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Effects of Infection by *Belonolaimus longicaudatus* on Rooting Dynamics among St. Augustinegrass and Bermudagrass Genotypes

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Abstract: Understanding rooting dynamics using the minirhizotron technique is useful for cultivar selection and to quantify nematode damage to roots. A 2-yr microplot study including five bermudagrass ('Tifway', *Belonolaimus longicaudatus* susceptible; two commercial cultivars [TifSport and Celebration] and two genotypes ['BA132' and 'PI 291590'], which have been reported to be tolerant to *B. longicaudatus*) and two St. Augustinegrass ('FX 313', susceptible, and 'Floratam' that was reported as tolerant to *B. longicaudatus*) genotypes in a 5 x 2 and 2 x 2 factorial design with four replications, respectively, was initiated in 2012. Two treatments included were uninoculated and *B. longicaudatus* inoculated. In situ root images were captured each month using a minirhizotron camera system from April to September of 2013 and 2014. Mixed models analysis and comparison of least squares means indicated significant differences in root parameters studied across the genotypes and soil depths of both grass species. 'Celebration', 'TifSport' and 'PI 291590' bermudagrass, and 'Floratam' St. Augustinegrass had significantly different root parameters compared to the corresponding susceptible genotypes ($P \leq 0.05$). Only 'TifSport' had no significant root loss when infested with *B. longicaudatus* compared to non-infested. 'Celebration' and 'PI 291590' had significant root loss but retained significantly greater root densities than 'Tifway' in *B. longicaudatus*-infested conditions ($P \leq 0.05$). Root lengths were greater at the 0 to 5 cm depth followed by 5 to 10 and 10 to 15 cm of vertical soil depth for both grass species ($P \leq 0.05$). 'Celebration', 'TifSport', and 'PI 291590' had better root vigor against *B. longicaudatus* compared to Tifway.

Key words: Belonolaimus longicaudatus, bermudagrass, minirhizotron, rooting dynamics, St. Augustinegrass, sting nematode, turfgrass.

Bermudagrass (*Cynodon* spp.) and St. Augustinegrass (*Stenotaphrum secundatum*) are the two most popular and widely used perennial warm-season turf species in the coastal tropics. Bermudagrass produces very high-quality turf with exceptional recuperative potential, and has excellent heat, drought, and wear tolerance compared to other cool- and warm-season turf species, making it the number one choice on sports fields (Beard, 1973; Taliaferro et al., 2004). St. Augustinegrass is primarily used as a lawn grass in residential and commercial landscapes including parks. Turfgrasses are vulnerable to numerous diseases, pests, and plant-parasitic nematodes; and nematodes are among the most important pests of turfgrasses in sandy areas of the coastal plains of the Southeastern United States (Crow, 2005). In this region, *B. longicaudatus* is the most destructive nematode species, impairing the ability of turf roots to take up water and nutrients from soil (Giblin-Davis et al., 1992b; Crow, 2005). In addition to direct root damage, *B. longicaudatus* predisposes turfgrass to adverse conditions like drought and heat stress that could lead to a poor turf quality (Lucas, 1982). *Belonolaimus longicaudatus* can reduce the growth and vigor, which could affect turfgrass quality as a playing surface and its aesthetic appearance.

Management of *B. longicaudatus* becomes extremely important for highly maintained turf, particularly in golf courses. Use of a limited number of chemical nematicides is the primary nematode management option in turfgrass (Crow et al., 2005). Reliance on chemical options as the only management strategy for any pest is not ideal. Bermudagrass lacks resistance against most plant-parasitic nematodes including *B. longicaudatus*. However, nematode tolerance may offer an acceptable alternative to resistance. Plant tolerance can be defined in several ways. D'Arcy et al. (2001) defined tolerance as the ability of a plant to endure an infectious or noninfectious disease without serious damage or yield loss. In another definition, crops tolerant to pests and diseases are able to produce higher yields or a better-quality end product than would be produced by a susceptible crop without the use of pesticides (Tolmay, 2007). Based on the aforementioned definition, in bermudagrass, Pang et al. (2011a) described two types of tolerance to *B. longicaudatus*, Type-I and Type-II, based on root production. Type I tolerance is present in a genotype that suffers minimal or no root loss from the nematode, and Type II tolerance is present in a genotype that is able to produce significantly more roots compared with a standard genotype in the presence of the nematode. Thus, priorities are increasingly focused on understanding turfgrass rooting dynamics that will help develop cultivars that are damaged less by nematodes than those used in the past. Deeper and denser rooting turfgrass species also could reduce the frequency of irrigation and fertilizer applications (McCarty and Miller, 2002) and facilitate sod installation. Turfgrasses that develop an extensive root system may tolerate nematode damage while having enhanced water and nutrient extraction efficiencies from deeper in the soil profile where nematode root damage is often decreased (Hurd, 1975; Qian et al., 1997). Evaluation and selection of turfgrass species with deep and extensive root systems that better capture available soil water should be emphasized.

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In general, nematode population densities and root mass are negatively correlated with rooting depth. Bermudagrasses offer deep, extensive, and fibrous rooting patterns and genetic tolerance against low mowing heights compared with other warm-season turfgrasses (Carrow, 1996). Turfgrasses are frequently maintained at low mowing heights, depending on their use, which limits most root growth to the upper 10 cm of the soil profile (Madison, 1971). Golfers prefer dense green fairways, and smooth and fast rolling putting green conditions; however, low mowing heights reduce shoot growth and photosynthesis, rooting, and rhizome development (Beard, 1973). In such conditions, the presence of nematodes that feed on and damage turfgrass roots could limit root growth presenting problems for turf managers.

In most field experiments, study of rooting behavior or root morphology is labor intensive and requires at least partial destructive sampling (Bohm, 1979). Minirhizotron root observations can be a good choice for estimating and evaluating in situ rooting dynamics of turfgrasses in a nondestructive manner over time (Murphy et al., 1994). The minirhizotron allows monitoring of rooting patterns with a unique technique that involves tracking individual roots growing adjacent to a clear plastic tube with the help of a miniature camera system (Liu and Huang, 2002).

Bermudagrass genotypes tolerance to *B. longicaudatus* were found in various sources. Tolerant bermudagrass genotypes include 'Celebration', 'Princess 77', 'TifSport', 'PI 291590', and 'BA132' (Pang et al., 2011a, 2011b). Similarly, 'Floratam' St. Augustinegrass and other polyploids were found to be tolerant of *B. longicaudatus* compared with 'FX 313' and other diploids (Busey et al., 1993; Giblin-Davis et al., 1992a). However, these earlier studies were conducted under greenhouse conditions and involved destructive root sampling techniques.

Identification of deeper and denser rooted turfgrass genotypes that have higher nutrient and water use efficiency would benefit the integrated approach of nematode management. The specific objectives of this study were the following: (i) to evaluate the effects of infection by *B. longicaudatus* on rooting dynamics among St. Augustinegrass and bermudagrass genotypes, (ii) to evaluate the vertical distribution of root density among St. Augustinegrass and bermudagrass genotypes under *B. longicaudatus*-infested conditions, and (iii) to evaluate the relative tolerance of five bermudagrass and two St. Augustinegrass genotypes against *B. longicaudatus*.

Materials and Methods

Experimental design and nematode inoculum: A 2-yr microplot experiment was conducted from September 2012 to September 2014 on the campus of the University of Florida, Gainesville, FL (29°39′ North, 82°19′ West). The experiment included a 5 × 2 and 2 × 2 factorial arrangement of treatments with four replications for bermudagrass and St. Augustinegrass, respectively. The five bermudagrass genotypes evaluated were the susceptible cultivar (Tifway), plus two commercial cultivars (TifSport and Celebration), and two experimental germplasm lines ('BA132' and 'PI 291590') that were previously identified as tolerant to *B. longicaudatus* (Pang et al., 2011a, 2011b). The two St. Augustinegrass genotypes included a known highly susceptible diploid germplasm ('FX 313') and a common commercial polyploid cultivar (Floratam) that demonstrated tolerance to *B. longicaudatus* in greenhouse trials (Busey et al., 1993). Two nematode treatments were an uninoculated control and *B. longicaudatus* inoculated. Inoculum for *B. longicaudatus was* obtained from greenhouse cultures maintained on 'FX 313' St. Augustinegrass grown in clay pots filled with United States Golf Association (USGA) specification putting green sand (USGA, 1993). All stages (adult and juvenile) of *B. longicaudatus* were obtained from pure cultures using Cobb's decanting and sieving technique (Cobb, 1918) and modified Baermann method (McSorley and Frederick, 1991). Nematodes were collected on a 25-μm (500-mesh) sieve. The average number of adults and juveniles was determined from five replicates of 1-ml aliquots. Final numbers of nematodes were extrapolated to the corresponding volume of the suspension and stored in a refrigerator until inoculation.

Minirhizotron tube installation and imaging: To study rooting dynamics of bermudagrass and St. Augustinegrass, a transparent (5.1 cm-inner diameter and 45-cm-long) minirhizotron tube was inserted through a hole made by a hole-cutter saw in each microplot (total 56; 20.32 cm inner diameter and 45-cm deep PVC pipe) at a 45° angle to the soil surface, with 25 cm of the tube extending past the upper hole in the microplot (Fig. 1) in August 2012. The portion of the minirhizotron tubes exposed outside of the microplots was spray-painted black and the end hole was covered with a cap to prevent the entry of light and moisture. Each microplot was filled with soil (2.8% clay, 3.14% silt, 94.06% sand, 4.4% organic matter, and pH 5.1). This soil was earlier tested for growth of bermudagrass and reproduction of *B. longicaudatus* and was found to be ideal for both (W. T. Crow, unpubl. data). In situ root images (1.3 × 1.8 cm in size) were taken at 1.3-cm intervals along the minirhizotron tube (0.95-cm intervals of vertical soil depth) starting at ~1 cm soil depth, and extending to ~21 cm in the tube from the first location of imaging, or up to ~15 cm vertical soil depth using a BTC I-CAP digital camera system (Bartz Technology Corporation, Carpinteria, CA). Images were captured at the middle of each month from April to September of 2013 and 2014. Every root in each image was manually traced to determine the individual root length, surface area, volume, and average root diameter using WinRHIZO Tron software (Regent Instruments Inc., Quebec City,

FIG. 1. Microplot study of rooting dynamics of bermudagrass and St. Augustinegrass genotypes using minirhizotron method.

Quebec, Canada). Final results were extrapolated into three vertical soil depths of 0 to 5 cm (Zone 1), 5 to10 cm (Zone 2), and 10 to 15 cm (Zone 3).

Grass propagation, transplantation, and inoculation: In September 2012, propagation material for each bermudagrass and St. Augustinegrass genotype was obtained from established sod at the University of Florida Forage Research Unit, Hague, FL. Sod was washed free of soil and immersed into 0.5% sodium hypochlorite solution (Clorox® Regular Bleach; The Clorox Company, Oakland, CA) for 5 min to obtain pathogen-free stolons and rhizomes. Seventy-two-celled plastic trays (each cell dimension: 580 mm × 280 mm × 55 mm) filled with soil-mix (diatomite + perlite + compost) were used for propagating each genotype. A single sprig with two to three nodes of stolon or rhizome of bermudagrass or a stolon of St. Augustinegrass was planted into each cell. A total of seven trays were maintained in the greenhouse facility at the University of Florida with temperature ranging from 24°C to 34°C with natural daylight. Every week grasses were fertilized with all-purpose plant food (Miracle-Gro®; N, P_2O_5, K_2O—24:8:16, The Scotts Miracle-Gro Company, Marysville, OH) at a rate of 50 kg N/ha and irrigated for 2 min three times a day for 6 wk of the plug establishment period, and hand-mowed as needed. Well-established plugs for each genotype were transplanted from the greenhouse into the respective microplots on 2 November 2012, with one plug per microplot. Irrigation was given for 2 min three times a day for the first 2 wk and thereafter as needed. Fertilizer (N, P_2O_5, K_2O—10:10:10) was applied monthly at a rate of 50 kg N/ha during active growing season. Grasses were mowed (bermudagrass at height of ~2.5 cm and St. Augustinegrass ~6 cm) with a hand-held clipper every week throughout the study and clippings were removed. Applications of bifenthrin (Bifen I/T; Control Solutions, Pasadena, TX) were made for management of insect pests as required throughout the experiment. Nematodes were inoculated into the respective microplots three times over the course of the experiment, in February 2013, August 2013, and April 2014. At each inoculation, a suspension containing ~150 *B. longicaudatus* was inoculated into three holes (3-cm deep) made in each microplot (~50 nematodes/hole). Population densities of *B. longicaudatus* among bermudagrass and St. Augustinegrass genotypes were assessed at the end of the experiment. A single core (1.27-cm wide × 15-cm deep) was taken from the center surface of each microplot and nematodes were extracted from 50 cm^3 soil by sugar flotation with centrifugation for counting.

Data analysis: Data from the active growing season (April through September) from each year were considered for statistical analysis. Specific root parameters such as total root length (mm), adjusted root diameter (mm/10), average volume (mm^3), and surface area (mm^2) were analyzed using the general linear mixed models procedure of SAS (SAS Institute Inc., Cary, NC). Data from both years were pooled, and year and replications within a trial were considered as random effects. Means were separated by comparison of Tukey-Kramer least squares means ($P \leq 0.05$). Adjusted root diameter was calculated using following formula:

$$\text{Adjusted diameter} = \frac{\sum^{(LxD)}}{\sum^{L}}$$

where L is the total root length and D is the average root diameter.

RESULTS

Genotypic effect on rooting dynamics and population density of B. longicaudatus: Analysis of variance showed that there were significant genotypic effects on root length, surface area, volume, and average diameter of bermudagrass and St. Augustinegrass (Tables 1 and 2). At the end of the experiment, there were no differences in population densities of *B. longicaudatus* (Fig. 2) among bermudagrass genotypes. 'Celebration' and 'PI 291590' had significantly greater root length, root surface area, root volume, and average root diameter compared with the standard bermudagrass cultivar Tifway throughout the active growing season, i.e., mid-April to mid-September, except for April ($P \leq 0.05$). 'TifSport' had greater root length from July to September, root surface area and root volume throughout the active growing season, and average root diameter from May to July and September compared to 'Tifway' (Table 5). 'BA132' was the poorest performer among the bermudagrass genotypes and had less root length compared with 'Tifway' (Table 5) although it had root surface area, root volume, and average root diameter (except July) similar to 'Tifway'. The St. Augustinegrass genotype 'Floratam' had significantly lower numbers of *B. longicaudatus* at

TABLE 1. Analysis of variance (Type III test of fixed effects) for rooting dynamics among bermudagrass genotypes. Data are combined from 2013 and 2014.

Effect	Month					
	April	May	June	July	August	September
	Total root length (mm)					
Genotype (G)	***	***	***	***	***	***
Nematode (N)	***	***	***	***	***	***
Vertical soil depth (D)	***	***	***	***	***	***
G × N	***	***	***	***	***	***
G × D	NS	NS	NS	NS	NS	NS
D × N	NS	NS	NS	NS	NS	NS
G × N × D	NS	NS	NS	NS	NS	NS
	Total root surface area (mm^2)					
Genotype (G)	***	***	***	***	***	***
Nematode (N)	***	***	***	***	***	***
Vertical soil depth (D)	***	***	***	***	***	***
G × N	***	***	***	***	***	***
G × D	NS	NS	NS	NS	NS	NS
D × N	NS	NS	NS	NS	NS	NS
G × N × D	NS	NS	NS	NS	NS	**
	Total root volume (mm^3)					
Genotype (G)	***	***	***	***	***	***
Nematode (N)	***	***	***	***	***	***
Vertical soil depth (D)	***	***	***	***	***	***
G × N	**	**	***	***	***	***
G × D	**	NS	NS	NS	NS	NS
D × N	NS	NS	NS	NS	NS	NS
G × N × D	NS	NS	**	**	**	**
	Adjusted average root diameter (mm/10)					
Genotype (G)	NS	**	***	***	**	***
Nematode (N)	***	***	***	***	***	***
Vertical soil depth (D)	**	***	**	***	**	**
G × N	**	**	**	**	NS	NS
G × D	NS	NS	NS	NS	NS	NS
D × N	NS	NS	NS	NS	NS	NS
G × N × D	**	**	**	**	**	**

Double asterisks and triple asterisks indicate level of significance at $P \leq 0.05$ and 0.01, respectively, and NS is not significant ($P > 0.05$).

TABLE 2. Analysis of variance (Type III test of fixed effects) for rooting dynamics of St. Augustinegrass genotypes. Data are combined from 2013 and 2014.

Effect	Month					
	April	May	June	July	August	September
	Total root length (mm)					
Genotype (G)	**	***	**	**	**	**
Nematode (N)	***	***	***	***	***	***
Vertical soil depth (D)	***	***	***	***	***	***
G× N	NS	NS	NS	**	**	NS
G × D	NS	NS	NS	NS	NS	NS
D × N	NS	NS	NS	NS	NS	NS
G × N*D	NS	NS	NS	NS	NS	NS
	Total root surface area (mm^2)					
Genotype (G)	***	***	***	***	***	***
Nematode (N)	***	***	***	***	***	***
Vertical soil depth (D)	***	***	***	***	***	***
G × N	NS	NS	NS	NS	NS	NS
G × D	NS	NS	NS	NS	NS	NS
D × N	NS	NS	NS	NS	NS	NS
G × N*D	NS	NS	NS	NS	NS	NS
	Total root volume (mm^3)					
Genotype (G)	***	***	***	***	***	***
Nematode (N)	***	***	***	***	***	***
Vertical soil depth (D)	**	***	***	***	***	***
G × N	NS	NS	NS	NS	NS	**
G × D	NS	NS	NS	NS	NS	NS
D × N	NS	NS	NS	NS	NS	**
G × N*D	NS	NS	NS	NS	NS	NS
	Adjusted average root diameter (mm/10)					
Genotype (G)	**	**	**	**	**	**
Nematode (N)	***	***	***	***	***	***
Vertical soil depth (D)	NS	NS	NS	NS	NS	NS
G × N	NS	NS	NS	NS	NS	NS
G × *D	NS	NS	NS	NS	NS	NS
D × N	NS	NS	NS	NS	NS	**
G × N*D	NS	NS	NS	NS	**	**

Double asterisks and triple asterisks indicate level of significance at $P \leq 0.05$ and 0.01, respectively, and NS is not significant ($P > 0.05$).

the end of the experiment (Fig. 2) and significantly greater root length, root surface area, root volume, and average root diameter during the study than 'FX 313' ($P \leq 0.05$) (Table 5).

Effect of B. longicaudatus on rooting dynamics: Infection by *B. longicaudatus* had a substantial effect on rooting behavior of bermudagrass and St. Augustinegrass across genotypes (Tables 1 and 2). Root parameters such as root length, root surface area, and root volume were significantly reduced when *B. longicaudatus* was inoculated onto either bermudagrass or St. Augustinegrass across genotypes throughout the active growing season ($P \leq 0.05$). However, *B. longicaudatus* infestation significantly increased the average root diameter of bermudagrass and St. Augustinegrass genotypes for the same period of time (Table 3).

Vertical distribution of root parameters: Rooting dynamics of bermudagrass and St. Augustinegrass were significantly affected by vertical depth in the soil profile across genotypes (Tables 1 and 2). Root length was greatest from 0 to 5 cm (Zone 1) followed by 5 to 10 cm (Zone 2) and 10 to 15 cm (Zone 3) for both grass species during the active growing season ($P \leq 0.05$). Bermudagrass root surface area was similar in Zones 1 and 2 but these Zones had greater root surface area than Zone 3 in April and May; however, from July through September root surface area was significantly higher in Zone 1 followed by Zones 2 and 3 (Table 4). Average root diameter of bermudagrass was lower in Zone 1 than in Zone 2 during the active growing season ($P \leq 0.05$); however, it was similar to Zone 3 in April, June and July.

St. Augustinegrass root surface area was similar in Zones 1 and 2, and these Zones were greater than Zone 3 in April, but from May through September surface area was highest in Zone 1 followed by Zones 2 and 3. Total root volume was similar in Zones 1 and 2, and these Zones were greater than Zone 3 for both grass species throughout the active growing season (Table 4). Average root diameter of St. Augustinegrass was not affected by the vertical soil depth (Table 4).

Host × nematode interaction on rooting dynamics: Statistical analysis identified a significant host × nematode

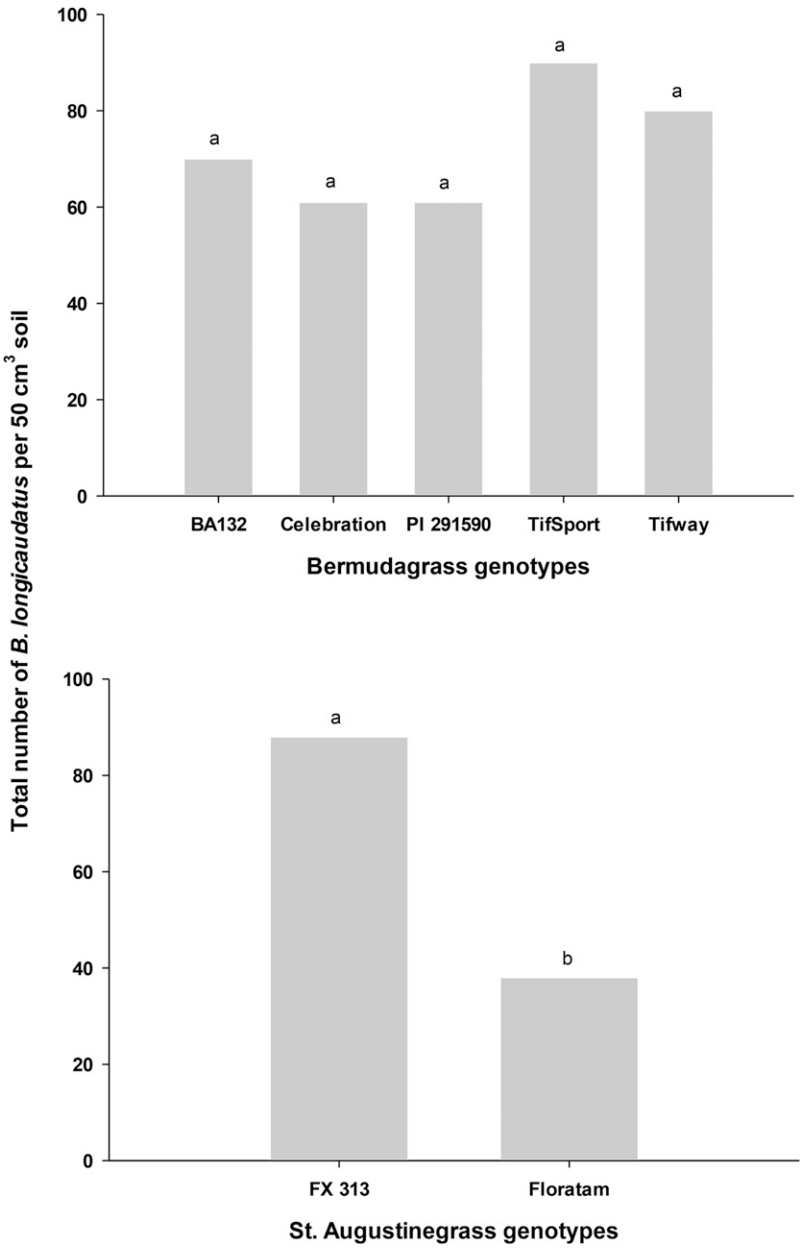

FIG. 2. *Belonolaimus longicaudatus* population densities among bermudagrass and St. Augustinegrass genotypes in September 2014. Means (four replications) with the same letter are not different according to comparison of Tukey-Kramer least squares means ($P \leq 0.05$).

interaction for bermudagrass but not for St. Augustinegrass. For Bermudagrass, each measured parameter had a significant genotype × nematode interaction ($P \leq 0.05$, Tables 1 and 2). Also, bermudagrass genotypes had significant three-way interaction among independent variables (genotype, nematode, and depth) (Table 1; data not shown). Root lengths of all bermudagrass genotypes except 'TifSport' were less in microplots inoculated with *B. longicaudatus* compared with the respective uninoculated microplots during the active growing season (Table 5). 'Celebration', 'PI 291590', and 'TifSport' all had greater root lengths than 'Tifway' and 'BA132' in microplots inoculated with *B. longicaudatus*. 'BA132' was the genotype most affected by *B. longicaudatus* infection, having reduced root lengths from June to September compared to the other four genotypes. *Belonolaimus longicaudatus* infestation caused reduction in root surface area of all bermudagrass genotypes except 'TifSport' throughout the active growing season, and 'Celebration' in April and May, compared to the uninoculated control (Table 5). Uninoculated 'BA132', 'TifSport', and 'Tifway' had similar root surface area compared with inoculated 'Celebration', 'PI 291590', and 'TifSport' throughout the growing season (Table 5). In uninoculated microplots, only 'PI 291590' had significantly greater surface area than 'Tifway'. However, *B. longicaudatus* infestation caused greater reduction of root surface area on 'Tifway' and 'BA132' than 'Celebration', 'PI 291590', and 'TifSport'.

Total root volume was similar and not affected by inoculation with *B. longicaudatus* for 'Celebration' and

TABLE 3. Effect of infection by *Belonolaimus longicaudatus* across all depths and genotypes on bermudagrass and St. Augustinegrass rooting dynamics. Data are combined from 2013 and 2014.

Nematode treatments	Month					
	April	May	June	July	August	September
			Total root length (mm)			
			Bermudagrass[a]			
Control	518 a[b]	559 a	547 a	570 a	566 a	595 a
B. longicaudatus	348 b	256 b	256 b	253 b	237 b	244 b
			St. Augustinegrass[c]			
Control	413 a	471 a	468 a	473 a	492 a	509 a
B. longicaudatus	100 b	101 b	99 b	101 b	96 b	92 b
			Total root surface area (mm^2)			
			Bermudagrass[a]			
Control	271 a	292 a	291 a	305 a	309 a	323 a
B. longicaudatus	148 b	158 b	161 b	162 b	158 b	159 b
			St. Augustinegrass[c]			
Control	199 a	233 a	237 a	251 a	267 a	277 a
B. longicaudatus	76 b	78 b	77 b	79 b	76 b	71 b
			Total root volume (mm^3)			
			Bermudagrass[a]			
Control	19.1 a	20.4 a	20.5 a	21.4 a	22.0 a	22.8 a
B. longicaudatus	11.3 b	11.6 b	11.9 b	12.2 b	12.1 b	12.4 b
			St. Augustinegrass[c]			
Control	13.7 a	16.6 a	17.0 a	18.5 a	20.0 a	6.1 b
B. longicaudatus	6.6 b	6.8 b	6.8 b	7.0 b	6.7 b	20.6 a
			Adjusted average root diameter (mm/10)			
			Bermudagrass[a]			
Control	1.60 b	1.64 b	1.66 b	1.66 b	1.70 b	1.72 b
B. longicaudatus	2.00 a	1.96 a	2.01 a	2.04 a	2.10 a	2.70 a
			St. Augustinegrass[c]			
Control	1.44 b	1.49 b	1.56 b	1.61 b	1.69 b	1.70 b
B. longicaudatus	2.34 a	2.37 a	2.40 a	2.38 a	2.43 a	2.46 a

[a] Means of 20 replications.
[b] Means in each column for each measured parameter followed by the same letters are not different for corresponding grass types according to comparison of least squares means ($P \leq 0.05$).
[c] Means of eight replications.

'TifSport' (Table 6). Nonetheless, 'BA132', 'PI 291590', and 'Tifway' had reduced root volume in microplots inoculated with *B. longicaudatus* compared to non-inoculated microplots throughout the active growing season ($P \leq 0.05$). In *B. longicaudatus*-inoculated microplots, reductions in root volume were greater for 'Tifway' and 'BA132' than for the other three genotypes. In uninoculated microplots, only 'PI 291590' had greater root volume than 'Tifway' (Table 6). *Belonolaimus longicaudatus* inoculation had no effect on average root diameter of 'PI 291590' and 'TifSport' throughout the active growing season (Table 6). An increase in average root diameter in microplots inoculated with *B. longicaudatus* was observed on 'BA132', 'Celebration' (except April), and 'Tifway' (except May, August, and September) during the active growing season compared to corresponding uninoculated microplots (Table 6).

DISCUSSION

Many root studies have used destructive methods of evaluating rooting dynamics that include ingrowth cores (Steingrobe et al., 2001) and sequential coring (Hertel and Leuschner, 2002). However, with the development of suitable camera technology for minirhizotrons in recent years, this technology is increasingly being used for characterizing the fate of individual roots over time (Taylor et al., 2014). Minirhizotron technology is now widely accepted and has become one of the preferred methods for direct evaluation of dynamic properties of fine roots. This could be a good method to study the in situ changes in rooting dynamics of turf roots damaged by plant-parasitic nematodes. However, despite the advantages, minirhizotron technology offers two disadvantages, i.e., labor-intensive tube installation and time-consuming image analysis on WinRHIZO Tron software (Vamerali et al., 2012).

Rooting dynamics and its parameters continue to be one of the most poorly understood phenomena in turf ecosystems. Furthermore, the evaluation of the effects of plant-parasitic nematodes on rooting dynamics may be misleading due to biases of inappropriate techniques. In the past, damage caused by *B. longicaudatus* to turfgrass roots has been quantified by measuring root depth (Crow et al., 2003), root dry weight

TABLE 4. Effect of vertical soil depth across all genotypes and treatments on bermudagrass and St. Augustinegrass rooting dynamics. Data are combined from 2013 and 2014.

Vertical soil depth (cm)	Month					
	April	May	June	July	August	September
			Total root length (mm)			
			Bermudagrass[a]			
0-5	506 a[b]	548 a	548 a	576 a	564 a	589 a
5-10	468 b	444 b	434 b	435 b	421 b	436 b
10-15	204 c	225 c	220 c	220 c	212 c	222 c
			St. Augustinegrass[c]			
0-5	310 a	339 a	339 a	352 a	358 a	367 a
5-10	233 b	254 b	260 b	266 b	265 b	261 b
10-15	160 c	177 c	165 c	159 c	162 c	167 c
			Total root surface area (mm^2)			
			Bermudagrass[a]			
0-5	260 a	285 a	293 a	310 a	313 a	324 a
5-10	259 a	270 a	267 a	269 b	269 b	273 b
10-15	114 b	125 b	125 b	127 c	124 c	130 c
			St. Augustinegrass[c]			
0-5	176 a	201 a	204 a	216 a	218 a	219 a
5-10	133 a	146 b	152 b	161 b	165 b	162 b
10-15	89 b	98 c	96 c	94 c	99 c	102 c
			Total root volume (mm^3)			
			Bermudagrass[a]			
0-5	17.9 a	19.6 a	20.5 a	21.8 a	22.3 a	22.9 a
5-10	19.7 a	20.4 a	20.2 a	20.4 a	20.7 a	20.7 a
10-15	7.9 b	8.6 b	8.7 b	8.9 b	8.9 b	9.2 b
			St. Augustinegrass[c]			
0-5	13.4 a	15.8 a	16.2 a	17.6 a	17.7 a	17.3 a
5-10	10.2 a	11.4 a	11.7 a	12.7 a	13.2 a	12.9 a
10-15	6.4 b	6.7 b	7.0 b	7.1 b	7.5 b	7.7 b
			Adjusted average root diameter (mm/10)			
			Bermudagrass[a]			
0-5	1.60 b	1.64 b	1.69 b	1.72 b	1.77 b	1.74 b
5-10	2.00 a	1.96 a	1.98 a	1.99 a	2.01 a	1.97 a
10-15	1.80 ab	1.80 a	1.84 ab	1.88 a	1.93 ab	1.90 ab
			St. Augustinegrass[c]			
0-5	1.85 a	1.93 a	1.96 a	1.98 a	1.99 a	1.87 a
5-10	1.93 a	1.96 a	1.98 a	2.07 a	2.16 a	2.22 a
10-15	1.82 a	1.85 a	1.88 a	1.87 a	1.96 a	2.07 a

[a] Means of 40 replications.
[b] Means in each column for each measured parameter followed by the same letters are not different for corresponding grass types according to comparison of least squares means ($P \leq 0.05$).
[c] Means of 16 replications.

(Schwartz et al., 2008), root ash dry weight (Giblin-Davis et al., 1991), root volume by water displacement (Pang et al., 2011c), and root length, diameter, surface area and volume using root scanning software on roots collected in soil cores (Schwartz et al., 2008; Pang et al., 2011b). All of these measurements can give good information, but only the use of minirhizotron allows the measurement of root parameters in situ.

Our bermudagrass results confirm those of Pang et al. (2011a, 2011b) such that 'Celebration' and 'PI 291590' exhibited a high degree of Type II tolerance, having a larger inherent root system that is likely to perform better when infested with *B. longicaudatus* than a standard cultivar (Tifway). We also confirm that 'TifSport' exhibits Type I tolerance, having minimal root loss from *B. longicaudatus*. Our results differed from Pang et al. (2011b) in that we found 'BA132' was a poor-rooting genotype whereas Pang et al. found it to produce significantly greater root lengths than Tifway. Pang et al. (2011b) conducted their experiments under greenhouse conditions and under a short time-frame (90 d) while the current experiment was conducted in microplots under field conditions for a 2-yr period. The 'BA132' genotype also has been evaluated in large field plots where it performed poorly after the first year of a 3-yr trial (Aryal, 2015). The different environmental conditions in the field versus the greenhouse may explain the different results in the studies, and highlights the need to go beyond greenhouse screening for genotype selection.

Our results on overall comparison of root parameters studied suggested that genotypic variability was the key factor that determines the rooting dynamics of a

TABLE 5. Effect of genotypes and *Belonolaimus longicaudatus* interaction on bermudagrass total root length and total surface area. Data are combined from 2013 and 2014.

Genotypes	Treatments	Month						
		April	May	June	July	August	September	Mean[a]
		Total root length (mm)[b]						
BA132	Control	491 ab[c]	511 abc	513 abc	511 bc	498 abc	523 b	508 b
BA132	Inoculated	107 e	104 f	95 f	89 g	78 f	82 f	92 f
Celebration	Control	567 a	643 a	607 a	635 a	644 a	697 a	632 a
Celebration	Inoculated	333 cd	367 de	358 d	345 e	342 d	362 cd	351 cd
PI 291590	Control	564 a	615 a	608 a	647 a	637 a	685 a	629 a
PI 291590	Inoculated	271 d	315 e	333 d	346 e	313 d	332 d	447 d
TifSport	Control	430 bc	452 bcd	441 bcd	487 cd	477 bc	477 bc	461 b
TifSport	Inoculated	328 cd	387 cde	397 cd	411 de	388 cd	380 cd	382 c
Tifway	Control	524 ab	588 ab	576 ab	580 ab	581 ab	607 ab	577 a
Tifway	Inoculated	178 e	175 f	173 e	162 f	151 e	153 e	165 e
		Total surface area (mm^2)[b]						
BA132	Control	226 bc	236 c	241 c	246 c	245 c	257 c	242 bc
BA132	Inoculated	69 e	65 d	61 d	59 d	54 d	54 d	60 f
Celebration	Control	308 ab	348 ab	342 a	362 ab	369 ab	396 ab	354 a
Celebration	Inoculated	222 bc	253 bc	258 bc	260 c	264 c	274 c	255 bc
PI 291590	Control	355 a	369 a	365 a	387 a	384 a	412 a	378 a
PI 291590	Inoculated	165 cd	191 c	206 c	217 c	209 c	223 c	201 d
TifSport	Control	238 bc	256 bc	256 bc	276 bc	274 bc	273 c	262 bc
TifSport	Inoculated	206 c	227 c	237 c	242 c	236 c	230 c	230 cd
Tifway	Control	240 bc	265 bc	263 bc	266 bc	288 bc	295 bc	269 b
Tifway	Inoculated	108 de	99 d	97 d	91 d	88 d	83 d	94 e

[a] Mean for across all dates.
[b] Means of four replications.
[c] Means in each column for each measured parameter followed by the same letters are not different according to comparison of least squares means ($P \leq 0.05$).

particular grass. The genotype and nematode interaction explains the genotypic variability among the bermudagrass genotypes. Bermudagrass genotypes 'Celebration', 'TifSport', and 'PI 291590' might have a genetic or physiological advantage by producing improved root architecture compared with 'Tifway'. Greater average root diameter results in greater root surface area and root volume. Also more fine roots results in greater root length and smaller average root diameter. Results on average root diameter suggested that 'Celebration', 'TifSport', and 'PI 291590' produced thicker roots than the standard susceptible genotype 'Tifway'. Similarly, the polyploid Floratam St. Augustinegrass had significantly greater values for every root parameter studied compared to the highly *B. longicaudatus*-susceptible diploid 'FX 313' which indicates 'Floratam' has clear evidence of tolerance (however, the reduced nematode reproduction on 'Floratam' compared to 'FX 313' indicates strong evidence of resistance) against *B. longicaudatus*. These findings should be important for future research and selection of bermudagrass and St. Augustinegrass genotypes relative to commercial standards for their use in a breeding program for nematode resistance/tolerance.

Little is known about depth distribution of bermudagrass and St. Augustinegrass roots in warm-season turf ecosystems. Understanding root distribution in the soil profile would allow selection of improved and more competitive genotypes of both grass types. It has been suggested that most turf roots are concentrated in the upper 10 cm of the soil profile (Madison, 1971) and our results agree. In our study, there was a significant effect of vertical soil depth on rooting dynamics across the bermudagrass and St. Augustinegrass genotypes. The greatest root length densities were recovered from 0 to 5 cm depths followed by the 5 to 10 and 10 to 15 cm vertical soil depths for both grass types. Approximately 78% and 71% of total root length densities were recovered from the first two zones for bermudagrass and St. Augustinegrass, respectively. The greater root length density in the upper 10 cm might be due to selective factors that have led to the evolution of the characteristically fine root production system of turfgrass (Murphy et al., 1994). Root surface area and root volume were also greater in the upper two zones of this study compared with deeper in the soil profile. In a crop-soil ecosystem, up to 90% of plant-parasitic nematodes were found in the top 90 cm of vertical soil depth (Westphal et al., 2004; Howland et al., 2014). In turf ecosystems, particularly in putting greens, the majority of plant-parasitic nematodes were found in the upper 5 cm of soil profile while others were more abundant deeper in the soil profile (Laughlin and Williams, 1971; Davis et al., 1994; Crow and Dant, 2014). These reports and our findings emphasize that most turf roots occur in the upper 10 cm of the soil profile and nematode management tactics must focus on protecting the fine roots produced in that Zone. Our findings further suggest that the extensive root systems of bermudagrass genotypes 'Celebration', 'TifSport', and 'PI 291590'; and the polyploid St. Augustinegrass

TABLE 6. Effect of genotypes and *Belonolaimus longicaudatus* interaction on bermudagrass total root volume and adjusted root diameter. Data are combined from 2013 and 2014.

Genotypes	Treatments	Month						
		April	May	June	July	August	September	Mean[a]
		Total root volume (mm^3)[b]						
BA132	Control	13.3 bc[c]	13.9 c	14.3 c	14.8 c	15.0 c	15.8 c	15.3 f
BA132	Inoculated	4.8 e	4.5 d	4.1 d	4.1 d	3.9 d	3.8 d	4.2 h
Celebration	Control	21.2 ab	24.3 ab	24.2 ab	25.9 ab	26.5 ab	28.8 ab	25.1 b
Celebration	Inoculated	16.7 bc	20.2 abc	21.3 abc	22.1 abc	22.8 abc	23.5 abc	20.2 c
PI 291590	Control	28.5 a	29.5 a	29.2 a	30.8 a	30.6 a	32.4 a	30.3 a
PI 291590	Inoculated	12.1 cd	14.1 c	15.7 bc	16.7 c	16.5 c	17.6 c	15.4 ef
TifSport	Control	16.2 bc	18.8 bc	19.1 bc	20.2 bc	20.2 bc	19.8 bc	19.3 cd
TifSport	Inoculated	16.1 bc	16.9 bc	17.7 bc	18.8 bc	17.7 bc	17.1 c	17.2 def
Tifway	Control	16.0 bc	17.4 bc	17.4 bc	17.6 bc	19.7 bc	20.0 bc	18.0 cde
Tifway	Inoculated	7.1 de	6.3 d	6.1 d	5.8 d	5.6 d	5.1 d	6.0 g
		Adjusted root diameter (mm/10)[b]						
BA132	Control	1.40 c	1.52 c	1.46 d	1.51 ef	1.53 d	1.54 d	1.50 e
BA132	Inoculated	2.21 a	2.10 ab	2.16 a	2.25 ab	2.10 ab	2.01 abc	2.14 ab
Celebration	Control	1.73 bc	1.72 bc	1.76 c	1.77 cd	1.80 cd	1.77 bcd	1.76 d
Celebration	Inoculated	2.10 ab	2.13 a	2.25 a	2.34 a	2.40 a	2.37 a	2.28 a
PI 291590	Control	1.90 ab	1.92 ab	1.87 c	1.88 cd	1.88 bc	1.88 bcd	1.90 cd
PI 291590	Inoculated	1.92 ab	1.93 ab	1.96 bc	2.04 bc	2.13 ab	2.16 ab	2.02 bc
TifSport	Control	1.74 bc	1.8 abc	1.82 c	1.79 cd	1.77 cd	1.77 bcd	1.78 d
TifSport	Inoculated	2.13 ab	1.93 ab	1.98 bc	1.97 cd	2.04 bc	2.04 abc	2.02 bc
Tifway	Control	1.54 c	1.44 c	1.44 d	1.23 f	1.56 d	1.54 cd	1.50 e
Tifway	Inoculated	1.90 ab	1.77 abc	1.74 c	1.71 de	1.80 dc	1.66 cd	1.77 d

[a] Mean for across all dates.
[b] Means of four replications.
[c] Means in each column for each measured parameter followed by the same letters are not different according to comparison of least squares means ($P \leq 0.05$).

'Floratam' may help to avoid nematode-initiated drought and nutrient deficiency by potentially increasing water and nutrient extraction efficiencies. This gives turfgrass managers a better selection of genotypic choices based on tolerance/resistance against nematodes.

The minirhizotron technology is a good method for understanding the root phenology and profile distribution in turfgrass ecosystems that justify its use for root quantification throughout the growing season. Further, this technology offers the advantage of recurrent sampling at the same location to understand the seasonal root growth patterns. This study, for the first time, provides detailed information about rooting dynamics and vertical distribution of warm-season bermudagrass and St. Augustinegrass root systems in a microplot setting. 'Celebration', 'TifSport', and 'PI 291590' (bermudagrass), and Floratam' (St. Augustinegrass) genotypes have consistently shown better root vigor (relative tolerance/resistance) against *B. longicaudatus* compared with other genotypes. Inclusion of such tolerant/resistant turfgrass species in an integrated approach could be vital to reduce reliance on nematicides in sustainable nematode management programs in turf ecosystems.

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