudatus* (Rhoades, 1985).

A 2-yr field study conducted to evaluate the responses of eight bermudagrass and three seashore paspalum cultivars to *B. longicaudatus* and *H. pseudorobustus* revealed that bermudagrass was a better host to *B. longicaudatus* than was seashore paspalum, and that seashore paspalum was the better host to *H. pseudorobustus* (Pang et al., 2011b). It was also noted that among bermudagrass cultivars TifSport was the poorest host to *B. longicaudatus* and the best host to *H. pseudorobustus*.

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These observations indicated the possibility of interactions between *B. longicaudatus* and *H. pseudorobustus* on turfgrass hosts. The objective of this study is to quantify the nematode-nematode interactions of *B. longicaudatus* and *H. pseudorobustus* on bermudagrass and seashore paspalum.

Materials and Methods

Database: The Florida Nematode Assay Lab (NAL) is the nematode diagnostic facility of the University of Florida in Gainesville, FL, that specializes in diagnosing nematode problems on turfgrasses. Since August 2005, NAL has used a custom Nematode Assay Database that utilizes Microsoft Access (Microsoft, Redmond, WA) to manage its sample information. The information contained in the database can be sorted based on various parameters including host plant and nematode genera detected. The content of the database from July 2005 to April 2012 was sorted to identify samples collected in Florida where *Belonolaimus* spp. and/or *Helicotylenchus* spp. were detected on bermudagrass (n = 10,484) and seashore paspalum (n = 3,436). For samples containing both nematodes, *Belonolaimus* and *Helicotylenchus* /100 cm^3 of soil were correlated and regressed using SAS software (SAS Institute, Cary, NC) for each of the two hosts individually and combined. Correlation was used to determine the degree to which the two nematodes affected one another, regression was used to define the relationship between them. While each genus was regressed on the other, the slopes, r^2 and P values were identical, only the X intercepts were different. Therefore, only the regression of *Helicotylenchus* on *Belonolaimus* was used to indicate relationships.

Field Study: Regression analysis was applied to the nematode data collected by Pang et al. (2011b) to quantify interrelationships between *B. longicaudatus* and *H. pseudorobustus* on bermudagrass and seashore paspalum. Eight bermudagrass cultivars (Champion, Floradwarf, Tifgreen, MiniVerde, TifEagle, Tifway, Celebration, and TifSport) and three seashore paspalum cultivars (Aloha, SeaDwarf, and SeaIsle 1) were evaluated. Each grass cultivar was grown in 2.25-m^2 plots in a randomized complete block design with five replications in a field naturally infested with both nematode species. Nematode samples were collected from these plots every 3 mon by taking nine soil cores (2.5-cm-diam.) from each plot and extracting nematodes from a 100-cm^3 subsample using a centrifugal flotation method (Jenkins, 1964). The plots were sampled nine times throughout the 2-yr field trial. The number of *H. pseudorobustus* recovered from each plot at each sampling date was regressed on the number of *B. longicaudatus* recovered for each cultivar individually, each species of grass individually, and all grasses combined.

Greenhouse Study: This experiment evaluated the reproduction of *B. longicaudatus* and *H. pseudorobustus* individually and in combination on 'Tifdwarf', 'Celebration', and 'TifSport' bermudagrass, and on 'Aloha' and 'SeaDwarf' seashore paspalum. The experiment was repeated in separate trials. Turf aerial sprigs for each cultivar were collected from greenhouse cultures and planted into UV-stabilized Ray Leach "Cone-tainers"™ (SC10, Stuewe & Sons, Inc., Tangent, OR; 3.8-cm diameter × 21-cm deep) filled with 100% United States Golf Association specification greens sand (USGA, 1993) as described by Pang et al. (2011a, 2011d). After 6 wk, the sprigs had developed enough root system for nematode inoculation. Nematodes for inoculum were reared in pot culture on creeping bentgrass (*Agrostis stolonifera*) and extracted using a decanting and sieving technique (Flegg, 1967). The average number of nematodes were counted from five 1-ml aliquots and extrapolated to the total volume of the stock suspension for each of the two nematodes. A series of *H. pseudorobustus* and *B. longicaudatus* inoculation rates, designed to reveal the effects of each nematode on the other, are shown in Table 1. The appropriate volume of each stock suspension was used to add nematodes in the desired quantities and combinations. Inoculum suspensions were pipetted into a 3-cm-deep hole made in the middle of the Cone-tainer. The holes were covered with a light layer of sand and moistened with a light mist. Cone-tainers were arranged in a randomized complete block design with six replications of each treatment. Experiments were harvested 90 d after inoculation with nematodes. Nematodes were extracted from the entire soil volume of each Cone-tainer by using a centrifugal flotation technique (Jenkins, 1964). The Pf were counted under a microscope. Regression analysis was used to evaluate the interactions between the two nematode species. From Cone-tainers inoculated with 15 *B. longicaudatus*, the Pf of *B. longicaudatus* was

Table 1. Inoculation rates (nematodes/Cone-tainer) for *Helicotylenchus pseudorobustus* and *Belonolaimus longicaudatus* used in the greenhouse study.

Treatment	*H. pseudorobustus*	*B. longicaudatus*
1	15	0
2	0	15
3	15	15
4	15	30
5	15	60
6	30	15
7	60	15

Table 2. Regression of *Helicotylenchus* spp. on *Belonolaimus* spp. from bermudagrass and seashore paspalum samples received by the Florida Nematode Assay Lab where both genera were detected.

Grass	n	Y	r^2	P
Paspalum	211	-0.00771x + 41	0.004	0.34
Bermuda	2,357	-0.01679x + 36	0.002	0.02
Combined	2,568	-0.00875x + 36	0.001	0.09

TABLE 3. Regression of *Helicotylenchus pseudorobustus* on *Belonolaimus longicaudatus* from field plots planted with different bermudagrass and seashore paspalum cultivars, replicated five times, sampled nine times over a 2-yr period.

Cultivar	*n*	*Y*	r^2	*P*
		Seashore paspalum		
Aloha	45	-8.23644x + 1044	0.37	0.0001
SeaDwarf	45	-4.45358x + 698	0.20	0.002
SeaIsle 1	45	-5.39539x + 816	0.32	0.0001
Combined	135	-5.72733x + 837	0.28	0.0001
		Bermudagrass		
Celebration	45	-0.27900x + 40	0.04	0.19
Champion	45	-0.03947x + 40	0.01	0.54
Floradwarf	45	-0.07429x + 13	0.03	0.25
MiniVerde	45	-0.05479x + 22	0.08	0.06
TifEagle	45	-0.12659x + 58	0.09	0.05
Tifgreen	45	-0.22017x + 110	0.06	0.12
TifSport	45	-2.74860x + 380	0.11	0.03
Tifway	45	-0.12749x + 13	0.01	0.51
Combined	360	-0.25100x + 91	0.04	0.0002
		Both grasses		
Combined	495	-1.05723x + 290	0.08	0.0001

regressed on the Pi of *H. pseudorobustus* (0, 15, 30, and 60 nematodes/Cone-tainer) and from Cone-tainers inoculated with 15 *H. pseudorobustus* the Pf of *H. pseudorobustus* was regressed on the Pi of *B. longicaudatus* (0, 15, 30, and 60 nematodes/Cone-tainer). These regressions were performed for each grass cultivar, each grass species, and all grasses combined.

RESULTS

Of the 10,484 bermudagrass submitted to the NAL containing either *Belonolaimus* or *Helicotylenchus*, 45% had contained only *Belonolaimus*, 33% contained only *Helicotylenchus*, and 22% contained both genera. Out of 3,436 seashore paspalum nematode assays that contained either *Belonolaimus* or *Helicotylenchus*, 34% contained only *Belonolaimus*, 59% contained only *Helicotylenchus*, and 6% contained both genera. From samples containing both genera, correlations between *Belonolaimus* and *Helicotylenchus* revealed a negative relationship on bermudagrass (R = 0.04; $P = 0.02$) and on both grasses (R = 0.03; $P = 0.09$). However, the relationship was not significant on seashore paspalum (R = 0.06; $P = 0.34$) (Table 2).

From the field study, *B. longicaudatus* had a negative relationship to *H. pseudorobustus* on bermudagrass ($P = 0.0002$), seashore paspalum ($P < 0.0001$), and on all grasses combined ($P < 0.0001$; Table 3). Relationships were significant on all three individual seashore paspalum cultivars evaluated ($P \leq 0.002$), and on three of the six bermudagrass cultivars evaluated ($P \leq 0.06$).

Results from the greenhouse study revealed that *B. longicaudatus* reduced the Pf of *H. pseudorobustus* on bermudagrass in both trials ($P < 0.0001$), but on seashore paspalum in only one trial ($P = 0.08$; Table 4). *Helicotylenchus pseudorobustus* reduced the Pf of *B. longicaudatus* on seashore paspalum ($P \leq 0.02$) and on bermudagrass ($P \leq 0.002$) in both trials (Table 5).

DISCUSSION

The NAL data found *Belonolaimus* spp. more frequently than *Helicotylenchus* spp. on bermudagrass, 67% and 55%, respectively. On seashore paspalum, *Helicotylenchus* spp. was found more frequently than *Belonolaimus* spp., 65% and 40%, respectively. This parallels the findings of Pang et al. (2011b, 2011d) that seashore paspalum is a better host to *H. pseudorobustus* and poorer host to *B. longicaudatus* than bermudagrass. Mixed populations were more frequently found on bermudagrass than on seashore paspalum. However, the NAL relies on samples submitted by clientele with varying degrees of professional knowledge and the turf species may not have been correctly identified in all cases. In addition, the results from the field and greenhouse experiments indicate that results vary among turfgrass cultivars within a species and the cultivar information is often not included in the information

TABLE 4. Regression of *Helicotylenchus pseudorobustus* Pf after 90 d on *Belonolaimus longicaudatus* Pi in greenhouse trials on multiple cultivars of seashore paspalum and bermudagrass.

	Trial 1			Trial 2		
Cultivar	*Y*	r^2	*P*	*Y*	r^2	*P*
			Seashore paspalum			
Aloha	-0.16710x + 94	0.01	0.6251	-1.04857x + 187	0.20	0.0272
SeaDwarf	-0.76060x + 123	0.13	0.0824	-0.44440x + 171	0.03	0.4399
Combined	-0.48087x + 109	0.06	0.0830	-0.30206x + 178	0.01	0.4259
			Bermudagrass			
Tifdwarf	-1.25240x + 138	0.15	0.0478	-1.72920x + 180	0.40	0.0061
Celebration	-1.60600x + 141	0.48	0.0002	-1.04860x + 187	0.20	0.0272
TifSport	-0.32950x + 72	0.12	0.0932	-0.89530x + 119	0.20	0.0305
Combined	-1.16911x + 125	0.23	0.0001	-1.70066x + 161	0.35	0.0001
			Both grasses			
Combined	-0.89219x + 119	0.16	0.0001	-1.14423x + 168	0.14	0.0001

TABLE 5. Regression of *Belonolaimus longicaudatus* Pf after 90 d on *Helicotylenchus pseudorobustus* Pi in greenhouse trials on multiple cultivars of seashore paspalum and bermudagrass.

Cultivar	Trial 1			Trial 2		
	Y	r^2	P	Y	r^2	P
			Seashore Paspalum			
Aloha	-0.30180x + 41	0.34	0.0033	-0.2467x+37	0.37	0.0016
SeaDwarf	-0.19940x + 44	0.05	0.2983	-0.2603x+34	0.33	0.0063
Combined	-0.25213x + 43	0.11	0.0209	-0.24990x+36	0.32	0.0001
			Bermudagrass			
Tifdwarf	-0.64890x + 83	0.32	0.0039	-0.83680x + 91	0.60	0.0001
Celebration	-0.32320x + 82	0.20	0.0276	0.20790x + 75	0.09	0.0682
TifSport	-0.32950x + 72	0.12	0.0932	-0.52140x + 75	0.23	0.0192
Combined	-0.43386x + 79	0.20	0.0001	-0.37602x + 80	0.14	0.002
			Both grasses			
Combined	-0.37098x + 65	0.11	0.0003	-0.3077x + 61	0.06	0.007

provided to the NAL. Therefore, the relationships between *H. pseudorobustus* and *B. longicaudatus* derived from the NAL data are not as obvious as those obtained from the field and greenhouse experiments.

It is noteworthy that while *B. longicaudatus* is generally considered more damaging to turfgrasses than *H. pseudorobustus*, the greenhouse experiment did not indicate that *B. longicaudatus* had a greater effect on *H. pseudorobustus* than vice versa. This indicates that the suppression mechanism is not simply because of root reductions caused by the more virulent species. It is possible that the mechanism is chemical in nature. Aryal et al. (2011a) observed elevated levels of catalase and peroxidases linked to plant defense responses in cotton infected by *R. reniformis* or *M. incognita*. Another possibility may be direct competition for feeding sites. *Belonolaimus longicaudatus* typically feeds on root tips of turfgrasses (Crow and Han, 2005). Although the feeding habits of *H. pseudorobustus* on turfgrasses has not been studied in detail, on *Z. mays* it feeds as a semiendoparasite on cortical food cells (Vovlas and Inserra, 1985), making direct competition for feeding sites unlikely.

The results of this study strongly support the hypothesis that *B. longicaudatus* and *H. pseudorobustus* are mutually inhibitory on bermudagrass and seashore paspalum hosts. However, the degree that these nematodes impact each other varies with the grass species and cultivar involved. The mechanism of this nematode-nematode interaction remains to be identified. Further research into these types of mechanisms could reveal phytochemicals that could be manipulated as a management tactic in the future.

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