Screening Bermudagrass Germplasm Accessions for Tolerance to Sting Nematodes

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Abstract. Breeding and improvement of new bermudagrass (*Cynodon* spp.) cultivars with superior nematode tolerance are essential because sting nematode (*Belonolaimus longicaudatus* Rau) is a major limitation for use of bermudagrass in the sandy coastal soils of the southeastern United States. The screening of both African (*Cynodon transvaalensis*) and common (*C. dactylon*) bermudagrass is necessary to develop triploid hybrid cultivars. Five commercial cultivars and 46 germplasm accessions of bermudagrass were tested for nematode responses in two greenhouse trials in 2009. Turfgrass was grown in sand-filled plastic conetainers and inoculated with 50 sting nematodes per conetainer. Nematode and root samples were collected 90 d after nematode inoculation. Fifteen bermudagrass accessions did not have measurable root loss from inoculation with sting nematode. Seven bermudagrass accessions, including ‘Celebration’, produced longer roots in sting nematode-infested soil than the standard ‘Tifway’. Differences in final nematode numbers were identified among the genotypes, and different relative responses were identified in variable ploidy levels and origins. This could aid a turfgrass breeding program by elucidating the genetic diversity available for breeding future bermudagrass cultivars for golf course cultivation.

Bermudagrass (*Cynodon* spp.) is the predominant turfgrass used in the southern United States and other warm regions in the world. A limitation for the use of bermudagrass in the southeastern United States is the sting nematode (*Belonolaimus longicaudatus*), which is frequently found in sandy coastal soils. It has been considered the most damaging plant-parasitic nematode on bermudagrass in Florida (Crow, 2005; Luc et al., 2007) where it causes damage to greens, fairways, and rough areas on golf courses as well as athletic fields and lawns (Crow and Han, 2005). With the cancellation of fenamiphos (Nemacur; Bayer CropScience, Research Triangle Park, NC), turfgrass managers are in need of new nematode management strategies. Use of resistant or tolerant cultivars would be the most desirable, least costly nematode management practice with the minimum number of ecological effects on non-target species (Giblin-Davis et al., 1992b). Breeding and improvement of new bermudagrass cultivars with superior nematode responses are essential. Giblin-Davis et al.

(1992b) tested the sting nematode tolerance and resistance of seven commercial bermudagrass cultivars and 30 experimental accessions. They found that 26 accessions showed a significant reduction in root dry weights compared with the uninoculated controls and that 25 supported reproduction of sting nematode. They also determined that ‘Tifway’ was among the most tolerant of the cultivars evaluated. Currently, there are few known sting nematode-resistant or -tolerant bermudagrass genotypes available. Except for ‘Tifway’, all bermudagrass cultivars used on greens are related to each other; therefore, environmental pressure exists for the development of a significant pest problem on bermudagrass greens. This highlights the need to select new sources of genetically superior bermudagrass accessions for use in a breeding program. Most bermudagrass cultivars that have been widely used on golf courses are triploids [*Cynodon dactylon* (L.) Pers. var. *dactylon* × *C. transvaalensis* Burt-Davy] derived from hybridizations of tetraploid common bermudagrass and diploid African bermudagrass. Previous development of sterile, triploid hybrids has focused primarily on the selection of a superior common bermudagrass parent. This was attributable, in part, to a lack of knowledge regarding the genetic diversity

and potential of improvement for African bermudagrass. Information is now available that indicates that improvement of African bermudagrass is possible for several turfgrass performance traits (Kenworthy et al., 2006). This necessitates the screening of both African and common bermudagrass. The University of Florida bermudagrass breeding program through multiyear evaluations has identified superior, Florida-adapted, experimental accessions of common and African bermudagrass to use in crosses to develop new sterile bermudagrass hybrids for use on golf courses. Sting nematode responses to these bermudagrass accessions remain uncharacterized and could provide valuable information in the selection of parents to develop progeny and cultivars resistant to this serious turfgrass pest. The objectives of this study were to test the responses caused by sting nematodes among superior University of Florida accessions of common and African bermudagrass and to select accessions with superior responses for future cultivar breeding and development.

Materials and Methods

Plant materials. Five commercial cultivars (Celebration, TifEagle, Tifway, TifSport, and TifGrand) and 46 germplasm accessions of bermudagrass were tested in two sequential experimental trials in 2009 in a greenhouse at the University of Florida Turfgrass Envirotron in Gainesville, FL. The bermudagrass accessions with origin information are listed in Table 1.

Inoculum preparation. Sting nematode was maintained on ‘FX-313’ st. augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] grown in clay pots filled with pure sand under greenhouse conditions (Giblin-Davis et al., 1992a). Juveniles and adults of sting nematode were extracted and collected from soil by using Cobb’s decanting and sieving technique (Cobb, 1918; Flegg, 1967). The average number of nematodes was counted from five 1-mL aliquots and extrapolated to the total volume of the suspension.

Nematode responses of germplasm accessions. Nematode-free aerial stolons of each cultivar/accession were vegetatively propagated into (3.8 cm diameter × 21 cm deep) ultraviolet-stabilized Ray Leach “Conetainers”™ (SC10; Stuewe & Sons, Inc., Tangent, OR) filled with 100% USGA specification greens sand (USGA, 1993). The bottom of conetainers was filled with Poly-fil (Fairfield Processing Corporation, Danbury, CT) to prevent sand from escaping from the drainage holes. Two pieces of terminal aerial stolons with one node each were planted into each conetainer. Two minutes of overhead mist irrigation was applied six times daily for 2 weeks to allow the sprigs to establish. From the third week onward, the irrigation was reduced to once a day in the morning for 6 min and 3 min a day from the beginning of the fifth week. Six weeks after establishment, grass was inoculated with no nematodes or 50 sting nematodes per conetainer. Before inoculation, suspensions of sting nematodes were

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Table 1. Mean root length of five cultivars and 46 germplasm accessions of bermudagrass at 90 d after inoculation with *Belonolaimus longicaudatus* in two experimental trials.

Genotype	Source ^w	Ploidy (2n)	Total root length (cm)					
			Trial 1			Trial 2		
			U	I	P =	U	I	P =
Tifway	CC	3x	1206	717	0.03	1690	1143	0.03
Celebration ^y	CC	4x	1535	1211 ^z	0.08	2358	1810 ^z	0.07
TifGrand	CC	3x	1561	1197 ^z	0.18	1635	1046	0.05
TifEagle ^x	CC	3x	939	758	0.51	1270	1246	0.95
TifSport	CC	3x	1420	735	0.01	1614	1354	0.39
131	FL	4x	1147	716	0.01	1906	1683 ^z	0.41
132 ^{y,x}	FL	4x	1367	1133 ^z	0.20	2042	1817 ^z	0.26
157 ^y	FL	4x	1405	1268 ^z	0.34	2115	1649 ^z	0.09
171 ^x	FL	4x	1198	1086 ^z	0.55	1650	1450	0.40
173 ^x	FL	3x	1126	991	0.26	1572	1444	0.56
227	FL	4x	1258	1111 ^z	0.54	1739	1122	<0.01
295 ^x	FL	6x	1322	1026 ^z	0.12	1594	1525	0.68
296 ^x	FL	3x	1062	805	0.28	1482	1187	0.40
299	FL	4x	912	782	0.28	1862	1028	0.04
301 ^x	FL	4x	689	490	0.14	1168	1165	0.99
304	FL	4x	1049	651	0.08	1832	1237	<0.01
334	FL	4x	1435	950	<0.01	1640	1369	0.28
343 ^x	FL	4x	1255	993	0.28	1371	1492	0.63
344	FL	6x	792	587	0.27	1339	927	<0.01
347	FL	4x	1292	839	0.14	1952	1305	0.06
355	FL	4x	1443	1167 ^z	0.39	1823	1247	0.10
445	FL	4x	1263	946	0.08	2175	1560	0.01
481 ^y	FL	4x	1577	1064 ^z	<0.01	2078	1664 ^z	0.21
525	HI	3x	777	745	0.86	1787	1307	0.08
528	HI	4x	1392	1142 ^z	0.10	1483	930	0.04
PI 289922	GRIN	5x	650	528	0.43	1406	618	0.02
PI 290868 ^x	GRIN	4x	757	594	0.36	920	717	0.12
PI 290872	GRIN	4x	872	747	0.58	1336	643	0.02
PI 290895	GRIN	3x	798	691	0.73	1210	500	0.10
PI 291590 ^y	GRIN	4x	1599	1061 ^z	<0.01	2182	1606 ^z	0.03
AB1 ^x	OK-GRIN	2x	1124	910	0.26	1580	1167	0.12
AB2	OK-GRIN	2x	1043	776	0.08	900	1075	0.55
AB21	OK-GRIN	2x	1605	1453 ^z	0.62	2220	1325	<0.01
AB3 ^{y,x}	OK-GRIN	2x	1563	1172 ^z	0.20	1844	1714 ^z	0.62
AB33 ^{y,x}	OK-GRIN	2x	1106	1063 ^z	0.77	1880	1607 ^z	0.34
AB37 ^x	OK-GRIN	2x	1327	2118 ^z	0.70	1501	1550	0.85
AB38	OK-GRIN	2x	1051	751	0.13	1420	1035	0.07
AB39	OK-GRIN	2x	1625	1434 ^z	0.34	2014	1333	<0.01
AB4	OK-GRIN	2x	1034	578	0.02	1231	1145	0.74
AB42	OK-GRIN	2x	1553	1052 ^z	0.02	2506	1561	<0.01
AB43	OK-GRIN	2x	1126	744	0.08	1236	1503	0.41
AB7	OK-GRIN	2x	1464	1040 ^z	0.03	1789	1465	0.21
UFC03	FL	4x	1010	730	0.07	1232	801	0.23
UFC06	FL	4x	1279	1126 ^z	0.55	1867	1306	0.02
UFC07	FL	6x	1277	1201 ^z	0.68	1450	1091	0.09
UFC11 ^x	FL	4x	764	679	0.64	1093	780	0.24
UFC12	FL	4x	1001	507	<0.01	1115	725	0.02
UFC25	FL	3x	1104	579	<0.01	1306	1143	0.45
UFC26 ^x	FL	3x	863	756	0.68	1214	1080	0.57
UFC29	FL	4x	1023	519	<0.01	1428	870	0.01
UFC30	FL	4x	939	821	0.27	1071	484	<0.01

^zDifferent from Tifway according to Fisher's protected least significant difference ($P \leq 0.1$).

^yGenotypes had greater root length than Tifway in the inoculated treatment in both trials ($P \leq 0.1$).

^xGenotypes did not show root reduction in either trial ($P \leq 0.1$).

^wSource of accessions are as follows: CC = commercial cultivar; FL = collected in Florida; HI = collected in Hawaii; GRIN = PIs available from Germplasm Resources Information Network–National Plant Germplasm System; OK-GRIN = African bermudagrass progeny that trace back to randomly mated GRIN PIs and Harlan/Huffine accessions (Harlan et al., 1970).

U = uninoculated and I = inoculated with 50 *B. longicaudatus*/conetainer. P = Probability associated with U versus I using linear contrasts.

Data are means of six replications.

concentrated to 10 nematodes/mL and set at room temperature for 3 h. None or a total of 5 mL of the suspensions were divided and inoculated into two 3-cm-deep holes made 1 cm from the surface center of the conetainer for the uninoculated and inoculated treatments, respectively. The holes were covered with a light layer of sand and moistened with

a light mist. Turfgrasses were maintained in a randomized complete block design with six replications for each genotype with a total of 51 bermudagrass genotypes. To provide insulation from temperature fluctuation, conetainers were placed in (60 × 35 × 15 cm) Beaver Plastics Styroblock™ (Stuewe & Sons, Inc., Tangent, OR). The experiments were

maintained under a temperature range of 24 to 34 °C with natural daylight in a greenhouse. Turfgrass was mowed once a week at a height of 2.5 cm and fertilized once a week using 24–8–16 (N-P₂O₅-K₂O) at a rate of 0.5 kg N/100 m² per growing month.

Experiments were harvested 90 d after the inoculation of nematodes. Root and soil samples were collected from each conetainer. Roots were collected by removing the shoots and Poly-fil. Roots were washed free of soil on an 853-μm (20-mesh) sieve and put into a 50-mL plastic tube submerged with water. Roots were digitally scanned using WinRHIZO root scanning equipment and software (Regent Instruments, Ottawa, Ontario, Canada). Root length was measured from the scanned images. Nematodes were extracted from the entire soil volume from the conetainer by using the modified centrifugal flotation technique (Jenkins, 1964). Nematode solution extracted from each conetainer was poured into a counting dish, and the final nematode population densities were counted under an inverted microscope at ×40.

Data analysis. To determine if a particular cultivar or accession was damaged by sting nematode, we compared total root length of the inoculated treatment with the non-inoculated control using linear contrasts at $P \leq 0.1$. Use of this P value level provided a more conservative threshold for identifying accessions that were not different in total root length between treatments. To determine if an accession performed better than a standard cultivar, the root lengths of the inoculated treatment from each accession were compared with 'Tifway' using the Fisher's protected least significant difference (LSD) test. Final nematode population densities were subjected to analysis of variance, and genotypes were compared by the Fisher's protected LSD test at $P \leq 0.1$. Statistical analysis was conducted by using the SAS program (SAS Institute, Cary, NC).

Results

Statistical analysis showed that there was a significant difference among the genotypes ($P < 0.0001$), treatment ($P < 0.0001$), and no interaction between them ($P = 0.8926$). A significant genotype × trial interaction ($P = 0.0139$) occurred, so results are presented separately for the two trials. For all genotypes, root lengths varied from 650 to 1599 cm (Trial 1) and 920 to 2358 cm (Trial 2) for the uninoculated controls and 490 to 1268 cm (Trial 1) and 426 to 1817 cm (Trial 2) for the inoculated treatments. Results of the linear contrast showed that genotypes 'Tifway', 'Celebration', 304, 445, 528, PI291590, AB42, UFC12, and UFC29 had significant reductions in root length from sting nematodes in both trials ($P \leq 0.1$), whereas 'TifEagle', 132, 171, 173, 295, 296, 301, 343, PI290868, AB1, AB3, AB33, AB37, UFC11, and UFC26 did not show root reduction in either trial (Table 1). Several genotypes were found to have greater root length in the inoculated treatment than 'Tifway' in both trials ($P \leq 0.1$). These

were 'Celebration', 132, 157, 481, PI291590, AB3, and AB33 (Table 1).

Accessions also showed differences in host status to sting nematode (Table 2). The final nematode population densities were significantly different among genotypes ($P \leq 0.001$). The final nematode numbers were

Table 2. Final mean population density of *Belonolaimus longicaudatus* on five cultivars and 46 germplasm accessions of bermudagrass 90 d after inoculation with 50 *B. longicaudatus*/conetainer in two experimental trials.

Genotype	Ploidy (2n)	Final population density	
		Trial 1	Trial 2
Celebration	4x	61 bcd ^{a,x}	8 j
TifGrand	3x	111 a	80 b
TifEagle ^z	3x	21 de	25 defghij
TifSport ^z	3x	25 cde	30 cdefghij
Tifway	3x	15 de	53 bcdefghi
131 ^z	4x	40 bcde	14 j
132 ^z	4x	29 cde	16 ij
157	4x	72 abc	62 bcde
171 ^z	4x	21 de	7 j
173 ^z	3x	32 cde	13 j
227 ^z	4x	35 cde	19 hij
295 ^z	6x	23 cde	25 defghij
296 ^z	3x	49 bcde	27 defghij
299 ^z	4x	39 bcde	10 j
301 ^z	4x	22 de	21 hij
304 ^z	4x	21 de	9 j
334 ^z	4x	8 e	5 j
343 ^z	4x	14 de	24 efghij
344 ^z	6x	29 cde	35 cdefghij
347	4x	49 bcde	60 bcdefg
355	4x	40 bcde	120 a
445	4x	86 ab	53 cdefghi
481	4x	50 bcde	60 bcdef
525 ^z	3x	4 e	38 cdefghij
528 ^z	4x	43 bcde	23 fghij
PI 289922 ^z	5x	15 de	14 j
PI 290868 ^z	4x	6 e	17 ij
PI 290872 ^z	4x	5 e	19 hij
PI 290895 ^z	3x	16 de	2 j
PI 291590 ^z	4x	36 cde	32 cdefghij
AB1 ^z	2x	17 de	15 ij
AB2 ^z	2x	2 e	17 ij
AB21	2x	112 a	68 bc
AB3	2x	31 cde	63 bcd
AB33 ^z	2x	11 e	15 ij
AB37 ^z	2x	26 cde	17 ij
AB38 ^z	2x	33 cde	6 j
AB39	2x	114 a	17 ij
AB4 ^z	2x	9 e	19 hij
AB42	2x	43 bcde	82 ab
AB43 ^z	2x	25 cde	30 bcdefghij
AB7 ^z	2x	24 cde	16 ij
UFC03	4x	10 e	55 bcdefgh
UFC06 ^z	4x	20 de	24 defghij
UFC07 ^z	6x	8 e	8 j
UFC11 ^z	4x	12 de	17 ij
UFC12 ^z	4x	14 de	17 ij
UFC25 ^z	3x	7 e	18 hij
UFC26 ^z	3x	21 de	22 ghij
UFC29 ^z	4x	43 bcde	37 cdefghij
UFC30 ^z	4x	30 cde	17 ij

^aThe final nematode number decreased on these genotypes (less than 50 sting nematodes/conetainer) in both trials.

^zData are means of six replications, and represent numbers of nematodes recovered from the whole conetainer.

^xMeans within a column followed by the same letter are not different ($P \leq 0.05$), Fisher's protected least significant difference test.

compared with the initial inoculum level of 50 sting nematodes/conetainer. Nematode numbers increased (more than 50 sting nematodes/conetainer) in 'Celebration', 'TifGrand', '157', '445', 'AB21', and 'AB39' in Trial 1 with the maximum increase of 2.3-fold in AB39. In Trial 2, 'TifGrand', 'Tifway', 157, '347', 355, 445, '481', AB21, AB42, and 'UFC03' increased the numbers of sting nematode, and the highest increase was 2.4-fold in 355. However, to select better nematode response, we considered accessions on which the nematode numbers decreased (less than 50 sting nematodes/conetainer) in both trials as potential good candidates for breeding. Based on this, nine African and 28 common bermudagrass accessions could move forward in the selection process (Table 2).

Discussion

Genotypes with better sting nematode tolerance, which either had no reduction in root length or had greater root length than 'Tifway', were found in various sources, and there was no relationship between nematode response and the origin of genotypes. According to Giblin-Davis et al. (1992b), 'Tifway' was relatively tolerant to sting nematode, whereas at the same time it was a host. Because 'Tifway' is the most commonly used bermudagrass cultivar in the southeastern United States (Burton, 1966), it was used as the standard cultivar for comparison in our trials. Nematode tolerance in bermudagrass has been defined as a genotype either suffering minimal root reduction from the nematodes or as being able to produce more roots than a standard genotype in the presence of the nematodes (Pang et al., 2011). The inoculated to non-inoculated comparisons allow us to determine the degree to which the genotype is damaged by nematodes. Comparisons to 'Tifway' allow us to determine the relative tolerance compared with a standard cultivar. Both types of comparisons are vital for genotype selection and exclusion of only one type of comparison can lead to improper conclusions. For example, UFC11 did not have root length differences between inoculated and non-inoculated. However, when we compare the root lengths of UFC11 with 'Tifway', we see that it had poor root production overall and would be a poor choice to move forward in the selection process. Conversely, PI291590 is a vigorously rooting accession compared with 'Tifway', but it was severely damaged by sting nematode. Genotypes whose roots were not significantly impacted by sting nematode and also were more vigorously rooting than 'Tifway' in both trials were 132, AB3, and AB33. Furthermore, the final nematode population densities could provide an additional reference for germplasm selection and cultivar breeding for nematode response. Accessions that maintained a final nematode density of less than 50 sting nematodes/conetainer in these studies would have a potential to suppress nematode reproduction and lower the number of nematodes in the field or golf courses, which can be used as a strategy for nematode management.

Genotypes 132 and AB33 not only maintained vigorous rooting under sting nematode-infested soil, but also were good candidates to reduce nematode population densities based on the fact that the final nematode numbers were 50% lower than the initial density in this study (Table 2).

Overall, differential responses to sting nematode were identified among bermudagrass accessions with diverse genetic backgrounds (diploid, triploid, tetraploid, pentaploid, and hexaploid) and sources. Accessions were identified with increased and decreased rooting under sting nematode pressure when compared with 'Tifway'. No obvious relationship was found to exist between rooting response and ploidy level or origin of the accessions. This information should be of value to select parents for future breeding and cultivar improvement. The results of this study should not be considered definitive because multiple nematode species or pathogens exist in natural fields, and the nematode or pest responses of these genotypes need to be assessed with future field studies.

Conclusions

This study indicated that bermudagrass germplasm accessions respond differently in host suitability and tolerance to sting nematodes and that selection of genotypes with improved responses to nematodes is possible. Accessions 132, AB3, and AB33 were not measurably affected by sting nematode and were more vigorously rooting than the standard cultivar, Tifway. Accessions that are relatively tolerant to sting nematode were identified with variable ploidy levels and origins. Accessions with larger root systems tend to be more tolerant, which might be potentially useful for turfgrass breeding and development for host tolerance against nematodes. This is the first report of sting nematode responses on African bermudagrass and variable responses were observed. The use of diploid and tetraploid genotypes identified in this research may prove to be highly valuable genetic resources for future improvement of bermudagrass and serve to reduce dependence on nematicides.

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