RESEARCH/INVESTIGACIÓN

EFFECT OF *BELONOLAIMUS LONGICAUDATUS* ON ROOT PARAMETERS OF ST. AUGUSTINEGRASS CULTIVARS

Kenneth H. Quesenberry[1], William T. Crow[2]* and Kevin E. Kenworthy[1]

[1]Department of Agronomy, University of Florida, Gainesville, FL 32611-0695 USA. [2]*Entomology and Nematology Department, University of Florida, PO Box 110620 Gainesville, FL 32611-0620 USA. *Corresponding author: wtcr@ufl.edu

ABSTRACT

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In Florida, St. Augustinegrass (*Stenotaphrum secundatum*) is the most commonly used turfgrass species. Sting nematode (*Belonolaimus longicaudatus*) is recognized as a limiting factor to St. Augustinegrass in Florida, where 'Floratam', a polyploid, is the predominant cultivar; although the use of new diploid cultivars is increasing. The objective of this study was to evaluate the root response of three cultivars, Floratam, and two diploid cultivars 'Palmetto' and 'Captiva' grown in plastic pots containing 650 cm^3 of sand in a greenhouse to inoculation with 0, 50, or 300 *B. longicaudatus*/pot. Ninety days after inoculation, nematodes were extracted from the complete soil volume of the pots and counted. Roots were washed clean of soil, scanned, and root lengths, mean root diameters, and mean root volume were measured. All cultivars were good hosts for *B. longicaudatus*, 'Palmetto' supported higher population densities than the other cultivars at the 50 nematode inoculation rate only. There were differences in reproductive factor (Rf) among the inoculation rates, where the 50 nematode inoculation rate (Pi) had an Rf of 0.52, and the 300 nematode inoculation rate had an Rf of 1.84. At Pi 300, the non-infested treatment had greater root lengths than inoculated for all cultivars, demonstrating that *B. longicaudatus* has a significant effect on St. Augustinegrass root development. Root diameter and volume were not affected by inoculation with *B. longicaudatus*. Root diameter and volume were higher for Floratam than for Palmetto or Captiva. Palmetto had greater root length than the other cultivars.

Key words: Belonolaimus longicaudatus, St. Augustinegrass, *Stenotaphrum secundatum*, sting nematode, susceptibility, turfgrass.

RESUMEN

Quesenberry, K. H., W. T. Crow, and K. E. Kenworthy. 2015. Efecto de *Belonolaimus longicaudatus* sobre parámetros radicales de cultivares de pasto de San Agustín. Nematropica 45:96-101.

En Florida, el pasto de San Agustín (*Stenotaphrum secundatum*) es la más común de las especies de césped usadas. El nematodo *Belonolaimus longicaudatus* constituye un factor limitante reconocido del pasto de San Agustín en Florida, siendo el cultivar poliploide 'Floratam', el predominante; aunque el uso de nuevos cultivares diploides está en aumento. El objetivo de este estudio fue evaluar la respuesta de las raíces de tres cultivares, Floratam y dos cultivares diploides 'Palmetto' y 'Captiva', cultivados en macetas de plástico con 650 cm^3 de arena en invernadero, a la inoculación con 0, 50 o 300 *B. longicaudatus*/maceta. Noventa días después de la inoculación, los nematodos fueron extraídos de todo el suelo contenido en las macetas y contados. La raíces fueron lavadas, para eliminar el suelo adherido, escaneadas y se midieron su longitud, diámetro medio y volumen medio. Todos los cultivares fueron buenos hospedantes de *B. longicaudatus*, 'Palmetto' mantuvo densidades de población mayores que otros cultivares cuando el inóculo fue de 50 nematodos por maceta. Hubo diferencias en los factores de reproducción (Rf) a los diferentes niveles de inóculo (Pi), el nivel de inóculo de 50 nematodos por maceta mostro un Rf de 0.52, y el de 300 nematodos por maceta tuvo un Rf de 1.84. A Pi 300, el tratamiento no-infestado mostró mayor longitud de las raíces que los inoculados en todos los cultivares, demostrando que *B. longicaudatus* tiene un efecto significativo sobre el desarrollo de las raíces del pasto de San Agustín. El diámetro y volumen de las raíces no se vieron afectados por la inoculación con *B. longicaudatus*. El diámetro y volumen de las raíces fueron mayores en Floratam que en Palmetto o Captiva. Palmetto tuvo mayor longitud de raíces que el resto de cultivares.

Palabras clave: Belonolaimus longicaudatus, Pasto de San Agustín, *Stenotaphrum secundatum*, nematodo, susceptibilidad, césped.

INTRODUCTION

The turfgrass industry in Florida generates estimated revenues of $7.82 billion and encompasses 3.94 million acres of maintained turfgrass (golf courses, sod farms, institutional grounds, and home lawns). St. Augustinegrass (*Stenotaphrum secundatum*) is the primary turfgrass species grown for commercial sod sales in Florida (>20,000 ha) (Hodges and Stevens, 2010). 'Floratam', a polyploid St. Augustinegrass, was released in 1973 (Horn *et al.*, 1973) and quickly became the predominant cultivar produced in Florida. Today, it accounts for over 70% of current St. Augustinegrass sod acres in Florida (Haydu *et al.*, 2006). Floratam was originally selected for resistance to panicum mosaic virus, which causes St. Augustine decline (SAD) and the southern chinch bug (*Blissus insularis*) (Horn *et al.*, 1973). However, in the 1980s a 'polyploid damaging population' (PDP) of southern chinch bug that overcomes the resistance of Floratam was documented in Florida (Busey and Center, 1987; Busey, 1990). Floratam spreads vigorously through rapid stolon production, exhibits poor shade tolerance, and has a coarse leaf texture with an upright growth habit that makes it unappealing to certain consumers. Therefore, consumer demand has led to the development of several newer cultivars with semi-dwarf to dwarf growth characteristics. One of the most common is 'Palmetto', a diploid cultivar characterized by semi-dwarf growth habit and better shade tolerance than Floratam. Recent research has led to the development of another diploid cultivar, NUF-76 [US Plant Patent 21,280, (Nagata and Busey, 2010)] released in 2007 and known as 'Captiva'. Captiva is resistant to the PDP southern chinch bug and to the plant hopper (*Liburnia pseudoseminigra*). However, once this cultivar was introduced into commercial production, several landscape installations experienced significant loss of sod quality and plant death. Efforts to identify the causes of the sod installation failures identified potential causes as fungal and nematode pathogens.

Sting nematode (*Belonolaimus longicaudatus* Rau) has been shown to be a significant pest on many warm-season turfgrasses (Crow and Han, 2005). *Belonolaimus longicaudatus* is common in the coastal plains of the southeastern United States and is considered a limitation for the use of turfgrasses in the state of Florida. Damage to St. Augustinegrass roots, and subsequent topgrowth chlorosis, has been attributed to *B. longicaudatus* in the field (Kelsheimer and Overman, 1953) and has been observed in controlled greenhouse studies (Rhoades, 1962; Busey *et al.*, 1991, 1993). Generally, diploid St. Augustinegrass genotypes have been characterized as susceptible (Busey *et al.*, 1991), but resistance was identified in some polyploid cultivars including Floratam (Busey *et al.*, 1993).

Since the nematicide options for lawn grasses are very limited, identification of genetic resistance to *B. longicaudatus* would be an important objective. The search for useful resistance or tolerance to *B. longicaudatus* in different turfgrass species was initiated over 25 years ago by screening available cultivars and a limited number of bermudagrass (*Cynodon* spp.) accessions (Giblin-Davis *et al.*, 1992b). More recently, Pang *et al.* (2011b, 2011c) evaluated the response of dwarf and non-dwarf cultivars and accessions of common bermudagrass (*C. dactylon*), African bermudagrass (*C. transvaalensis*), and seashore paspalum (*Paspalum vaginatum*), and Schwartz *et al.* (2010) of zoysiagrass to *B. longicaudatus*. This work and additional research has developed methods for greenhouse screening of vegetatively propagated warm season turfgrasses to *B. longicaudatus* (Schwartz, *et al.*, 2008; Pang *et al.*, 2011a).

The objectives of this research were to compare Floratam, Palmetto, and Captiva St. Augustinegrass cultivars for susceptibility to *B. longicaudatus*. Nematode reproductive factor (Rf) was used to compare resistance among cultivars, whereas root scanning technology was used to compare plant tolerance.

MATERIALS AND METHODS

Inoculum of *B. longicaudatus* was maintained on 'FX-313' St. Augustinegrass grown in clay pots filled with pure sand under greenhouse conditions (Giblin-Davis *et al.*, 1992a). Juveniles and adults of *B. longicaudatus* were extracted and collected from soil using a decanting and sieving technique (Flegg, 1967). The mean number of nematodes was counted from five 1-ml aliquots and extrapolated to the total volume of the suspension.

The plant growth containers were 6-cm-diam., 25-cm-deep black plastic pots (Deepot D40L; Stuewe & Sons, Tangent, OR, USA) that were filled with 650 cm^3 USGA specification greens grade sand (USGA, 1993). Drainage holes in the bottom of the pots were covered with polyester fiberfill to keep sand from leaking out. Each rack held 20 pots. The experiment was initiated by planting two nematode free, three node, stolon cuttings of Floratam, Captiva or Palmetto into each pot. Two minutes of overhead irrigation were applied three times daily to allow the cuttings to develop roots. At 3 wk after propagation, each pot was inoculated with 0 (water only), 50, or 300 mixed life stages *B. longicaudatus*. Inoculation was accomplished by pipetting an appropriate volume of water with nematodes into 2 holes approximately

3-cm-deep in the top of the soil in each pot. The holes were covered with sand after inoculation and lightly moistened. Plants were grown for 90 d after inoculation and clipped every 2 to 3 wk to maintain stolon length of approximately 7 to 10 cm. The experiment was repeated in consecutive years, from September to December of 2010 and from March to June of 2011. The greenhouse temperature controls were set to be heated when temperature was below 21°C and fans and evaporative coolers engaged when temperatures were above 29°C. The experimental design for each individual experiment was a randomized complete block, split plot with nematode levels as main plots and cultivars as sub plots. There were 10 replications of each cultivar/nematode level combination. Data were analyzed according to general linear mixed models with replications and experiments treated as random effects and means were separated using orthogonal contrasts using SAS software (SAS Institute, Cary, NC, USA).

Experiments were harvested 90 d after inoculation. Root and soil samples were collected from each pot. Roots were collected by removing sand with roots from the shoots and polyester fiberfill. Roots were washed free of sand using a 853-μm sieve and then submerged into water in 50-ml plastic centrifuge tubes for storage in a refrigerator until analysis. Roots were digitally scanned using WinRHIZO root scanning equipment and software (Regent Instruments, Ottawa, Ontario, Canada) as described by Pang *et al.* (2011a). Root length, average diameter, and volume were measured from the scanned images. Nematodes were extracted from the entire soil volume of each pot using a modified centrifugal flotation technique (Jenkins, 1964) and counted using an inverted microscope at ×40. Reproduction factor (Rf) was calculated by dividing the final population density (Pf) by the inoculation density (Pi).

RESULTS

Cultivar effects on reproductive factor

There were no effects of cultivars or years, or any effects of the interactions of these factors with each other or with inoculation levels on *B. longicaudatus* Pf at the highest inoculation rate (300/pot). However, *B. longicaudatus* Pf was significantly higher on Palmetto than on the other cultivars at 50/pot (Table 1). There was a significant effect of inoculation level on *B. longicaudatus* Pf. When inoculated at 300 *B. longicaudatus*/pot, the mean Pf decreased to 155, an Rf of 0.52. When inoculated at 50 *B. longicaudatus*/pot, the mean Pf increased to 92, an Rf of 1.84 (Table 1).

Cultivar effects on root diameter, length, and volume

Analysis of the mean root diameter as determined from root scans revealed significant differences between the polyploid cultivar, Floratam, and the two diploid cultivars Captiva and Palmetto. The analysis also showed effects of year, but no effect of nematode inoculation levels and no interaction of years with cultivars or inoculation levels with cultivars. Floratam had larger mean root diameter than either Captiva or Palmetto (Table 2), which were not different from each other. Analysis of total root length showed effects of cultivar, year, and a year × cultivar interaction ($P \leq 0.05$) and an effect of inoculation level ($P = 0.07$). The year by cultivar interaction appeared to be due to changes in magnitude rather than rankings, and results are presented for cultivars averaged over years. Total root length was higher for Palmetto than Captiva or Floratam, which were not different from each other (Table 2). The data were subset only to evaluate differences among cultivars in the 0 inoculation treatment, but the cultivar rankings were unchanged (data not shown). This finding suggests that the observed differences in total root length were primarily genotype effects of the cultivars and not impacted by nematode inoculation.

Root volume is a function of both diameter and length. Evaluation of root volume showed effects of cultivar and year, but unlike root diameter there was an effect of inoculation level, and an interaction of year with inoculation level and cultivar with inoculation level. Similar to root length, these interactions appeared to be due to changes in magnitude rather than changes in rank. Floratam had larger total root volume (6.30 cm^3/pot) than Captiva (4.27 cm^3/pot) or Palmetto (4.17 cm^3/pot), which were not different from each other (Table 2). Root volume averaged over cultivars was not different between the 0 and 50 inoculation level, but the 300 inoculation level resulted in a decrease in root volume compared to the 50 inoculation level (Table 3). Root length averaged over cultivars was not different between the 0 and 50 inoculation level, but the 300 inoculation level resulted in a decrease in root length compared to the 0 inoculation level (Table 3).

DISCUSSION

While Busey *et al.* (1993) found Floratam and other polyploid cultivars of St. Augustinegrass to be resistant to *B. longicaudatus* relative to diploid cultivars, our results indicate that Floratam is similar in susceptibility to the tested diploids, Palmetto and Captiva. The reason for this difference is unknown, but may be related to the carrying capacity of Floratam under the different conditions between the

Table 1. Effects of St. Augustinegrass cultivar and inoculation rate on population density of *Belonolaimus longicaudatus* per 650 cm^3 pot 90 d after inoculation averaged across two trials.

Cultivar	Inoculation rate (*B. longicaudatus*/pot)[y]			
	0	50	300	Mean
Floratam	0	81[y]Bb[z]	160 Aa	121 a
Palmetto	0	112 Ba	137 Aa	125 a
Captiva	0	82 Bb	168 Aa	125 a
Mean	0	92 B	155 A	---

[y]*Belonolaimus longicaudatus*/pot. Data are means of 20 replications.
[z]Means within a row followed by common capital letter are not different, and means within a column followed by common lower case letters are not different as determined using orthogonal contrasts ($P \leq 0.05$).

Table 2. Root parameters of three cultivars of St. Augustinegrass averaged across *Belonolaimus longicaudatus* inoculation rates (0, 50, 500 nematodes/650 cm^3 pot) and across two repetitions.

Cultivar	Root diameter (mm)	Root length (cm)	Root volume (cm^3)
Floratam	0.55[y]a[z]	2604 b	6.30 a
Palmetto	0.40 b	2965 a	4.17 b
Captiva	0.43 b	2757 b	4.27 b

[y]Data are means of 60 replications.
[z]Means within columns followed by common letters are not different as determined using orthogonal contrasts ($P \leq 0.05$).

Table 3. Effect of inoculation rate (0, 50, 500 nematodes/650 cm^3 pot) of *Belonolaimus longicaudatus* on root parameters of three cultivars of St. Augustinegrass averaged across cultivars and across two repetitions.

Inoculation rate	Root diameter (mm)	Root length (cm)	Root volume (cm^3)
0	0.45[y]a[z]	2859 a	5.03 ab
50	0.48 a	2796 ab	5.24 a
300	0.45 a	2670 b	4.48 b

[y]Data are means of 60 replications.
[z]Means within columns followed by common letters are not different as determined using orthogonal contrasts ($P \leq 0.05$).

two studies.

The greatest differences observed among cultivars were between Floratam and the dipoloids Palmetto and Captiva with regard to root diameter and volume. Similarly, Busey *et al.* (1993) reported increased root dry weights of polyploid cultivars compared to diploids. These findings are in agreement with the general trend that increases in ploidy level results in increases in plant morphological traits (Burnham, 1962). The lack of effects from *B. longicaudatus* on root diameter suggests that this genotype morphological characteristic is in fact more related to ploidy than to any effects of nematode feeding. The greatest impact from *B. longicaudatus* was on root length indicating that this root measurement is the most valuable for evaluating plant tolerance to ectoparasitic nematodes on turfgrasses, confirming the results of earlier studies (Schwartz *et al.*, 2008; Pang *et al.*, 2011a).

It is of interest that in these 650 cm^3 pots the Rf of *B. longicaudatus* was >1 for the 50 nematode inoculation rate and <1 for the 300 nematode inoculation rate. These results suggest that, under the study conditions, the carrying capacity for *B. longicaudatus* was between 100 and 150 nematodes per pot for all three cultivars. Conversely, effects of *B. longicaudatus* on root volume and length were only observed at the 300 nematode inoculation rate. If the Sasser *et al.* (1984) method for assignment of host suitability for root-knot nematodes was applied to these results, the St. Augustinegrass cultivars would be classified as tolerant to *B. longicaudatus* at the 50 nematode inoculation rate, but hypersusceptible at the 300 nematode inoculation rate. These conclusions highlight that care should be taken when applying terms and definitions developed for one nematode-host system to another. Differences in Rf and tolerance to *B. longicaudatus* with different inoculation rates have been reported previously on zoysia (Schwartz *et al.*, 2008), and negative Rf values for *B. longicaudatus* resulting from high inoculation densities have been observed on bermudagrass and seashore paspalum (Pang *et al.*, 2011b, c). For an ectoparasitic nematode such as *B. longicaudatus*, inoculation with lower numbers are better suited for quantifying plant resistance and higher numbers are more useful for quantifying plant tolerance in short-term studies. Preliminary studies with the specific nematode-host combination and growing conditions used are necessary to estimate what inoculation rates should be used.

Floratam has been a highly successful cultivar for over 40 years and has a reputation for rapidly establishing from sod and developing a deep root system. Our findings of larger diameter roots for Floratam than with the diploid cultivars suggests that attention should be given to selecting for larger root diameters in diploid germplasm to enhance sod establishment. This study found that Captiva was not more susceptible to *B. longicaudatus* than other common cultivars. Therefore, while *B. longicaudatus* is damaging to Captiva, it is probably not the primary cause of increased incidence of sod establishment failure.

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