METHODOLOGY FOR SCREENING FOR RESISTANCE TO *BELONOLAIMUS LONGICAUDATUS* IN TURFGRASS[1]

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ABSTRACT

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Efforts to screen large numbers of turfgrass genotypes would likely result in the discovery of genes associated with resistance or tolerance to plant-parasitic nematodes. The identification of germplasm with these characteristics will become increasingly important as stringent regulations regarding the use of soil fumigants and nematicides are put into place. Therefore, a glasshouse study was initiated to investigate plant-parasitic nematode evaluation methods on 'TifEagle' hybrid bermudagrass to identify a high throughput, accurate and repeatable greenhouse screen useful to turfgrass breeding programs during sequential trials in 2007. Three establishment methods, classified as: i) conetainers grown in for 45 days (45-d conetainers), ii) conetainers grown in for 90 days (90-d conetainers), and iii) clay pots grown in for 90 days (90-d clay pots) before inoculation with sting nematodes, respectively, were assessed. Two inoculation rates, 50 and 100 mixed life stages of *Belonolaimus longicaudatus*/100 cm^3 of soil, were compared to an uninoculated control within each establishment method. Total dry root weights were 38% and 28% larger for uninoculated treatments when compared to an average of the two sting nematode inoculated treatments in the 45-d conetainers and 90-d conetainers, respectively. Root weights were not significantly reduced by sting nematode pressure in the 90-d clay pots. Total root lengths of uninoculated treatments were 57%, 55%, and 31% greater than an average of the two inoculated treatments in the 45-d conetainers, 90-d conetainers, and 90-d clay pots, respectively. Results were generally more variable for treatments grown in 90-d clay pots and some root length characteristics were not consistent between trials in the 90-d conetainers. Quantifying root damage using 45-d conetainers inoculated with 50 sting nematodes provided reproducible results characteristic of those reported in other greenhouse and field evaluations.

Key words: *Belonolaimus longicaudatus*; bermudagrass, *Cynodon* spp., germplasm screening, sting nematode, turf breeding.

RESUMEN

Schwartz, B. M., K. E. Kenworthy, W. T. Crow, J. A. Ferrell, G. L. Miller, and K. H. Quesenberry. 2008. Metodología para la evaluación de *Belonolaimus longicaudatus* en un programa de mejoramiento de pastos. 38:163-175.

Cuando se evalúan grandes cantidades de genotipos de pastos existe el potencial de encontrar genes asociados con resistencia o tolerancia a nematodos fitoparásitos. La identificación de germoplasma con estas características es cada vez más importante debido al aumento en las restricciones al uso de fumigantes y nematicidas. Por esta razón, se llevo a cabo un estudio en invernadero para comparar algunos métodos de evaluación de respuestas a nematodos fitoparásitos en el híbrido 'TifEagle' de pasto bermuda, con el fin de identificar un método preciso, reproducible y útil para eva-

luar altos volúmenes de germoplasma en programas de mejoramiento. Se evaluaron tres métodos de establecimiento de las plantas antes de la inoculación con los nematodos: i) "conetainers" (recipientes plásticos con base cónica) establecidos durante 45 días, ii) "conetainers" establecidos durante 90 días y iii) macetas de barro establecidas durante 90 días. También se evaluaron dos niveles de inóculo, 50 y 100 individuos de *Belonolaimus longicaudatus*/100 cm^3 de suelo, en conjunto con cada método de establecimiento. El peso seco total de las raíces fue 38% y 28% más alto en los tratamientos sin inocular que en el promedio de los tratamientos con ambos niveles de inóculo en los "conetainers" establecidos 45 y 90 días, respectivamente. No se observó reducción significativa en el peso de las raíces en las macetas establecidas durante 90 días. La longitud total de las raíces en los controles sin inocular fue 57%, 55%, y 31% mayor que el promedio de los tratamientos con los dos niveles de inóculo en los "conetainers" establecidos durante 45 y 90 días y en las macetas de 90 días, respectivamente. Se observó mayor variabilidad en los resultados obtenidos en las macetas establecidas durante 90 días. La longitud de las raíces en los "conetainers" de 90 días no fue consistente entre ensayos. La cuantificación del daño a las raíces utilizando "conetainers" establecidos durante 45 días y 50 individuos de *B. longicaudatus* como inóculo brindó resultados reproducibles y similares a los obtenidos en otros ensayos de invernadero o de campo.

Palabras clave: *Belonolaimus longicaudatus*, *Cynodon* spp., evaluación de germoplasma, mejoramiento de pastos, pasto bermuda.

INTRODUCTION

Sting nematode (*Belonolaimus longicaudatus*) is a parasite of many warm-season turfgrasses. This nematode feeds primarily on the actively growing root tips of their hosts which over time results in shallow, necrotic roots (Crow and Han, 2005). A variety of management techniques have been evaluated in an attempt to eliminate or suppress plant-parasitic nematodes where they are damaging. The most common, and often only, method of nematode management in susceptible cultivars has been the use of nematicides (Crow *et al.*, 2003; Giblin-Davis *et al.*, 1991; Johnson, 1970a; Perry *et al.*, 1970). The recent loss of fenamiphos, the most commonly used nematicide on turf in the United States, has heightened the need for new nematode management strategies. This has renewed interest in the development of turfgrass cultivars with improved resistance and tolerance to the sting nematode.

The search for useful resistance or tolerance to the sting nematode has been limited to readily available cultivars and a limited number of germplasm accessions (Bekal and Becker, 2000; Giblin-Davis *et al.*, 1992b; Johnson, 1970b; Tarjan and Busey, 1985). In bermudagrass, more effort has been dedicated to breeding and selecting for root-knot nematode resistance (Burton *et al.*, 1946; Riggs *et al.*, 1962; Sledge, 1962). Burton (1974) stated that several radiation induced mutants of 'Tifgreen', 'Tifway' and 'Tifdwarf' were more resistant to root-knot nematodes than their respective parental clones. Root-knot nematode resistant bermudagrasses often lower the population of root-knot nematodes, but may serve as a host for ectoparasitic nematode species (Good *et al.*, 1965). The forage bermudagrass 'Coastcross-1' exhibited sting nematode resistance (Burton, 1972). A greater effort to screen broader germplasm of susceptible turfgrass species would likely result in the discovery of more resistant or tolerant individuals. Improvement through breeding should be possible because genetic variability for

these characteristics has been demonstrated (Giblin-Davis *et al.*, 1992b). As regulations regarding the use of soil fumigants and nematicides become stricter, it would be valuable to identify any genetic sources for effective control of this pest.

For breeding programs, current methods have limited the efficient screening of broader germplasm pools due to the space constraints associated with larger clay pots (Crow and Welch, 2004; Hixson *et al.*, 2004; Winchester and Burt, 1964) or the extended cycle times needed to observe root reductions (Giblin-Davis *et al.*, 1992b; Tarjan and Busey, 1985). Damage induced by plant-parasitic nematodes becomes more apparent on golf courses when secondary abiotic stresses are present (Crow, 2005a). Simulating field conditions by reducing the frequency of fertilizer application and limiting irrigation may result in more effective screening. Schwartz *et al.* (2006) found that weekly fertilization and twice daily irrigation hindered the detection of genetic potential for resistance or tolerance to sting nematodes when zoysiagrasses were grown in shorter, uninsulated conetainers.

Many turfgrass species are suitable hosts for sting nematode reproduction (Bekal and Becker, 2000; Robbins and Barker, 1973). Identifying an appropriate plant standard for use as a control when evaluating sting nematode response could increase the accuracy of screening large numbers of germplasm lines. Plant standards will need to respond to sting nematode populations in the greenhouse in a manner characteristic of the host-pathogen relationship seen in the field. Damaging numbers of sting nematodes have been associated with subjectively measured declines in turf quality and root lengths on ultradwarf bermudagrasses in Florida (Crow, 2005b). Reductions in total and fine root lengths of the ultradwarf bermudagrass, TifEagle, also corresponded to increasing sting nematode population densities when evaluated in the greenhouse (Crow and Welch, 2004).

Nematode and root characteristics reported for turfgrasses in the literature were evaluated to select the most informative and repeatable combinations of establishment method and inoculation treatment used in this study. Dry root weights were the most widely used measure of plant-parasitic inflicted root damage before the advent of digital scanners and root length analysis software. Plant breeders should first focus on the primary symptom of nematode damage, reduction of root lengths, and not the correlated decrease in root weight. Root weight can only be used to indirectly measure the problem if associations between root weight and root length are strong. Qualitative turfgrass quality ratings and clipping weights have not been consistently associated with root damage in bermudagrass (Giblin-Davis *et al.*, 1992b; Hixson *et al.*, 2004; Tarjan and Busey, 1985) and would probably not make effective selection criteria for estimating plant-parasitic nematode damage. This research was initiated to investigate evaluation methods to identify a high throughput, accurate and repeatable greenhouse screen useful to turfgrass breeding programs in comparing response to *B. longicaudatus*, an ectoparasitic nematode.

MATERIALS AND METHODS

Two experimental trials were conducted sequentially during the 2007 growing season in a glasshouse at the University of Florida Turfgrass Envirotron in Gainesville, FL. Planting materials were

nematode-free, aerial stolons of 'TifEagle' hybrid bermudagrass (Hanna and Elsner, 1999). Three establishment protocols were evaluated using (3.8 cm diameter × 21 cm deep) UV stabilized Ray Leach "Cone-tainers"™ (SC10, Stuewe & Sons, Inc., Tangent, OR) and (10 cm diameter × nine cm deep) tapered clay pots. Turf establishment methods were classified as conetainers grown in for 45 days (45-d conetainers), conetainers grown in for 90 days (90-d conetainers), and clay pots grown in for 90 days (90-d clay pots) before inoculation with sting nematodes, respectively. Two inoculation rates, 50 and 100 mixed life-stages of *B. longicaudatus*/ 100 cm^3 of soil, were compared to an uninoculated nematode free control within each turf establishment method. The experimental design was a split-plot with establishment methods arranged as whole-plots and inoculation treatments as sub-plots with six replications. Upon inoculation, conetainers were placed in (60 × 35 × 15 cm) Beaver Plastics Styroblock™ containers (77/170, Stuewe & Sons, Inc., Tangent, OR) to simulate the environment provided by the thicker-walled clay pots. The daily average high and low air temperatures in the glasshouse were 33.6 ± 2.9°C and 23.9 ± 1.3°C, respectively over the course of both trials.

Conetainers and clay pots were filled with 100 and 280 cm^3 of autoclaved United States Golf Association (USGA) root-zone specification sand (Anonymous, 1993), respectively. Poly-fil (Fairfield Processing Corporation, Danbury, CT) was placed in the bottom of each to prevent sand from escaping from the drainage holes. Conetainers were planted with one terminal aerial stolon approximately 5 cm long. Clay pots had approximately seven times the growing surface area of the conetainers, and were therefore planted with seven equivalent stolons. One minute of overhead mist was applied eight times daily for one week to allow the rootless sprigs to establish. The frequency of irrigation was reduced to four times daily during the second week of growth. Beyond the second week, a single application of two minutes/day of irrigation was scheduled. Peters Professional 20-20-20 General Purpose Water Soluble Fertilizer (Scotts-Sierra Horticultural Products Co., Marysville, OH) was applied weekly at a rate of 2.4 g/m^2 for the first month after planting. Subsequent applications were made at the time of inoculation, and again 45 days later. Leaf canopies were trimmed weekly for the duration of the experiments.

Nematode inoculum was extracted using a modified Baermann funnel method (McSorley and Frederick, 1991) from a pure population of *B. longicaudatus* which was maintained on 'FX-313' St. Augustinegrass. The population density of nematodes in the extract was estimated by quantifying the number of sting nematodes in one ml aliquots on a counting slide (Hawksley and Sons Limited, Lancing, Sussex, UK). Nematode counts were replicated five times with 83 ± 6 and 85 ± 4 sting nematodes/ml present in the extracted solutions for the first and second experiments, respectively. The solutions were diluted to deliver aliquots of 50 sting nematodes/3 ml of solution. Experimental units within establishment methods were sorted by canopy density, and groups of three with similar densities were assigned to the same replication. Inoculation proceeded sequentially according to replication. An aliquot of inoculum for conetainers receiving the 50 sting nematode/100 cm^3 soil treatment was pipetted into a single hole (1 cm diameter x 3 cm deep), which was then pressed closed. Those receiving the 100 sting nematode/ 100 cm^3 soil treatment were inoculated

with two aliquots in separate holes. Inoculum aliquot size was adjusted to contain 140 and 280 sting nematodes to account for the larger soil volume in the clay pots for the 50 and 100 sting nematodes/100 cm^3 soil treatments, respectively.

Experiments were terminated after 90 days and brought to the laboratory for destructive analysis. The plant and corresponding soil volume were isolated from each conetainer and clay pot. Shoots were trimmed off at the soil level and the Poly-fil was removed. Roots and nematodes were extracted from the entire experimental unit in the conetainer treatments, and from a 100 cm^3 soil core serving as a representative sample from each clay pot.

Nematodes were extracted from the soil medium using a centrifugal-sugar flotation technique (Jenkins, 1964), modified by adding five cm^3 of clay to keep the sediment plug intact as the supernatant liquid was removed after the first centrifugation. Counts were made to determine final sting nematode population densities (Pf) on an inverted light microscope at ×40 magnification and to verify nematode pressure was present.

Roots were collected from uninoculated and inoculated treatments, submersed underwater in 50 ml plastic centrifuge tubes, and stored at -23°C for later analysis. The root samples were thawed after sting nematode counts had been completed. Individual root systems were placed into a clear acrylic glass tray where a digital image was created using an Epson Perfection V700 Photo scanner (Epson America, Inc., Long Beach, CA). Lengths of five root diameter classifications (< 0.125 mm, 0.125 to 0.250 mm, 0.250 to 0.500 mm, 0.500 to 1.000 mm, and > 1.000 mm) were individually quantified using WinRHIZO Pro v2007d software (Regent Instruments, Inc., Quebec, QC) and then summed to determine total root length (TRL) of each sample. Roots were later dried at 75°C for 48 h to obtain total dry root weights (TDRW). Reproduction factor (Rf), sting nematode numbers on a total root length basis (Pf/TRL), sting nematode numbers on a total dry root weight basis (Pf/TDRW), total root dry weight percent reduction (TDRW % red.), total root length percent reduction (TRL % red.), and fine root (diameter < 0.125 mm) length percent reduction (FRL % red.) were calculated with the measured observations.

$$Rf = \frac{PF}{\#\ of\ sting\ nematodes\ inoculated}$$

$$TDRW\ \%\ red. = \left[\frac{(TDRW\ of\ inoculated - TDRW\ of\ uninoculated)}{TDRW\ of\ uninoculated} \right] \text{x } 100$$

$$TRL\ \%\ red. = \left[\frac{(TRL\ of\ inoculated - TRL\ of\ uninoculated)}{TRL\ of\ uninoculated} \right] \text{x } 100$$

$$FRL\ \%\ red. = \left[\frac{(FRL\ of\ inoculated - FRL\ of\ uninoculated)}{FRL\ of\ uninoculated} \right] \text{x } 100$$

The distribution of data for each characteristic was assessed with a histogram and normal probability plot for normality. Transformed datasets were utilized where conditions of normality were not met. An analysis of variance was performed on each trait to test whether establishment methods and inoculation treatments varied. To further study the precision of each method, data were analyzed separately even where establishment method interactions were not significant. Where appropriate, differences between the inoculated treatments and uninoculated controls were tested with orthogonal coefficients. Pearson correlation coefficients were computed using the CORR procedure in SAS software (SAS Institute Inc., Cary, NC) for 45-d conetainers and 90-d clay pots to test whether any traits were associated with, or could be predictors of, other characteristics in the two dissimilar pots.

RESULTS

Variation between establishment methods was detected ($P \leq 0.05$) in all four sting nematode population characteristics (Pf, Rf, Pf/TRL, and Pf/TDRW), but inoculation treatment × establishment method interactions were significant ($P \leq 0.05$) for final nematode populations and sting nematode numbers on a total dry root weight basis (Table 1). Nematode populations increased in every treatment except for 90-d clay pots inoculated with 100 sting nematodes/100 cm^3 soil. Reproduction factors were approximately two times larger ($P \leq 0.01$) in the low inoculation treatment than in the higher treatment for both conetainer establishment methods. Final sting nematode numbers on a total root length and total dry root weight basis were not different for inoculation treatments within the three establishment methods. Results for all population characteristics, except nematode reproduction, were less variable with conetainer treatments than when evaluated in clay pots (Table 2). Data collected from 45-d conetainers was more consistent between trials than that collected from 90-d conetainers. Therefore, 45-d conetainer data was used for correlation analysis with the 90-d clay pot data. Sting nematode reproduction and final population density were correlated ($P \leq 0.05$) with all root characteristics, excluding fine root length percent reduction, in the 45-d conetainer establishment method. Alternatively, there were no associations detected between nematode count data and root measurements in the 90-d clay pots (Table 3).

Total dry root weights were different ($P \leq 0.01$) for establishment methods and inoculation treatments (Table 1), but no treatment differences were found for total dry root weight percent reduction due to variable observations in the 90-d clay pot establishment method. Total dry root weights were 38% and 28% larger ($P \leq 0.01$) for uninoculated treatments when compared to an average of the two inoculated treatments in the 45-d and 90-d conetainers, respectively. Root weights were not significantly reduced by sting nematode pressure in the 90-d clay pots (Table 4). Total dry root weights were good predictors of total ($r = 0.94$, $P \leq 0.01$) and fine ($r = 0.85$, $P \leq 0.01$) root lengths in the 45-d conetainer establishment method. Correlation between total dry root weight percent reduction and total root length percent reduction ($r = 0.89$, $P \leq 0.01$) was also observed. Associations between total dry root weights and total root lengths ($r = 0.79$, $P \leq 0.01$), total dry root weights and fine root lengths ($r = 0.69$, $P \leq 0.01$), and total dry root weight percent reduction and fine root length percent reduction ($r = 0.55$, $P \leq 0.01$) were also present in the 90-d clay pots, but the relationships were not as strong (Table 3).

Table 1. Mean squares for *Belonolaimus longicaudatus* reproduction factor (Rf), final population density (Pf), population density on a total root length basis (Pf/TRL), population density on a total dry root basis (Pf/TDRW), total dry root weight (TDRW), total dry root weight percent reduction (TDRW % red.), total root length (TRL), total root length percent reduction (TRL % red.), fine root length (FRL), and fine root length percent reduction (FRL % red.) of TifEagle bermudagrass evaluated in three establishment (Est.) methods with different inoculation treatments (Inoc. TRT) in two experimental trials

		Mean squares									
Source	df	Rf	Pf	Pf/TRL	Pf/TDRW	TDRW[z]	TDRW % red.	TRL[c]	TRL % red.	FRL[c]	FRL % red.
Trial (T)	1	1.488**	95.4**	0.4960**	11789**	0.0866**	7.91	5729209**	1.03	711290**	4.77
Error A: Rep (T)	10	0.034	7.4	0.0135	163	0.0028	2.99	349298	1.44	53090	2.02
Est. Method (E)[x]	2	1.874**	137.1**	0.1377*	1292*	0.0314**	13.08	6132352**	6.03	1358829**	7.67
E × T	2	0.499	31.5	0.0449	1144	0.0140**	5.93	1623045**	0.29	250491**	0.85
Error B: Rep × E (T)	20	0.177	23.3	0.0276	333	0.0011	3.86	86198	2.15	15915	2.34
Inoc. TRT (I)[y]	1	6.778**	66.2	0.0094	237	0.0058**	2.58	3946964**	7.29*	874549**	9.30**
I × T	1	0.101	0.0	0.0000	1	0.0001	0.92	265678*	0.00	89535**	0.10
I × E	2	0.290	91.1*	0.0563	946*	0.0015	0.18	283354**	0.74	63174**	0.84
I × T × E	2	0.178	12.5	0.0201	77	0.0010	1.44	63695	2.02	22437	2.22
Error C: MSE	30	0.141	23.5	0.0230	265	0.0008	2.07	58618	1.19	12990	1.05
% CV		26.3	35.0	34.8	32.9	26.0	16.0	17.9	13.2	18.4	12.4

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

[x]Conetainers grown in for 45 days, conetainers grown in for 90 days, and clay pots grown in for 90 days before inoculation with sting nematodes, respectively

[y]Inoculated with 0, 50, or 100 *B. longicaudatus*/100 cm^3 soil.

[z]Analysis included comparison to uninoculated controls, therefore df for I, I × T, I × E, I × T × E, and Error C were 2, 2, 4, 4, and 60, respectively.

Table 2. Mean reproduction factor (Rf), final population density (Pf), population density on a total root length basis (Pf/TRL), population density on a total dry root basis (Pf/TDRW), of *Belonolaimus longicaudatus* on TifEagle bermudagrass 90 days after inoculation evaluated in three establishment methods with two inoculation treatments in two experimental trials.

Treatment[y]	Rf (nematodes)	Pf (nematodes)	Pf/TRL (nematodes/cm)	Pf/TDRW (nematodes/g)
	45-d conetainers			
I (50)	2.7 ± 1.5**[z]	134 ± 75	0.17 ± 0.10	2790 ± 1976
I (100)	1.4 ± 0.6	138 ± 58	0.22 ± 0.14	3569 ± 3203
	90-d conetainers			
I (50)	4.2 ± 1.6**	210 ± 80	0.14 ± 0.07	1750 ± 863
I (100)	2.2 ± 1.0	223 ± 101	0.15 ± 0.07	1850 ± 789
	90-d clay pots			
I (50)	2.8 ± 1.5**	391 ± 213	0.36 ± 0.17	4518 ± 2597
I (100)	0.8 ± 0.7	221 ± 193	0.29 ± 0.39	3154 ± 4088

**Inoculated treatments significantly different at the 0.01 probability level, according to orthogonal coefficient analysis.

[y]Inoculated (I) with 50 or 100 *B. longicaudatus*/100 cm^3 soil.

[z]Data are means of two trials with six replications each ± standard deviations.

Total root lengths and fine root lengths were highly correlated ($r \approx 0.98$, $P \leq 0.01$), as were the corresponding percent reductions ($r \approx 0.97$, $P \leq 0.01$), in the 45-d conetainer and 90-d clay pot establishment methods (Table 3). Analysis of variance indicated a significant interaction ($P \leq 0.01$) between inoculation treatments and establishment methods for total and fine root lengths. Total and fine root length percent reductions did not vary with establishment method, but inoculation treatments differed ($P \leq 0.05$) for both characteristics in the combined analysis (Table 1). Total root lengths of uninoculated treatments were 57%, 55%, and 31% greater ($P \leq 0.01$) than an average of the two inoculated treatments in the 45-d conetainers, 90-d conetainers, and 90-d clay pots, respectively (Table 4). Similar results were found for fine root lengths, except in trial two where these lengths were not reduced as greatly in the higher inoculation treatment within the 90-d conetainer method. Related inoculation treatment x trial interactions for total and fine root length percent reductions were also evident ($P \leq 0.05$) within 90-d conetainers. Differences in root length reduction between inoculation treatments were only significant ($P \leq 0.05$) in the 90-d conetainer method at the conclusion of trial one, but results were inconsistent between trials. Greater numerical differences in root length percent reduction were found between the higher and lower inoculation treatments in the clay pots than with either conetainer method, but within treatment variability prevented significant detection of these differences (Table 5).

Table 3. 45-d conetainer (above diagonal) and 90-d clay pot (below diagonal) correlation coefficients of *Belonolaimus longicaudatus* reproduction factor (Rf), final population density (Pf), population density on a total root length basis (Pf/TRL), population density on a total dry root weight basis (Pf/TDRW), total dry root weight (TDRW), total dry root weight percent reduction (TDRW % red.), total root length (TRL), total root length percent reduction (TRL % red.), fine root length (FRL), and fine root length percent reduction (FRL % red.) of TifEagle bermudagrass.

Nematode and root data	Rf	Pf	Pf/TRL	Pf/TDRW	TDRW	TDRW % red.	TRL	TRL % red.	FRL[z]	FRL % red.
	count	count	count/cm	count/g	g	%	cm	%	cm	%
Rf		0.90**	0.71**	0.62**	-0.42*	-0.38*	-0.48**	-0.39*	-0.45**	-0.30 ns
Pf	0.94**		0.85**	0.74**	-0.49**	-0.50**	-0.58**	-0.54**	-0.56**	-0.44**
Pf/TRL	0.64**	0.78**		0.97**	-0.63**	-0.67**	-0.71**	-0.62**	-0.69**	-0.51**
Pf/TDRW	0.67**	0.77**	0.97**		-0.62**	-0.67**	-0.67**	-0.56**	-0.64**	-0.43**
TDRW	0.03 ns[y]	0.02 ns	-0.29 ns	-0.38*		0.60**	0.94**	0.40*	0.85**	0.30 ns
TDRW % red.	0.13 ns	0.07 ns	-0.22 ns	-0.25 ns	0.51**		0.65**	0.89**	0.66**	0.80**
TRL	0.01 ns	-0.05 ns	-0.36*	-0.39*	0.79**	0.30 ns		0.58**	0.98**	0.51**
TRL % red.	0.10 ns	-0.01 ns	-0.28 ns	-0.19 ns	0.08 ns	0.55**	0.32 ns		0.66**	0.98**
FRL	0.05 ns	-0.01 ns	-0.32 ns	-0.33*	0.69**	0.25 ns	0.97**	0.37*		0.61**
FRL % red.	0.16 ns	0.06 ns	-0.23 ns	-0.13 ns	0.06 ns	0.45**	0.34*	0.96**	0.41*	

*, **Significant at the 0.05 and 0.01 probability levels, respectively.
[y]Not significant at the 0.05 probability level.
[z]Fine root (diameter < 0.125 mm) length.

Table 4. Mean total dry root weight (TDRW), total dry root weight percent reduction (TDRW % red.), and total root length (TRL) of TifEagle bermudagrass 90 days after inoculation evaluated in three establishment methods with uninoculated and inoculated treatments in two experimental trials.

Treatment[y]	TDRW (g)	TDRW % red. (g)	TRL (cm)
	45-d conetainers		
U	0.09 ± 0.04**[z]	—	1394 ± 523**
I (50)	0.07 ± 0.06	-24 ± 23	950 ± 455
I (100)	0.06 ± 0.04	-33 ± 24	828 ± 338
	90-d conetainers		
U	0.16 ± 0.03**	—	2393 ± 260**
I (50)	0.13 ± 0.03	-19 ± 27	1608 ± 352
I (100)	0.12 ± 0.02	-23 ± 19	1478 ± 260
	90-d clay pots		
U	0.11 ± 0.05	—	1401 ± 609**
I (50)	0.10 ± 0.05	4 ± 46	1153 ± 472
I (100)	0.11 ± 0.07	-3 ± 45	994 ± 483

**Uninoculated controls significantly different from both inoculated treatments at the 0.01 probability level, according to orthogonal coefficient analysis.

[y]Uninoculated (U); Inoculated (I) with 50 or 100 *B. longicaudatus*/100 cm^3 soil.

[z]Data are means of two trials with six replications each ± standard deviations.

DISCUSSION

There were sufficient sting nematodes in both conetainer and clay pot inoculation treatments to establish reproducing populations. Lautz (1959) found that inoculum treatments of only 10 sting nematodes/pot resulted in no population increases even though good reproduction was observed when 40 sting nematodes/pot were artificially inoculated onto the same host. Final population densities within the 45-d and 90-d conetainers were equal for both low and high inoculation treatments even though the reproduction factors were approximately two times larger in the lower treatment. This indicates that sting nematode carrying capacities may have been met for the respective size of the root systems in each establishment method. Total root lengths in the 90-d clay pots were reduced by the high inoculation treatment when compared to the uninoculated control despite a reproduction factor below one, suggesting that the carrying capacity of the root systems were exceeded and a resulting nematode maximum population density occurred. Therefore, if differences in nematode reproduction (resistance) are the primary objective of the research, an inoculation rate of 50 *B. longicaudatus* per 100 cm^3 of soil and an evaluation period of 90 days appear adequate. However, if the amount of damage caused by the nematodes (tolerance) is the primary objective of the research a higher inoculation rate of 100 *B. longicaudatus* per 100 cm^3 of soil or a longer evaluation period will likely yield better data.

Table 5. Mean total root length percent reduction (TRL % red.), fine root length (FRL), and fine root length percent reduction (FRL % red.) of TifEagle bermudagrass 90 days after inoculation evaluated in three establishment methods with uninoculated and inoculated treatments in two experimental trials.

Treatment[x]	TRL % red. (cm)	FRL (cm)	FRL % red. (cm)
	45-d conetainers		
U	—	670 ± 224**	—
I (50)	-31 ± 16[y]	438 ± 170	-32 ± 19
I (100)	-41 ± 13	383 ± 122	-42 ± 13
	90-d conetainers (Trial 1)		
U	—	1130 ± 162**	—
I (50)	-31 ± 11[z]	787 ± 118[zz]	-30 ± 11[zz]
I (100)	-46 ± 10	573 ± 90	-49 ± 9
	90-d conetainers (Trial 2)		
U	—	1074 ± 107**	—
I (50)	-34 ± 19	721 ± 142	-32 ± 16
I (100)	-30 ± 10	776 ± 103	-28 ± 6
	90-d clay pots		
U	—	610 ± 250*	—
I (50)	-9 ± 39	518 ± 193	-6 ± 39
I (100)	-28 ± 25	425 ± 184	-28 ± 27

*, **Uninoculated controls significantly different from both inoculated treatments at the 0.05 and 0.01 probability levels, respectively, according to orthogonal coefficient analysis.

[x]Uninoculated (U); Inoculated (I) with 50 or 100 *B. longicaudatus*/100 cm^3 soil.

[y]Data are means of two trials with six replications each ± standard deviations for 45-d conetainers and 90-d clay pots, and means of one trial with six replications ± standard deviations for 90-d conetainers.

[z, zz]Inoculated treatments significantly different at the 0.05 and 0.01 probability levels, respectively, according to orthogonal coefficient analysis.

Differences in the number of nematodes on a total root length and total dry root weight basis between establishment methods may not be meaningful when the only treatments are within the same cultivar. Standardized population descriptors such as these could be more useful when making comparisons between different grasses within, or among species. Correlation between root length percent reductions and sting nematode population density would be expected on a putting green under stress. These traits were significantly correlated in the 45-d conetainer establishment method with no detectable association found in the 90-d clay pots. This may indicate that conditions in the 45-d conetainers were more representative of field conditions.

Differences were not found between total dry root weights of uninoculated and inoculated treatments in the 90-d clay pot establishment method even though total root lengths were reduced by the sting nematode pressure. Johnson (1970b) noted that root systems of uninoculated

bermudagrass controls were more dense and fibrous than those of inoculated plants. He concluded that visual symptoms were much more apparent than indicated by root weight differences. Results found in the conetainer establishment methods contrasted those from the clay pots. Identification and selection of superior genotypes in conetainers using only total dry root weight reductions could be possible due to significant differences found between uninoculated and inoculated treatments and the high correlation of root weights and lengths in these establishment methods.

Total and fine root lengths of 'TifEagle' bermudagrass were reported to be 57% and 69% greater, respectively, in uninoculated treatments than inoculated when evaluated in large (15 cm) clay pots (Crow and Welch, 2004). These measurements and the corresponding root length percent reductions were very similar to the results found in the 45-d conetainer method evaluated herein. When fine root length is highly associated with total root length it may not need to be reported. However, no additional effort is required for its measurement and it may be informative when explaining root damage caused by plant-parasitic nematodes in other turfgrass species with variable root architecture. Results across trials were not consistent for fine root length or total and fine root length percent reductions in the 90-d conetainers suggesting a potential lack of stability for this establishment method.

Plant breeding methodologies are purposefully designed to only be as comprehensive as required to gather enough information to make genetic gains from selection. The balance between greater investments in time and complexity must be maintained with screening larger population sizes in the search for genes of interest. Plant-parasitic nematode populations and root systems are inherently variable. Therefore it is particularly necessary to reduce outside variation when experimenting with nematodes in the greenhouse so that the genetic potential of each turfgrass being evaluated can be reached and quantified. Conetainer treatments were not root-bound at the completion of the experiments. Sting nematode and bermudagrass measurements were characterized from the entire experimental unit rather than from a sample core as necessary in the clay pots. Sampling error may have contributed to the variability observed in characteristics measured within the 90-d clay pot establishment method because the soil at the pot edge may not be conducive to sting nematode survival, but very favorable for root growth.

Based on this research, TifEagle bermudagrass can be effectively used as a susceptible plant standard to verify pathogenicity of sting nematode inoculum and quantify root injury of untested genotypes during initial screening of large sets of turfgrass germplasm. Use of conetainers will increase the number of germplasm lines that can be evaluated in a single trial because they require less bench space in the greenhouse and the extraction of roots and nematodes does not require sampling as with larger clay pots. Inoculation levels should be no higher than required to establish reproducing populations of nematodes because availability of inoculum can be a limiting factor to the number of genotypes that can be screened in a particular trial. Our results support establishing turfgrasses for 45 days in deep, insulated conetainers before inoculation with 50 *B. longicaudatus*/100 cm^3 soil to determine initial plant response. Further testing of plant response/tolerance may require higher inoculum levels or longer evaluation intervals.

LITERATURE CITED

Anonymous. 1993. USGA recommendation for a method of putting green construction: The 1993 revision. USGA Green Section Record 31:1-3.

Bekal, S., and J. O. Becker. 2000. Host range of a California sting nematode population. HortScience 35:1276-1278.

Burton, G. W. 1972. Registration of 'Coastcross-1' bermudagrass. Crop Science 12:125.

Burton, G. W. 1974. Breeding bermudagrass for turf. Pp. 18-22, *in* E. C. Roberts, Ed. Proceedings of the 2nd International Turfgrass Research Conference. International Turfgrass Society, Blacksburg, VA.

Burton, G. W., C. W. McBeth, and J. L. Stephens. 1946. The growth of 'Kobe' Lespedeza as influenced by the root-knot nematode resistance of the bermuda grass strain with which it is associated. Journal of the American Society of Agronomy 38:651-656.

Crow, W. T. 2005a. Plant-parasitic nematodes on golf course turf. Outlooks on Pest Management 16:10-15.

Crow, W. T. 2005b. How bad are nematode problems on Florida's golf courses. Florida Turf Digest 22:10-12.

Crow, W. T., and H. Han. 2005. Sting nematode. Plant Health Instructor: DOI: 10.1094/PHI-I-2005-1208-01.

Crow, W. T., and J. K. Welch. 2004. Root reductions of St. Augustinegrass (*Stenotaphrum secundatum*) and hybrid bermudagrass (*Cynodon dactylon* × *C. transvaalensis*) induced by *Trichodorus obtusus* and *Paratrichodorus minor*. Nematropica 34:31-37.

Crow, W. T., R. M. Giblin-Davis, and D. W. Lickfeldt. 2003. Slit injection of 1,3-dichloropropene for management of *Belonolaimus longicaudatus* on established bermudagrass. Journal of Nematology 35:302-305.

Giblin-Davis, R. M., P. Busey, and B. J. Center. 1992a. Dynamics of *Belonolaimus longicaudatus* parasitism on a susceptible St. Augustinegrass host. Journal of Nematology 24:432-437.

Giblin-Davis, R. M., J. L. Cisar, F. G. Bilz, and K. E. Williams. 1991. Management practices affecting phytoparasitic nematodes in 'Tifgreen' bermudagrass. Nematropica 21:59-69.

Giblin-Davis, R. M., J. L. Cisar, F. G. Bilz, and K. E. Williams. 1992b. Host status of different bermudagrasses (*Cynodon* spp.) for the sting nematode, *Belonolaimus longicaudatus*. Journal of Nematology 24:749-756.

Good, J. M., N. A. Minton, and C. A. Jaworski. 1965. Relative susceptibility of selected cover crops and 'Coastal' bermudagrass to plant nematodes. Phytopathology 55:1026-1030.

Hanna, W. W., and J. E. Elsner. 1999. Registration of 'TifEagle' bermudagrass. Crop Science 39:1258.

Hixson, A. C., W. T. Crow, R. McSorley, and L. E. Trenholm. 2004. Host status of 'Sealsle 1' seashore paspalum (*Paspalum vaginatum*) to *Belonolaimus longicaudatus* and *Hoplolaimus galeatus*. Journal of Nematology 36:493-498.

Johnson, A. W. 1970a. Control of lance nematode, *Hoplolaimus galeatus*, on 'Tifdwarf' bermudagrass by chemical dips. Journal of Nematology 2:179-181.

Johnson, A. W. 1970b. Pathogenicity and interaction of three nematode species on six bermudagrasses. Journal of Nematology 2:36-41.

Lautz, W. H. 1959. Increase of *Belonolaimus longicaudatus* on various plant species in artificially inoculated soil. Plant Disease Reporter 43:48-50.

McSorley, R., and J. J. Frederick. 1991. Extraction efficiency of *Belonolaimus longicaudatus* from sandy soil. Journal of Nematology 23:511-518.

Perry, V. G., G. C. Smart, Jr., and G. C. Horn. 1970. Nematode problems of turfgrasses in Florida and their control, p. 489-492 Proceedings of the 83rd Annual Meeting of the Florida State Horticultural Society. Miami Beach, USA.

Riggs, R. D., J. L. Dale, and M. L. Hamblen. 1962. Reaction of bermuda grass varieties and lines to root-knot nematodes. Phytopathology 52:587-588.

Robbins, R. T., and K. R. Barker. 1973. Comparisons of host range and reproduction among populations of *Belonolaimus longicaudatus* from North Carolina and Georgia. Plant Disease Reporter 57:750-754.

Schwartz, B. M., K. E. Kenworthy, and W. T. Crow. 2006. Methods for screening zoysiagrass cultivars for sting nematode response. *in* ASA-CSSA-SSSA Abstracts 68:23. American Society of Agronomy, Madison, WI.

Sledge, E. B. 1962. Preliminary report on a *Meloidogyne* sp. parasite of grass in Florida. Plant Disease Reporter 46:52-54.

Tarjan, A. C., and P. Busey. 1985. Genotypic variability in bermudagrass damage by ectoparasitic nematodes. HortScience 20:675-676.

Winchester, J. A., and E. O. Burt. 1964. The effect and control of sting nematodes on 'Ormond' bermuda grass. Plant Disease Reporter 48:625-628.

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