RESPONSES OF BERMUDAGRASS AND SEASHORE PASPALUM TO STING AND SPIRAL NEMATODES

By

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To my mother and father and who have helped me in my education throughout my lifetime, making this miracle possible

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TABLE OF CONTENTS

page

ACKNOWLEDGMENTS 4

LIST OF TABLES 8

LIST OF FIGURES 10

ABSTRACT 12

CHAPTER

1 INTRODUCTION 14

2 LITERATURE REVIEW 18

Cynodon spp. 18
Distribution and Utility 18
Taxonomy and Species 18
Growth Habit and Establishment 19
Adaptation and Cultivation 19
Cultivars 20
Paspalum vaginatum 21
Distribution and Utility 21
Growth Habit and Establishment 22
Adaptation and Cultivation 22
Cultivars 24
Belonolaimus longicaudatus 24
Taxonomy 24
Distribution and Hosts 24
Life Cycle and Biology 25
Damage and Symptoms 25
Management 25
Helicotylenchus spp. 26
Taxonomy 26
Distribution and Hosts 27
Life Cycle and Biology 27
Damage and Symptoms 28
Management 28

3 BERMUDAGRASS CULTIVAR RESPONSES TO STING NEMATODES 29

Introduction 29
Materials and Methods 31
Plant Materials 31
Inoculum Preparation 32

Nematode rDNA Analysis....32
Cultivar Nematode Responses....33
Data Analysis....35
Results....35
Discussion....38

4 TOLERANCE AND RESISTANCE OF SEASHORE PASPALUM CULTIVARS TO STING AND SPIRAL NEMATODES....46

Introduction....46
Materials and Methods....47
Plant Materials....47
Inoculum Preparation....48
Nematode Species Identification....48
Greenhouse Studies....48
Data Analysis....50
Results....51
Responses to *Belonolaimus longicaudatus*....51
Responses to *Helicotylenchus pseudorobustus*....52
Discussion....52

5 FIELD RESPONSES OF BERMUDAGRASS AND SEASHORE PASPALUM CULTIVARS TO STING AND SPIRAL NEMATODES....57

Introduction....57
Materials and Methods....59
Two-year Field Study....59
One-year Field Study....61
Statistical Analysis....62
Results....63
Two-year Field Study....63
Bermudagrass cultivars....63
Seashore paspalum cultivars....65
One-year Field Study....66
Bermudagrass cultivars....66
Seashore paspalum cultivars....66
Discussion....66

6 DNA CONTENT OF BERMUDAGRASS ACCESSIONS IN FLORIDA....84

Introduction....84
Materials and Methods....86
Plant Materials....86
Flow Cytometry....86
Results and Discussion....87

7 SCREENING BERMUDAGRASS GERMPLASM ACCESSIONS FOR RESPONES TO STING NEMATODES....96

Introduction....96
Materials and Methods....97
Plant Materials....97
Inoculum Preparation....98
Nematode Responses of Germplasm Accessions....98
Data Analysis....100
Results....100
Discussion....102
Conclusions....103

8 SUMMARY....109

LIST OF REFERENCES....112

BIOGRAPHICAL SKETCH....121

LIST OF TABLES

Table		page
3-1	Mean total root length of 17 bermudagrass cultivars 90 days after inoculation with *Belonolaimus longicaudatus*.	41
3-2	Mean fine root length of 17 bermudagrass cultivars 90 days after inoculation with *Belonolaimus longicaudatus*.	42
3-3	Mean final population density (Pf) and reproductive factor (Rf) of *Belonolaimus longicaudatus* on 17 bermudagrass cultivars 90.	43
4-1	Mean total root length of seven seashore paspalum cultivars 90 days after inoculation with *Belonolaimus longicaudatus*.	55
4-2	Final mean population density (Pf) and reproduction factor (Rf) of *Belonolaimus longicaudatus* on seven seashore paspalum cultivars.	55
4-3	Mean total root length of seven seashore paspalum cultivars 90 days after inoculation with *Helicotylenchus pseudorobustus*	56
4-4	Mean population density (Pf) and reproduction factor (Rf) of *Helicotylenchus pseudorobustus* on seven seashore paspalum cultivars.	56
5-1	Population density of *Belonolaimus longicaudatus* on eight bermudagrass cultivars	71
5-2	Population density of *Helicotylenchus pseudorobustus* on eight bermudagrass cultivars.	71
5-3	Total root length of eight bermudagrass cultivars in nematode infested field plots.	72
5-4	Aboveground percent green cover of eight bermudagrass cultivars in nematode infested field plots.	72
5-5	Population density of *Belonolaimus longicaudatus* on three seashore paspalum cultivars.	73
5-6	Population density of *Helicotylenchus pseudorobustus* on three seashore paspalum cultivars.	73
5-7	Total root length of three seashore paspalum cultivars in nematode infested field plots.	73
5-8	Aboveground percent green cover of three seashore paspalum cultivars in nematode infested field plots.	74
5-9	Population density of *Belonolaimus longicaudatus* and *Helicotylenchus pseudorobustus* on bermudagrass and seashore paspalum.	74

5-10 Total root length and aboveground percent green cover of bermudagrass and seashore paspalum in nematode infested field plots. 74

5-11 Aboveground percent green cover of two bermudagrass cultivars in nematode infested field plots. 75

5-12 Nematode population density of *Belonolaimus longicaudatus* and *Helicotylenchus pseudorobustus* on two bermudagrass cultivars. 75

5-13 Aboveground percent green cover of seven seashore paspalum cultivars in nematode infested field plots. 75

5-14 Nematode population density of *Belonolaimus longicaudatus* on seven seashore paspalum cultivars. 76

5-15 Nematode population density of *Helicotylenchus pseudorobustus* on seven seashore paspalum cultivars. 76

6-1 Nuclear DNA content and ploidy level of 47 University of Florida bermudagrass accessions and three commercial cultivars. 91

7-1 Mean total root length of five cultivars and 46 germplasm accessions of bermudagrass 90 days after inoculation with *Belonolaimus longicaudatus*. 105

7-2 Final mean population density (Pf) and reproductive factor (Rf) of *Belonolaimus longicaudatus* on five cultivars and 46 germplasm accessions of bermudagrass. 107

LIST OF FIGURES

Figure page

3-1 Regression relationship between the root volumes of bermudagrass measured by scanning method and graduated cylinder method in test one. 44

3-2 Regression relationship between the root volumes of bermudagrass measured by scanning method and graduated cylinder method in test two. 45

5-1 Regression relationship between population density of *Helicotylenchus pseudorobustus* and *Belonolaimus longicaudatus* in 'Celebration' and 'TifSport' bermudagrass. 77

5-2 Regression relationship between the total root length of 'Celebration' bermudagrass and population density of *Belonolaimus longicaudatus*. 78

5-3 Regression relationship between the percent green cover in 'Aloha' seashore paspalum and 'Celebration' bermudagrass and the population densities of *Belonolaimus longicaudatus*. 79

5-4 Regression relationship between the total root length of 'Floradwarf' and 'Tifgreen' bermudagrass and the population densities of *Helicotylenchus pseudorobustus*. 80

5-5 Regression relationship between percent green cover in 'Floradwarf' and 'TifEagle' bermudagrass and the population density of *Helicotylenchus pseudorobustus*. 81

5-6 Regression relationship between population density of *Helicotylenchus pseudorobustus* and *Belonolaimus longicaudatus* in 'Aloha', 'SeaDwarf', and 'SeaIsle 1' seashore paspalum. 82

6-1 Flow cytometric histogram of diploid, triploid, and tetraploid bermudagrass and trout erythrocyte nuclei. 92

6-2 Flow cytometric histogram of diploid and triploid bermudagrass and trout erythrocyte nuclei. 92

6-3 Flow cytometric histogram of diploid and tetraploid bermudagrass and trout erythrocyte nuclei. 93

6-4 Flow cytometric histogram of diploid bermudagrass accession and trout erythrocyte nuclei. 93

6-5 Flow cytometric histogram of triploid bermudagrass cultivar control and trout erythrocyte nuclei. 94

6-6 Flow cytometric histogram of tetraploid bermudagrass and trout erythrocyte nuclei. 94

6-7 Flow cytometric histogram of hexaploid bermudagrass cultivar control and trout erythrocyte nuclei.....95

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Bermudagrass (*Cynodon* spp.) and seashore paspalum (*Paspalum vaginatum* Swartz) are commonly used warm-season turfgrasses on golf courses in Florida. *Belonolaimus longicaudatus* is the most serious nematode pests on turfgrass and *Helicotylenchus* spp. are often found to have high population densities in seashore paspalum in Florida. Greenhouse and field experiments were conducted to test 17 bermudagrass cultivars and seven seashore paspalum cultivars for their resistance and tolerance to *Belonolaimus longicaudatus* or *Helicotylenchus pseudorobustus*.

Greenhouse studies showed that no dwarf bermudagrass cultivars were both tolerant and resistant to *B. longicaudatus*. 'Tifdwarf' and 'Emerald Dwarf' were tolerant and 'Champion', 'TifEagle', and 'Floradwarf' were damaged by *B. longicaudatus*. Non-dwarf cultivars 'TifSport' and 'Riviera' were both tolerant and resistant to *B. longicaudatus*. Differences in tolerance among seashore paspalum were found although all cultivars were susceptible to *B. longicaudatus* but resistant to *H. pseudorobustus*. 'Salam', 'SeaDwarf', and 'SeaIsle Supreme' were tolerant to *B. longicaudatus*, and 'SeaSpray' and 'SeaIsle 2000' were tolerant to *H. pseudorobustus*.

Field studies indicate that bermudagrass is a better host to *B. longicaudatus*, and seashore paspalum is a better host to *H. pseudorobustus*. A negative logarithmic relationship was found

between the population densities of *B. longicaudatus* and *H. pseudorobustus* in two bermudagrass and three seashore paspalum cultivars. TifSport bermudagrass and SeaDwarf seashore paspalum were the most resistant to *B. longicaudatus* in field.

Flow cytometry was used to determine the nuclear DNA content and ploidy levels of 47 University of Florida bermudagrass germplasm accessions. Twenty triploid, 24 tetraploid, one pentaploid, and three hexaploid accessions were identified. Most of these, along with 12 diploid African bermudagrass (*Cynodon transvaalensis* Burtt-Davy) accessions were further screened for nematode responses under greenhouse conditions. Results indicated that four African and seven common bermudagrass accessions were tolerant to *B. longicaudatus*, and eight African and 22 common bermudagrass accessions were resistant to *B. longicaudatus*, all of which could be used for future cultivar breeding for nematode tolerance or resistance. Accessions that are both resistant and tolerant to *B. longicaudatus* were identified in both common and African bermudagrass accessions, which would aid in increasing the genetic diversity of future bermudagrass cultivars on golf courses.

CHAPTER 1
INTRODUCTION

Golf is important to Florida's economy. It was reported that in 2000, golf course revenues were $4.44 billion in Florida (Haydu and Hodges, 2002). The most widely used warm-season turfgrasses on putting greens, tees, and fairways of golf courses in Florida are bermudagrass (*Cynodon* spp.) and seashore paspalum (*Paspalum vaginatum* Swartz). Hybrid bermudagrass cultivars (*C. dactylon* [L.] Pers. var. *dactylon* × *C. transvaalensis* Burtt-Davy) that produce fine-textured and dense turf have become the standards for use on golf courses in Florida and other regions where warm-season turfgrasses are utilized. As the most salt tolerant warm-season turfgrass, seashore paspalum is increasingly used in the southern coastal areas.

Based on current estimates, 87% of Florida golf courses are under the risk of nematode damage (Crow, 2005a). Sting (*Belonolaimus longicaudatus* Rau) and spiral nematodes (*Helicotylenchus* spp.) are common nematode pests occurring on turf in Florida. Sting nematodes are frequently found in the sandy coastal soil of the southeastern areas of the United States and are considered as the most damaging plant-parasitic nematode on bermudagrass in Florida (Crow, 2005a; Luc *et al*., 2007). Sting nematodes are ectoparasites that feed from the outside of the roots by inserting the stylet into the roots. Previous studies showed that sting nematode could lead to reduced drought tolerance (Trenholm *et al*., 2005) and increased potential for nitrate leaching in turf (Luc *et al*., 2006). Sting nematodes damage turf roots and their feeding usually causes the root tip to stop growing and results in stunted roots. Aboveground symptoms include wilting, chlorosis, and thinning of the turf, and these symptoms typically occur in irregular patches in the field.

Spiral nematodes were found on >84% of golf courses (Crow, 2005a) and 85% of seashore paspalum lawns in Florida (Hixson and Crow, 2004). Population densities of spiral nematodes in

the moderate or high risk categories (more than 500 nematodes/100 cm³ soil) in seashore paspalum were often found in Florida (Hixson and Crow, 2004). Spiral nematodes are found throughout the southeastern United States on turfgrasses and cause more damage on sandy soils (UOIE, 2000). *Helicotylenchus* spp. is smaller in size than *B. longicaudatus*, and the body is spiral shaped when dead. *Helicotylenchus* spp. are ectoparasitic or semi-endoparasitic on plant roots depending on the species. The underground symptoms include shortened and discolored roots, with necrotic lesions, and the aboveground symptoms on turf include a delayed spring green-up, chlorosis and necrosis of leaf blades, stunting, reduced vigor, and gradual thinning of the turf (UOIE, 2000).

Nematode management on golf courses can be challenging. Warm-season turfgrasses are perennial, so use of crop rotation or cover crops is not available. Recent cancellation of fenamiphos (Nemacur®, Bayer CropScience, Research Triangle Park, NC) has resulted in the need for alternative nematode management tactics. Currently, 1,3-dichloropropene (Curfew Soil Fumigant®, Dow AgroSciences, Indianapolis, IN) is the most effective chemical management option for plant-parasitic nematodes on turf. However, Curfew provides only short-term control, is expensive, and environmental restrictions highlight the need for alternative options. A bionematicide with *Pasteuria* sp. as the active ingredient (EcoNem™, Pasteuria BioScience, Alachua, FL) is being used for management of *B. longicaudatus* on turf, but to date it has not been found to be effective in field trials (Crow, W. T., personal communication). Utilization of resistant or tolerant grass cultivars is the most efficient and least costly practice with minimal ecological effects on non-targeted species (Giblin-Davis *et al*., 1992b). Information about the resistance and tolerance to *B. longicaudatus* of several newer bermudagrass cultivars used on golf course putting greens and fairways is not available. Except for ‘TifEagle’, all bermudagrass

cultivars used on greens share a common genetic background. Therefore, there is intense environmental pressure for pest development on greens-type bermudagrass cultivars due to a lack of genetic diversity (K. E. Kenworthy, personal communication). This highlights the need to select new sources of genetically superior bermudagrass. Most bermudagrass cultivars that are widely used on golf courses are triploid bermudagrass (*C. dactylon* var. *dactylon* × *C. transvaalensis*) resulting from hybridizations between tetraploid, common bermudagrass and diploid, African bermudagrass (Burton, 1974). Previous development of hybrids tended to focus on the selection of a superior common bermudagrass parent. This was because it had previously been assumed that there was limited genetic variation among available accessions of African bermudagrass. However, Kenworthy *et al*. (2006) provided evidence that variation does exist for many traits in African bermudagrass. This necessitates the screening of both African and common bermudagrass for nematode responses. Not much is known about the range of responses of commercial seashore paspalum cultivars to *B. longicaudatus* or *Helicotylenchus* spp., but this information is needed by golf course superintendents or consumers to make decisions on which cultivar should be planted in a nematode infested site.

The objectives of this project were: 1) to determine the range of resistance and tolerance of newer commercial bermudagrass cultivars to *B. longicaudatus*, and to compare these to standards such as ‘Tifway’ and ‘Tifdwarf’; 2) to determine the range of responses of seashore paspalum cultivars to *B. longicaudatus* and *Helicotylenchus* spp., respectively; 3) to determine if an alternative method proposed by Quesenberry and Dunn (1977) for assessing sting nematode damage on bermudagrass roots is as effective, or more efficient, than conventional methods; 4) to conduct field evaluations of bermudagrass and seashore paspalum cultivars to identify cultivars with superior turf characters and nematode responses; 5) to compare the relative

resistance and tolerance of seashore paspalum to bermudagrass; 6) to confirm the ploidy level of bermudagrass germplasm accessions with good turf characters in the field so that ploidy levels can be grouped for nematode response screening and breeding; 7) to screen selected bermudagrass germplasm accessions from the ploidy level tests for their resistance and tolerance to *B. longicaudatus*.

CHAPTER 2
LITERATURE REVIEW

Cynodon spp.

Distribution and Utility

Bermudagrass (*Cynodon* spp.) originated in Africa and was introduced to the United States in 1751 (Hanson, 1972). It is now distributed in over 100 countries throughout the tropical and subtropical areas of the world, and is well adapted to Asia, Africa, Australia, India, South America and the southern region of the United States (Harlan *et al*., 1970). In the United States, bermudagrass is distributed throughout warmer regions: from Florida northward to Maryland and New Jersey along the east coast, and westward along the southern border to California (Harlan *et al*., 1970). Bermudagrass is not only a forage grass but also a major turfgrass used on golf courses, sport fields, lawns, and parks.

Taxonomy and Species

Bermudagrass belongs to kingdom Plantae, subkingdom Tracheobionta, superdivision spermatophyta, division Magnoliophyta, class Liliopsida, subclass Commelinidae, order Cyperales, family Poaceae, subfamily Eragrostoideae, tribe Chloridea, and genus *Cynodon* (USDA, 2010).

There are nine species in the genus *Cynodon* and the basic chromosome number is nine (Harlan and de Wet, 1969; Wu *et al*., 2006). Tetraploid *Cynodon dactylon* [L.] Pers. var *dactylon* is known as common bermudagrass (2n = 4x = 36) and is the most widespread species (de Silva and Snaydon, 1995). *Cynodon transvaalensis* Burtt-Davy (2n = 2x = 18), known as African bermudagrass, is a diploid species (Forbes and Burton, 1963; Wu *et al*., 2006). Triploid bermudagrass (*C. dactylon* [L.] Pers. var. *dactylon* × *C. transvaalensis* Burtt-Davy) (2n = 3x = 27) produces fine-textured, dense bermudagrass cultivars that have become the standards for use

on golf courses in regions where warm season turfgrasses are utilized. Pentaploid (2n = 5x = 45) and hexaploid (2n = 6x = 54) plants have been previously identified (Johnston, 1975; Hanna *et al*., 1990; Burton *et al*., 1993; Wu *et al*., 2006; Kang *et al*., 2007). 'Tifton 10', a hexaploid cultivar, has been used on golf courses, athletic fields, and home lawns (Hanna *et al*., 1990).

Growth Habit and Establishment

Bermudagrass is a sod forming grass, which establishes quickly and spreads by stolons, rhizomes and seed. Most commercial cultivars are vegetatively propagated by planting of sprigs, sod, or plugs. Bermudagrass has a perennial root system with vigorous rhizomes. Roots are produced at the nodes of stolons after new leaves or tillers are produced (Burton and Hanna, 1995).

Adaptation and Cultivation

Bermudagrass is well adapted to tropical and subtropical climates. It grows best under high temperatures, moderate to high rainfall, and mild winters (Turgeon, 2005). Temperature is the key factor that limits its adaptation in the world. Optimum daytime temperature for bermudagrass is between 35° and 38°C and soil temperature for root growth is near 27°C (Turgeon, 2005). Bermudagrass prefers moist climates and needs more than 50 cm of rainfall per year (Turgeon, 2005). During a long drought, bermudagrass can go dormant, but will restart growth with sufficient irrigation or rainfall (Trenholm, *et al*., 2003).

Bermudagrass grows well on a wide variety of soils. All fertilization should be based on a soil test, but Florida soils are high in phosphorous, and typically require little if any phosphorus input (Trenholm, *et al*., 2003). It was reported that 489 to 733 g N/100 m^2 per month is usually applied to bermudagrass in the field during the growing season (Trenholm, *et al*., 2003). Bermudagrass on lawns is usually mowed at the height of 1.9 to 3.8 cm, but can be maintained at a lower mowing height of 1.3 cm with a high level of management if no more than 1/3 of the

total blade is mowed (Trenholm, *et al*., 2003). While bermudagrass used on golf courses can be mowed low as 3 mm (Beard, 2002). Higher mowing heights could encourage a deeper root system and reduce weeds and pest problems.

Bermudagrass has good drought and salt tolerance, but poor cold and shade tolerance. It is poorly tolerant to many diseases, insects, and nematode pests (Trenholm, *et al*., 2003). Arthropods such as mole crickets (*Scapteriscus* spp.) are major pests on bermudagrass (Trenholm, *et al*., 2003). However, several genera of nematodes are among the most serious pests of bermudagrass in Florida (Crow, 2005b). Nematodes cause yellowing, wilting, and stunting of growth in turf, and symptoms usually appear as patches in the field. Nematode damage is more serious on sandy soils (Crow, 2005b).

Cultivars

'Tifway' (Tifton 419), 'TifSport', 'Celebration', 'Riviera', 'Patriot', 'Midlawn', 'Midiron', 'Princess 77', and Tifton10 are non-dwarf cultivars usually used on fairways and tees of golf courses. These cultivars are usually maintained at a height of 0.6 to 2.5 cm (Beard, 2002). Tifway is the most popular bermudagrass cultivar used on golf course fairways and sports fields in the southern United States (Burton, 1966). This cultivar has good disease resistance and a very dark green color compared with other cultivars (Burton, 1966). TifSport is an induced mutant from cold tolerant Midiron and it produces better quality turf than Midiron and has improved cold tolerance compared to Tifway (Bouton *et al*., 1997). Patriot has excellent cold hardiness compared with other hybrid bermudagrasses (Wu *et al*., 2009). Midiron and Midlawn are cold tolerant triploid cultivars with dark color and medium texture that are used on golf course fairways and lawns (Polomski *et al*., 2010). Tifton 10 is a vegetatively propagated hexaploid cultivar released in 1988. It has moderate salt tolerance and better turf quality than Midiron, and has good winter survival in Georgia (Hanna *et al*., 1990). Princess 77 and Riviera are improved

seeded common bermudagrass cultivars. Princess 77 was released in 1995 with the characteristics of dark-green color, good turf quality and density, and fine leaf texture (Morris, 2002; Rodgers and Baltensperger, 2005). It is now used on home lawns, parks, athletic fields, and golf courses. Riviera is a cold tolerant, medium-dark green, medium textured, and traffic-tolerant cultivar, which forms a traffic-tolerant turf similar to Tifway (Wu *et al*., 2009).

'Champion', 'MiniVerde', 'TifEagle', 'Jones Dwarf', 'Floradwarf', 'Tifgreen', 'Tifdwarf', and 'Emerald Dwarf' are triploid, hybrid bermudagrass cultivars used on putting greens. Champion, MiniVerde, TifEagle, and Floradwarf are ultra-dwarf cultivars that can tolerate mowing height as low as 3 mm (Beard, 2002). Tifgreen was the first cultivar released on golf course putting greens in 1956, and its offtype Tifdwarf, with darker green color and smaller leaves was released in 1965 (Burton, 1966). TifEagle, and induced mutant from Tifway II, was released in August 1997. It produces better quality turf than Tifdwarf under mowing height of 4 mm or less. TifEagle is vegetatively propagated and produces more stolons and thatch than Tifdwarf (Hanna and Elsner, 1999). Floradwarf is a natural mutant from Tifgreen and released in 1995. It is a dwarf, dense, fine-textured cultivar that has greater turf density than Tifdwarf due to its short stolons and internodes (Dudeck and Murduch, 1998). Emerald Dwarf bermudagrass was selected in 1992, and it has greater shoot density and rhizome development than Tifgreen (CTF, 2010). Its shoot density and leaf morphology are similar to that of Tifdwarf, but forms deeper roots. Emerald Dwarf is usually used on golf course putting greens and high quality tees (CTF, 2010).

Paspalum vaginatum

Distribution and Utility

Seashore paspalum (*Paspalum vaginatum* Swartz) is originally from tropical and subtropical areas of North and South America (Duncan and Carrow, 2000; Morton, 1973). This

grass is widely used for turf in South Africa, Australia and New Zealand. In the United States, seashore paspalum is distributed from Florida northward to North Carolina along the east coast, and westward along the southern coast border from Florida to Texas (Duncan and Carrow, 2000; Morton, 1973). This grass is mainly adapted for turf use, and improved cultivars have been developed for golf courses, sports fields, and other landscape uses.

Growth Habit and Establishment

Similar to bermudagrass, seashore paspalum is also a creeping grass that establishes and spreads by stolons and rhizomes. Most commercial cultivars are vegetatively propagated by planting of sprigs, sod, stolons, or plugs. Seashore paspalum grows vigorously and the leaves expand more rapidly than most bermudagrass cultivars, but it roots less deeply than bermudagrass (Beard *et al*., 1991).

Adaptation and Cultivation

Seashore paspalum has excellent salt tolerance and can grow in soils with salt levels as high as 54 dSm^{-1}, at which most horticultural crops cannot survive (Lee *et al*., 2004). This character makes it well adapted to coastal areas subjected to salt spray and poor water quality (Duncan and Carrow, 2000). Seashore paspalum grows well in a wide range of soil types, including heavy and poorly drained soils ((Duncan and Carrow, 2000). Compared with bermudagrass, seashore paspalum is highly tolerant to various environmental stresses. It can maintain acceptable turf quality with less nitrogen fertilizer. It forms a good quality turf in soils ranging in pH from 3.6 to 10.2 and in waterlogged soils (Duncan, 1999b; Duncan and Carrow, 2000).

Seashore paspalum grows best under warm temperatures and long day length conditions. It is watered on an as needed basis due to its good drought tolerance, and over watering can reduce its stress tolerance and predispose it to diseases (Duncan and Carrow, 2000). Actively growing

seashore paspalum cultivars require 2.5 to 3.8 cm of water per week (Duncan, 1999b). To promote root development, deep, infrequent irrigation is usually applied for established seashore paspalum. Watering during the early morning will reduce the chance of disease development. Optimum annual nitrogen fertilizer rates range from 24 to 39 g/m^2 for golf courses, athletic fields, and landscape areas (Trenholm *et al*., 2001). Florida soil is rich in phosphorous, so little or no supplemental phosphorous is typically needed. An equal amount of potassium and nitrogen is best for seashore paspalum (Trenholm and Unruh, 2002). Seashore paspalum should be mowed below 2.5 cm and reductions in mowing height will produce denser turf. Golf course putting greens are maintained between 3 and 5 mm, while tees and fairways kept between 1.3 and 1.9 cm, and athletic fields are maintained between 1.3 and 2.5 cm (Duncan, 1999b; Trenholm and Unruh, 2003). Beard *et al*. (1991) reported that the best mowing height for seashore paspalum is 1.3 cm, which results in the best turf quality, shoot density, and competitiveness against weeds.

Various turfgrass pests can affect seashore paspalum. Insects such as spittlebugs (*Aphrophora saratogensis* Fitch), sod webworms (*Herpetogramma phaeopteralis* Guenée), billbugs (*Sphenophorus venatus vestitus* Chittenden) and mole crickets (*Scapteriscus* spp.) are usually observed on seashore paspalum (Duncan and Carrow, 2000; McCarty and Miller, 2002). Susceptibility to dollar spot (*Sclerotinia homoeocarpa* Benn), leaf spot diseases (*Helminthosporium* spp., *Bipolaris* spp., *Drechslera* spp.) and fairy ring has been observed in seashore paspalum cultivars (McCarty and Miller, 2002). Incidences of fusarium blight (*Fusarium* sp.) and take-all patch (*Gaeumannomyces graminis* var. *graminis* [Sacc.] Arx and D. L. Olivier) in seashore paspalum have been reported in Florida (Trenholm and Unruh, 2003). However, nematodes also have been shown to damage this grass (Hixson *et al*., 2004).

Cultivars

'Aloha', 'SeaDwarf', 'Salam', 'SeaIsle 1', 'Sea Isle 2000', 'SeaSpray', and 'SeaIsle Supreme' are common cultivars of seashore paspalum, all of which were released in the past 10 to 20 years. SeaSpray is the only seeded cultivar. SeaIsle 1 is a fine-leaved, dense-growing cultivar used on golf course fairways (Trenholm and Unruh, 2002). SeaIsle 2000 was the first cultivar of seashore paspalum recommended for use on greens. SeaDwarf is a dwarf cultivar for putting greens and fairway on golf courses (McCarty and Miller, 2002).

Belonolaimus longicaudatus

Taxonomy

Sting nematode (*Belonolaimus longicaudatus* Rau) belongs to the kingdom Animalia, subkingdom Metazoa, branch Eumetazoa, division Bilateralia, subdivision Protostomia, section Pseudocoelomata, superphylum Aschelminthes, phylum Nematoda, class Secernentea, subclass Tylenchia, order Tylenchida, suborder Tylenchina, superfamily Dolichodoroidea, family Belonolaimidae, subfamily Belonolaiminae, genus *Belonolaimus*, species *longicaudatus* (Siddiqi, 2000). Based on SSU rDNA, De Ley and Blaxter (2004) classified sting nematodes into class Chromadorea, subclass Chromadoria, order Rhabditida, suborder Tylenchina, infraorder Tylenchomorpha, superfamily Tylenchoidea, family Dolichodoridae, subfamily Belonolaiminae, genus *Belonolaimus*, and species *longicaudatus*.

Distribution and Hosts

Belonolaimus longicaudatus is not only distributed in the sandy coastal plains of the Atlantic and gulf coasts but also occurs naturally in sandy areas of Kansas and Nebraska in the United States. Through infested turf sod, *B. longicaudatus* has been introduced to California and internationally to the Caribbean islands, Puerto Rico, and Bermuda (Perry and Rhoades, 1982). Sting nematodes are found in sandy soils and prefer at least 80% sand content to survive

(Robbins and Barker, 1974). Sting nematodes have a wide host range including grains, turf and forage grasses, vegetables, and fruits. Plants such as beans, cabbage, carrots, millet, oat, rye, strawberry, and tomatoes are often attacked by sting nematodes in Florida (Crow and Han, 2005).

Life Cycle and Biology

Sting nematodes are large in size and adults are 2 to 3 mm long and 29 to 34 µm wide (Rau, 1958; Mai *et al*., 1996; Luc, 2004). They are ectoparasites that feed from the outside of the roots by use of a protracted stylet. The reproduction style of sting nematodes is amphimixis. Sting nematodes are bisexual and their life cycle is simple: second-stage juveniles hatch from the eggs and feed on the roots and molt through the third- and fourth- stage juveniles to become adults. Eggs are laid in pairs and about 128 eggs were laid by one female in 90 days (Huang and Becker, 1999). The total life cycle from egg to egg is about 24 days at 28°C (Huang and Becker, 1999).

Damage and Symptoms

Sting nematodes damage turf roots and their feeding usually causes the root tip to stop growing. They also affect the roots' absorption of water and nutrients, reduce plant photosynthesis, and finally affect the plant biomass (Crow and Han, 2005). It was also reported that sting nematodes lead to reduced drought tolerance (Trenholm *et al*., 2005) and increased nitrate leaching in turf (Luc *et al*., 2006). Aboveground symptoms include wilting, chlorosis, and thinning of the turf, and these symptoms appear as irregular patches in the field. High population densities of sting nematodes may cause plant death (Crow and Han, 2005).

Management

Management of sting nematodes on golf courses is difficult. The nematicide 1, 3-dichloropropene (Curfew Soil Fumigant™ Dow AgroSciences, Indianapolis, IN) is the only

effective and currently available chemical product for nematode management on turfgrass. However, the nematode population densities may rebound following an application because this chemical does not have residual activity (Crow, 2010). A bionematicide with the active ingredient *Pasteuria* sp., which parasitizes and kills *B. longicaudatus* (EcoNem™, Pasteuria BioScience, Alachua, FL) has been labeled for use on turfgrasses in Florida (Crow, 2010). However, consistent efficacy on sting nematodes in field studies has not been shown (W. T. Crow, unpublished data). Cultural practices could also help reduce the damage of sting nematodes on turf. Frequent and light irrigation and fertilization could keep the damaged grass from wilting. Mowing height also has an effect on the grass's responses to nematodes. Research has shown that "raising mowing height slightly can reduce nematode damage considerably" (Crow, 2010). Maintenance of a healthy root system is essential to improve the nematode tolerance or resistance of turfgrass. Cultural practices such as soil amendments and aeration encourages a healthy root system and enhances tolerance to nematodes (Crow, 2010). Little information is available about the efficacy of utilization of resistant or tolerant turfgrass cultivars for *B. longicaudatus* management.

***Helicotylenchus* spp.**

Taxonomy

Helicotylenchus spp. (spiral nematode) belongs to the kingdom Animalia, subkingdom Metazoa, branch Eumetazoa, division Bilateralia, subdivision Protostomia, section Pseudocoelomata, superphylum Aschelminthes, phylum Nematoda, class Secernentea, subclass Tylenchia, order Tylenchida, suborder Tylenchina, superfamily Tylenchoidea, family Hoplolaimidae, subfamily Hoplolaiminae, genus *Helicotylenchus* (Steiner, 1914; Golden, 1956). However, based on SSU rDNA, De Ley and Blaxter (2004) recently classified it into class Chromadorea, subclass Chromadoria, order Rhabditida, suborder Tylenchina, infraorder

Tylenchomorpha, superfamily Tylenchoidea, family Hoplolaimidae, subfamily Rotylenchoidinae, and genus *Helicotylenchus*.

Distribution and Hosts

Spiral nematodes are found throughout the southeastern United States on turfgrasses but cause more damage on sandy soils (O'Bannon and Inserra, 1989). They are worldwide in distribution and well adapted to tropical and subtropical climates. *Helicotylenchus multicinctus* (Cobb, 1893) Golden, 1956 causes a serious decline of bananas; *H. dihystera* (Cobb, 1893) Sher, 1961 and *H. digonicus* (Perry, 1959) have been reported to damage Kentucky bluegrass in Australia (O'Bannon and Inserra, 1989); *H. pseudorobustus* (Steiner, 1914) Golden, 1956 can cause damage to a lot of crops including corn, grasses, and some vegetables (O'Bannon and Inserra, 1989). *Helicotylenchus* spp. also can attack cocoa, sugarcane, coffee, corn, tea, turfgrasses, and weeds (Wouts and Yeates, 1994).

Life Cycle and Biology

Spiral nematodes are smaller in size than sting nematodes. The female and male body lengths range from 0.47 to 0.53 mm and 0.43 to 0.55 mm, respectively. The dead body of this nematode is generally spiral in shape, and this character is very helpful for identification. Their feeding habits are ectoparasitic or semi-endoparasitic depending on the species. *Helicotylenchus pseudorobustus* is a migratory semi-endoparasite, whose anterior penetrates into the roots with the posterior remaining outside (O'Bannon and Inserra, 1989). *Helicotylenchus multicinctus* is endoparasitic and the whole body penetrates inside the roots (O'Bannon and Inserra, 1989). Ectoparasitic species feed only in the outer cortex of roots but do not migrate through cortex (Wouts and Yeates, 1994). *Helicotylenchus multicinctus* is bisexual and reproduces by cross-fertilization, but for some *Helicotylenchus* species, males are rare and they reproduce by

parthenogenesis (O'Bannon and Inserra, 1989). The life cycle of *Helicotylenchus* spp. is similar to that of *B. longicaudatus*, but it takes 30 to 45 days to go from egg to egg (Ferris, 2010).

Damage and Symptoms

There are mainly four species of *Helicotylenchus* spp. that cause economic damage in plants: *H. multicinctus*, *H. pseudorobustus*, *H. digonicus*, and *H. dihystera* (O'Bannon and Inserra, 1989). *Helicotylenchus dihystera*, *H. digonicus*, and *H. pseudorobustus* have been reported to damage turf or pasture in United States, New Zealand, and Australia (O'Bannon and Inserra, 1989; Davis *et al*., 2004). In Florida, *H. pseudorobustus* occurs on turf and can cause damage to turfgrass (W. T. Crow, personal communication). The aboveground symptoms of spiral nematode damage on turfgrass include a slow green-up in the spring, chlorosis and dieback of the grass blades, stunting, reduction in vigor, and gradual thinning of the turf (UOIE, 2000). However, symptoms may not be obvious in other crops. Parasitism by *H. multicinctus* can cause shortened and discolored roots with necrotic lesions and dieback, and sometimes can lead to the stress of the entire host plant (O'Bannon and Inserra, 1989).

Management

Similar chemical and cultural strategies as used for sting nematode management can be used for spiral nematode management on turfgrass. As one of the most cost effective, as well as environmentally friendly practices, development and utilization of nematode resistant or tolerant turfgrass cultivars is worthwhile to evaluate as a method for managing spiral nematodes.

CHAPTER 3
BERMUDAGRASS CULTIVAR RESPONSES TO STING NEMATODES

Introduction

Golf is a major component of Florida's economy. It was reported that in 2000, golf course revenues were $4.44 billion in Florida (Haydu and Hodges, 2002). Bermudagrass is the most widely used warm-season turfgrass on putting greens, tees, and fairways of golf. Crow (2005a) found that 87% golf courses in the state of Florida were at risk for nematode damage, with the most damaging species being the sting nematode (*Belonolaimus longicaudatus* Rau). Research has shown that sting nematodes lead to reduced drought tolerance (Trenholm *et al*., 2005) and increased potential for nitrate leaching in turf (Luc *et al*., 2006). Furthermore, their damage can result in premature wilt, chlorosis, and even death of turfgrass when found in combination with other biotic or abiotic stresses (Johnson, 1970; Busey *et al*., 1991). Nematode-damaged turfgrass requires increased irrigation and fertilizer, which may result in waste of valuable water resources and increased risk of groundwater contamination with nitrates (Luc *et al*., 2006; Crow, 2005b).

In recent years, environmental concerns have focused on the overuse of water, fertilizer, and pesticides (Haydu and Hodges, 2002). The recent cancellation of fenamiphos (Nemacur®, Bayer CropScience, Research Triangle Park, NC) has resulted in the need for new nematode management tactics. Currently, 1,3-dichloropropene (Curfew® Soil Fumigant, Dow AgroSciences, Indianapolis, IN) is the only nematicide available for nematode management on turfgrass with established efficacy (Crow, 2010). However, it provides only short-term control, is expensive, and alternative options are needed due to environmental restrictions (Crow, 2010). A biopesticide containing *Pasteuria* sp. (EcoNem™, Pasteuria Bioscience, Alachua, FL) has been labeled for sting nematode management on turfgrasses in Florida (Crow, 2010). However, it has not been effective in University of Florida turfgrass field trials (Crow, W. T., personal

communication). Utilization of resistant or tolerant cultivars could be one of the most environmentally friendly and least costly practices for nematode management on turf.

Research has been conducted to test the responses of bermudagrass to sting nematodes. The University of Florida Cooperative Extension Service classifies that moderate and high risk of damage from sting nematode on bermudagrass occurs at 10 and 25 sting nematodes/100 cm^3 soil, respectively (Crow, 2010). Giblin-Davis *et al*. (1992b) tested the host status of 37 bermudagrass accessions to sting nematodes, and recommended an arbitrary standard of "sensitive" if more than an 11% reduction in root dry weight occurred. Of the 37 accessions tested, 26 were sensitive and suitable hosts to *B. longicaudatus*, and commercial cultivars 'Midiron', 'Tifdwarf', 'Tifgreen', 'Tifgreen II', and 'Tifway II' all supported the reproduction of *B. longicaudatus* and were sensitive to their infection. Tifway had better tolerance but also supported the reproduction of *B. longicaudatus*. Severe damage to Tifway caused by *B. longicaudatus* in the field is common (W. T. Crow, personal communication).

Johnson (1970) tested the pathogenicity and interaction of *Criconemoides ornatus* Raski (now *Mesocriconema ornatum* (Raski, 1952) Loof and De Grisse, 1989), *Tylenchorhynchus martini* Fielding (now *T. annulatus* (Cassidy, 1930) Golden, 1971), and *Belonolaimus longicaudatus* on six bermudagrass cultivars ('Common', 'U-3', 'Tufcote', 'Continental', 'Tiffine', and Tifdwarf). He found that the number of fibrous roots decreased as the number of nematode species and total number of nematodes increased. All bermudagrass cultivars supported high population densities of the three nematode species but *B. longicaudatus* was a better competitor than *C. ornatus* and *T. martini* since population densities of *C. ornatus* and *T. martini* were suppressed more by *B. longicaudatus* than *B. longicaudatus* was by the other nematode species. Good *et al*. (1965) also reported that coastal bermudagrass supported the

reproduction of *B. longicaudatus*. However, other studies showed that the forage bermudagrass 'Coastcross-1' was resistant to sting nematodes (Burton, 1972).

Nematologists have used root growth parameters to assess nematode damage in plant roots. Quesenberry and Dunn (1977) used a graduated cylinder method to measure root volume to evaluate nematode damage in plant roots. With the development of technology in recent years, WinRhizo root scanning equipment and software (Regent Instruments Inc., Ottawa, Canada) has been developed and used to measure root length, diameter, volume, surface area, root tips and crossings, etc. (Bauhus and Messier, 1999). However, the efficiency of the graduated cylinder and root scanning methods have not been compared, and is warranted to identify the method most efficient for screening a large number of diverse genotypes. Although a range in responses of bermudagrass to sting nematodes has been studied, information about the responses of several more recently released cultivars is lacking. One objective of this research was to evaluate the relative resistance or tolerance of commercial bermudagrass cultivars to *B. longicaudatus* to help growers and turfgrass managers with cultivar selections where *B. longicaudatus* is present. Another objective was to determine if a proposed alternative method used by Quesenberry and Dunn (1977) for assessing sting nematode damage on bermudagrass roots is as effective as or more efficient than the WinRhizo root scanning method.

Materials and Methods

Plant Materials

Eight dwarf and nine non-dwarf bermudagrass cultivars were tested in two sequential experimental trials in 2009 in a glasshouse at the University of Florida Turfgrass Envirotron in Gainesville, FL. The dwarf cultivars were 'Champion', 'MiniVerde', 'TifEagle', 'Jones Dwarf', 'Floradwarf', Tifgreen, Tifdwarf, and 'Emerald Dwarf'; and the non-dwarf cultivars included

Tifway, ‘TifSport’, ‘Celebration’, ‘Riviera’, ‘Patriot’, ‘Midlawn’, Midiron, ‘Princess 77’, and ‘Tifton10’.

Inoculum Preparation

Belonolaimus longicaudatus was maintained on ‘FX-313’ St. Augustinegrass (*Stenotaphrum secundatum* Kuntze) grown in clay pots filled with United States Golf Association (USGA) specification putting green sand under greenhouse conditions (Giblin-Davis *et al*., 1992a; USGA, 1993). Nematodes were extracted from soil using Cobb’s decanting and sieving technique (Cobb, 1918; Flegg, 1967). All stages of nematodes including juveniles and adults were collected. Nematode suspensions were concentrated using a 25-µm (500-mesh) sieve. The average number of juveniles and adults were counted from five replicates of 1-ml aliquots and results were extrapolated to the total volume of the suspension. Suspensions were stored in a refrigerator until needed.

Nematode rDNA Analysis

Molecular analysis was conducted to aid in sting nematode species identification. Isohair extraction kit (Nippon Gene Co. LTD., Toyama, Japan) was used to extract DNA from individual females. Ribosomal DNA of the ITS (Internal Transcribed Spacer) was amplified by PCR using the 18S (5’-TTG ATT ACG TCC CTG CCC TTT-3’) (forward) and 26S (5’-TTT CAC TCG CCG TTA CTA AGG -3’) (reverse) primers (Vrain, 1993). The following components were added to each polymerase chain reaction tube: 15.0 µL of GoTag® Green Master Mix (Promega Corp., Madison, WI), 1.5 µL of 10 pM forward primer, 1.5 µL of 10 pM reverse primer (Intergrated DNA Technologies, Coralville, IA), 1 µL of DNA and 11.0 µL of distilled water, with the total volume of 30 µL. All PCR reactions were run in an icycler (BioRad Laboratories, Inc., Hercules, CA), with the cycling sequence as: one cycle of 94°C for 7 min, 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, and 72°C for 10 min. Montage® PCR

centrifugal filter kit (Millipore Corp., Billerica, MA) was used to purify the PCR products. All products were sequenced on Perkin Elmer/Applied Biosystems automated DNA sequencers at the University of Florida ICBR sequencing core facility, and the same primers were used for sequencing as for PCR amplification. Sequences were edited using Sequencher (4.1.2 Gene Codes Corporation). Using the default parameters of Clustal X 1.83 (Thompson *et al.*, 1997), the sequences gained in this study were aligned to each other and the outgroup taxon *Pratylenchus coffeae* (Zimmermann) Filipjev & Schuurmans Stekhoven (LSU GenBank accession #AF170443), and the alignments were adjusted manually in MacClade 4.0 (Maddison and Maddison, 2000).

Cultivar Nematode Responses

Nematode-free aerial stolons of each cultivar were vegetatively propagated into 3.8-cm-diameter × 21-cm-deep (volume = 150 cm^3) UV-stabilized Ray Leach "Cone-tainers"™ (SC10, Stuewe & Sons, Inc., Tangent, OR) filled with 100% USGA specification greens sand. The bottom of the 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 irrigation mist was applied six times daily for two weeks to allow the sprigs to establish roots. Beginning at the third week, the irrigation was reduced to once a day in the morning for six minutes, and three minutes a day from the fifth week. Six weeks after establishment, cultivars were inoculated with 0 or 50 *B. longicaudatus* per conetainer. Before inoculation, suspensions of *B. longicaudatus* were taken out of the refrigerator, concentrated to 10 nematodes/ml, and set at room temperature for three hours. A total of 5 ml of the suspension was divided into two 3-cm deep holes made 1 cm from the center of the pot in the inoculated treatments, and the uninoculated controls receive no solution. The holes were covered with a light layer of sand, and moistened with a light mist.

The experiment was arranged as a randomized complete block design with six replications. To provide insulation from temperature extremes, conetainers were placed in (60 × 35 × 15 cm) Beaver Plastics Styroblock™ (Stuewe & Sons, Inc., Tangent, OR). The experiments were conducted under a temperature range of 24 to 34^{o}C with natural daylight in a greenhouse at the University of Florida in Gainesville, FL. Treatments were fertilized once a week using 24-8-16 (N-P_2O_5-K_2O) at a rate of 0.5 kg N/100 m^2 per growing month and clipped once a week at 2.5 cm. Bermudagrass mites (*Eriophyes cynodoniensis* Sayed) and rhodesgrass mealybugs (*Antonina graminis* Maskell) were monitored and sprayed with bifenthrin (Bifen I/I Insecticide/Termiticide, Control Solutions, Inc., Pasadena, TX) when needed, and all conetainers were treated equally.

Experiments were harvested 90 days after the inoculation of nematodes. Root and nematode samples were collected from each conetainer. Roots were collected by removing the shoots and Poly-fil. The roots were washed free of soil on an 853-µm sieve, put into a 50-ml plastic tube, and submerged with water. Finer roots were separated from soil and collected into the plastic tube by submerging and shaking the 853-µm sieve in tap water. Roots were scanned using WinRhizo root scanning equipment and software (Regent Instruments Inc., Ottawa, Canada). Length of fine roots (≤ 0.5 mm-diameter), length of all roots, and root volume were measured from the scanned images. After scanning, roots in each sample were collected and dried by rolling them into a paper towel. For the graduated cylinder method the roots were then submerged into a 10-ml graduated cylinder prefilled with 5 ml water. The volumes of the water in the cylinder were recorded before and after submerging the roots into the cylinder. The volume of roots was calculated as follows: root volume = volume of the water after submerging the roots into the cylinder - volume of the water before submerging the roots. Percent reduction in the root length or volume of inoculated plants compared with the non-inoculated control plants

was calculated by the following formula: [(root measurement of non-inoculated control – root measurement of inoculated plant) / root measurement of non-inoculated control] * 100.

Nematodes were extracted from the total soil in the conetainer using the modified centrifugal flotation technique (Jenkins, 1964). The final nematode population densities (Pf) were counted under an inverted light microscope. The reproductive factor (Rf), which is an indicator of resistance (Oostenbrink, 1966) was calculated by the following formula: Rf = final nematode density (Pf) / initial inoculum density (Pi).

Data Analysis

Data of the two trials were analyzed separately. Total root lengths and fine root lengths of the inoculated treatment and the non-inoculated control of each cultivar were compared by a linear contrast at $P \leq 0.1$. Tolerance was determined by the difference in root length between the two treatments. A cultivar was considered tolerant if there was no difference ($P \leq 0.1$) in total root length between the two treatments; otherwise, the cultivar was classified as intolerant. Resistance was determined by the nematode reproductive factor (Rf) at harvest. A cultivar was designated as resistant if Rf ≤ 1 and susceptible if Rf > 1. Final nematode population densities were subjected to analysis of variance (ANOVA), and the differences among cultivars were compared by the Fishers protected least significant difference test at $P \leq 0.05$.

The root volumes were measured digitally using root scans and by water displacement (graduated cylinder). Regression analysis was conducted to compare the two methods. All statistical analyses were conducted using SAS programs (SAS Institute, Cary, NC).

Results

The sting nematode species used in this study was identified as *B. longicaudatus* by the rDNA analysis. Differences in responses to sting nematodes were observed among the cultivars

evaluated (Table 3-1). A significant interaction was found between the experimental trials and turfgrass cultivars, so the root lengths of each trial were analyzed separately. The total root length reductions for individual cultivars ranged from 1 to 40% and 3 to 46% in trials one and two, respectively. Linear contrasts showed that dwarf cultivars Champion, MiniVerde, TifEagle, Jones Dwarf, Floradwarf, and Tifgreen had significant reductions in total root length from *B. longicaudatus* compared with the non-inoculated controls in both trials. The non-dwarf cultivars, Princess 77, Midlawn, Celebration, and Midiron had significant reductions in the total root length in both trials. However, Tifton10 and Tifway showed significant reductions in root length from *B. longicaudatus* in trial two, but not in trial one. Based on both tests, the dwarf cultivars Tifdwarf and Emerald Dwarf did not suffer significant reductions in root length, therefore, they were considered tolerant to *B. longicaudatus*. Among the non-dwarf cultivars, TifSport, Patriot, and Riviera were consistently tolerant to *B. longicaudatus* in both trials.

The fine root lengths of the cultivars were also compared and similar damage of *B. longicaudatus* as in the total root length was found. Differences in the fine root lengths between the inoculated and uninoculated treatments were identified (Table 3-2). The linear contrasts indicated that Champion, MiniVerde, TifEagle, Jones Dwarf, Floradwarf, and Tifgreen had significant reductions in fine root length from *B. longicaudatus*.

Treatment differences in fine root lengths also were detected among the non-dwarf cultivars (Table 3-2). The reductions were in the range of 1 to 29% and 5 to 37%, respectively for the two trials. We found that Midlawn, Celebration, and Midiron had significant reductions in the fine root length in both trials. Tifton 10, Tifway, and Riviera suffered significant reductions in fine root length from *B. longicaudatus* only in trial two.

Population densities of *B. longicaudatus* increased on some bermudagrass cultivars and decreased on others. Final nematode population densities per conetainer of *B. longicaudatus* were in the range of 22 to 343 for trial one and 20 to 220 for trial two (Table 3-3). In trial one, compared to the initial inoculum density of 50 nematodes/conetainer, the population densities of *B. longicaudatus* increased on Jones Dwarf, Tifdwarf, and Emerald Dwarf with the corresponding Rf values of 2.5, 4.8, and 4.7, respectively. The most susceptible cultivar was the non-dwarf cultivar Princess 77 with a 6.9-fold increase in the population density. In trial two, more cultivars showed susceptibility. MiniVerde, Jones Dwarf, Tifgreen, Tifdwarf, Emerald Dwarf, Princess 77, Celebration, and Patriot all supported the reproduction of *B. longicaudatus*. As in trial one, the highest Rf of 4.4 was on Princess 77, and this cultivar served as a very good host to *B. longicaudatus*. Some moderate hosts with Rf values close to one were identified, for example Tifway and TifSport.

Overall, the dwarf cultivars Tifdwarf and Emerald Dwarf were tolerant but susceptible to *B. longicaudatus*. Champion, TifEagle and Floradwarf were resistant but intolerant to *B. longicaudatus*. No dwarf cultivars were both tolerant and resistant to *B. longicaudatus*. Non-dwarf cultivars TifSport, Patriot and Riviera were tolerant to *B. longicaudatus*, however, only TifSport and Riviera showed resistance. Most non-dwarf cultivars did not support the reproduction of *B. longicaudatus*, for example, Midlawn, Midiron, TifSport, Tifton 10, Tifway, and Riviera.

The root volume of each sample was compared by the root scanning method and the graduated cylinder method. Results from both methods were similar and produced a highly significant linear regression relationship between the two methods in trial one ($r^2 = 0.9968$, $P < 0.0001$) (Figure 3-1) and trial two ($r^2 = 0.9988$, $P < 0.0001$) (Figure 3-2). The results provided by

the two methods were extremely consistent with similar lines and slopes near 1.0 (Figure 3-1, Figure 3-2), which indicated that both methods can provide consistent root volume measurements.

Discussion

Although both methods mentioned above might give easy measurements of root volume, for roots with very small volume (less than 1 cm^3), the estimated error brought by reading the graduated cylinder (the minimum unit is 0.1 cm^3) could make the measurements less accurate than the root scanning method. Moreover, it took about the same amount of time using either method to do the root measurement. The graduated cylinder method can only measure the total volume of a sample, but the root scanning method also can measure root length, root surface area, and root volume in different diameter ranges. Additionally, the root scanning method also can measure the number of root tips and crossings. Therefore, the root scanning method provided more details in root measurement than the graduated cylinder method in the same amount of time. However, the root-scanning method is initially more expensive since special software and equipment are needed. Therefore, the root scanning method is preferred for root measurement when the facilities are available and the graduated cylinder method is an economical method to gain limited information, if root scanning equipment and software are not available.

Among the dwarf cultivars, those with shorter roots might support a lower nematode population density. The average total root lengths of the inoculated treatment in two trials were as low as 938, 1076, and 1094 cm, respectively for Champion, TifEagle, and Floradwarf; while in these cultivars the final nematode population densities fell to half of the initial inoculum level. On the other hand, cultivars Jones Dwarf, Tifgreen, Tifdwarf, and Emerald Dwarf all supported higher reproduction of *B. longicaudatus* and had root lengths of 1474, 1969, 2241, and 1959 cm,

respectively. The greater amount of roots probably provided more food source and feeding sites, which could support more nematodes. The most susceptible cultivar Princess 77 with an average Rf of 5.7 in two trials also had the largest root length of 2226 cm for the inoculated treatment. Therefore, what could be considered "resistance" based on low Rf may not be true resistance, but rather an indicator of differences in carrying capacity among cultivars. The carrying capacity is the population level at which the nematodes decrease root production to the extent that they limit nematode reproduction. The damage function model for *B. longicaudatus* on cotton was determined to be quadratic, where high inoculation densities resulted in fewer nematodes due to the reduction in feeding sites (Crow *et al*., 2000). The inoculation density used in the current experiment (50 *B. longicaudatus*/conetainer) may have resulted in population levels that were too high, limiting root production and further nematode reproduction. Cultivars defined as resistant might be defined susceptible if a lower inoculation density was used. Further research should use multiple inoculation densities to verify this hypothesis.

The average percent reduction of root length in TifEagle was 32%, which was similar to the previously reported results (Schwartz *et al*., 2010a). Another previous study (Giblin-Davis *et al*., 1992b) found that Tifgreen, Tifdwarf, Midiron, and Tifway were susceptible cultivars to *B. longicaudatus* under controlled conditions at 140 days after inoculation. In this study Tifgreen and Tifdwarf were observed to support the reproduction of *B. longicaudatus* 90 days after inoculation. Giblin-Davis *et al*. (1992b) found that Tifway was relatively more tolerant than other bermudagrass cultivars listed above, with a mean root dry weight reduction of 4%. Results of this study indicated an 8% reduction in total root length in Tifway in the first trial and a 24% reduction in the second trial.

Tifdwarf is the most widely used bermudagrass cultivars in golf course putting greens and Tifway is the most widely used on fairways. Tifdwarf and Tifgreen were very suitable hosts to *B. longicaudatus* and Tifgreen had significant reductions in root length when inoculated with sting nematodes. These results suggest that where *B. longicaudatus* is present, Tifdwarf and Emerald Dwarf might be good cultivar choices for putting greens because they were more tolerant of nematode damage. Similarly, TifSport and Riviera are non-dwarf cultivars that were both resistant and tolerant to *B. longicaudatus*, suggesting that they might be good cultivar selections for *B. longicaudatus* infested tees and fairways. Future studies will be needed to verify results under field conditions on golf courses and with different geographical and host isolates of *B. longicaudatus*. The results of our study are preliminary but the screening methods and some of these resistant or tolerant cultivars can be used as standards for future turfgrass cultivar and germplasm screening.

Table 3-1. Mean total root length of 17 bermudagrass cultivars 90 days after inoculation with *Belonolaimus longicaudatus* in two experiment trials. U = uninoculated and I = inoculated with 50 *B. longicaudatus*/conetainer.

	Total root length (cm)					
	Trial 1			Trial 2		
Cultivar	U	I	% reduction	U	I	% reduction
	Dwarf cultivars					
Champion	1373*	810 c[ab]	40 a	1524[#]	1066 de	31 ab
MiniVerde	2219*	1413 b	35 ab	1995*	1096 de	34 ab
TifEagle	2017*	1320 b	26 abc	1509*	832 e	37 ab
Jones Dwarf	2258[#]	1557 b	21 abc	1976[#]	1391 cd	25 ab
Floradwarf	1871[#]	1365 b	24 abc	1972*	822 e	46 a
Tifgreen	2178[#]	1635 b	20 abc	2650*	2302 a	13 b
Tifdwarf	2876	2527 a	10 bc	2454	1954 ab	21 ab
Emerald Dwarf	2387	2173 a	5 c	2089	1744 bc	16 ab
Mean	2147	1600	23	2021	1401	28
	Non-dwarf cultivars					
Princess 77	2637[#]	2139 a	15	2568[#]	2312 a	10[c]
Midlawn	2006*	1252 de	28	1551*	1201 c	20
Celebration	2786*	1992 ab	28	2267[#]	1908 b	14
Midiron	1930*	1452 de	22	1882*	1263 c	26
TifSport	2173	1931 abc	7	2204	1952 ab	3
Tifton 10	1272	1189 e	1	1938*	1195 c	36
Tifway	1622	1548 cde	8	1764[#]	1332 c	24
Patriot	1379	1322 de	12	2265	2093 ab	4
Riviera	1683	1625 bcd	7	1604	1219 c	18
Mean	1943	1606	14	2005	1608	17

[#], *Uninoculated treatments significantly different from inoculated treatments at $P \leq 0.1$, and $P \leq 0.05$, respectively, according to the linear contrast analysis.

[a]Data are means of six replications.

[b]For each cultivar type (dwarf, non-dwarf) means within a column followed by the same letter or by no letter are not different ($P \leq 0.05$), by Fishers protected least significant difference test.

Table 3-2. Mean fine root length of 17 bermudagrass cultivars 90 days after inoculation with *Belonolaimus longicaudatus* in two experiment trials. U = uninoculated and I = inoculated with 50 *B. longicaudatus*/conetainer.

	Fine root length (cm)					
	Trial 1			Trial 2		
Cultivar	U	I	% reduction	U	I	% reduction
	Dwarf cultivars					
Champion	1273*	750 c[ab]	40 a	1335[#]	944 cd	30 ab
MiniVerde	2001*	1262 b	35 a	1736*	994 cd	30 ab
TifEagle	1819*	1194 b	26 ab	1328*	765 d	35 ab
Jones Dwarf	1910*	1301 b	24 ab	1643*	1181 bc	23 ab
Floradwarf	1698*	1252 b	24 abc	1694*	757 d	43 a
Tifgreen	1944[#]	1463 b	19 abc	2051	1807 a	10 b
Tifdwarf	2215	1937 a	10 bc	2052	1640 a	20 ab
Emerald Dwarf	1977	1842 a	2 c	1769	1484 ab	16 ab
Mean	1855	1375	23	1701	1197	26
	Non-dwarf cultivars					
Princess 77	2093	1764 a	10	1984	1899 a	6
Midlawn	1768*	1104 cd	29	1383[#]	1064 c	20
Celebration	2328*	1646 ab	28	1822[#]	1556 b	12
Midiron	1706*	1267 cd	23	1585*	1112 c	25
TifSport	1870	1633 ab	8	1634	1591 b	13
Tifton 10	1113	1039 d	1	1721*	1058 c	37
Tifway	1449	1309 cd	13	1534*	1135 c	25
Patriot	1280	1204 cd	14	1767	1652 ab	5
Riviera	1543	1411 bc	3	1437[#]	1025 c	23
Mean	1683	1375	14	1652	1344	18

[#], *Uninoculated treatments significantly different from inoculated treatments at $P \leq 0.1$, and $P \leq 0.05$, respectively, according to the linear contrast analysis.

[a]Data are means of six replications.

[b]For each cultivar type (dwarf, non-dwarf) means within a column followed by the same letter or by no letter are not different ($P \leq 0.05$), by Fishers protected least significant difference test.

Table 3-3. Mean final population density (Pf) and reproductive factor (Rf) of *Belonolaimus longicaudatus* on 17 bermudagrass cultivars 90 days after inoculation with 50 *B. longicaudatus*/conetainer in two experimental trials.

	Final population density (Pf)[a]		Reproductive factor (Rf)	
Cultivar	Trial 1	Trial 2	Trial 1	Trial 2
	Dwarf cultivars			
Champion	22 c[bc]	23 c	0.4 c[a]	0.5 c
MiniVerde	49 c	56 bc	1.0 c	1.1 bc
TifEagle	25 c	49 bc	0.5 c	1.0 bc
Jones Dwarf	124 b	170 a	2.5 b	3.4 a
Floradwarf	31 c	32 c	0.6 c	0.6 c
Tifgreen	43 c	176 a	0.9 c	3.5 a
Tifdwarf	240 a	192 a	4.8 a	3.8 a
Emerald Dwarf	237 a	89 b	4.7 a	1.8 b
Mean	96	99	1.9	2.0
	Non-dwarf cultivars			
Princess 77	343 a	220 a	6.9 a	4.4 a
Midlawn	44 b	47 c	0.9 b	0.9 c
Celebration	47 b	130 b	0.9 b	2.6 b
Midiron	37 b	50 c	0.7 b	1.0 c
TifSport	43 b	52 c	0.9 b	1.0 c
Tifton 10	29 b	29 c	0.6 b	0.6 c
Tifway	50 b	44 c	1.0 b	0.9 c
Patriot	39 b	135 b	0.8 b	2.7 b
Riviera	40 b	35 c	0.8 b	0.7 c
Mean	75	82	1.5	1.6

[a]Numbers represent numbers of nematodes recovered from the whole conetainer.
[b]Data are means of six replications.
[c]For each cultivar type (dwarf, non-dwarf) means within a column followed by the same letter are not different ($P \leq 0.05$), by Fishers protected least significant difference test.

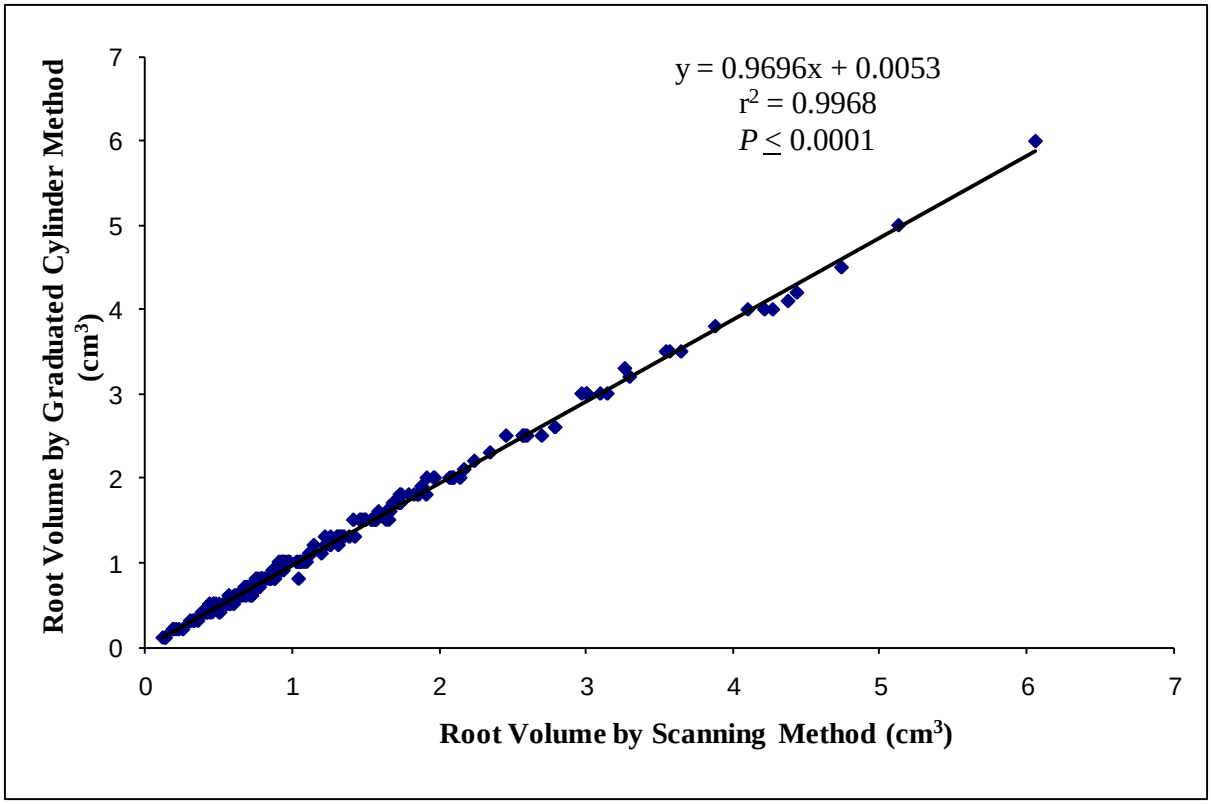

Figure 3-1. Regression relationship between the root volumes of bermudagrass measured by scanning method and graduated cylinder method in test one.

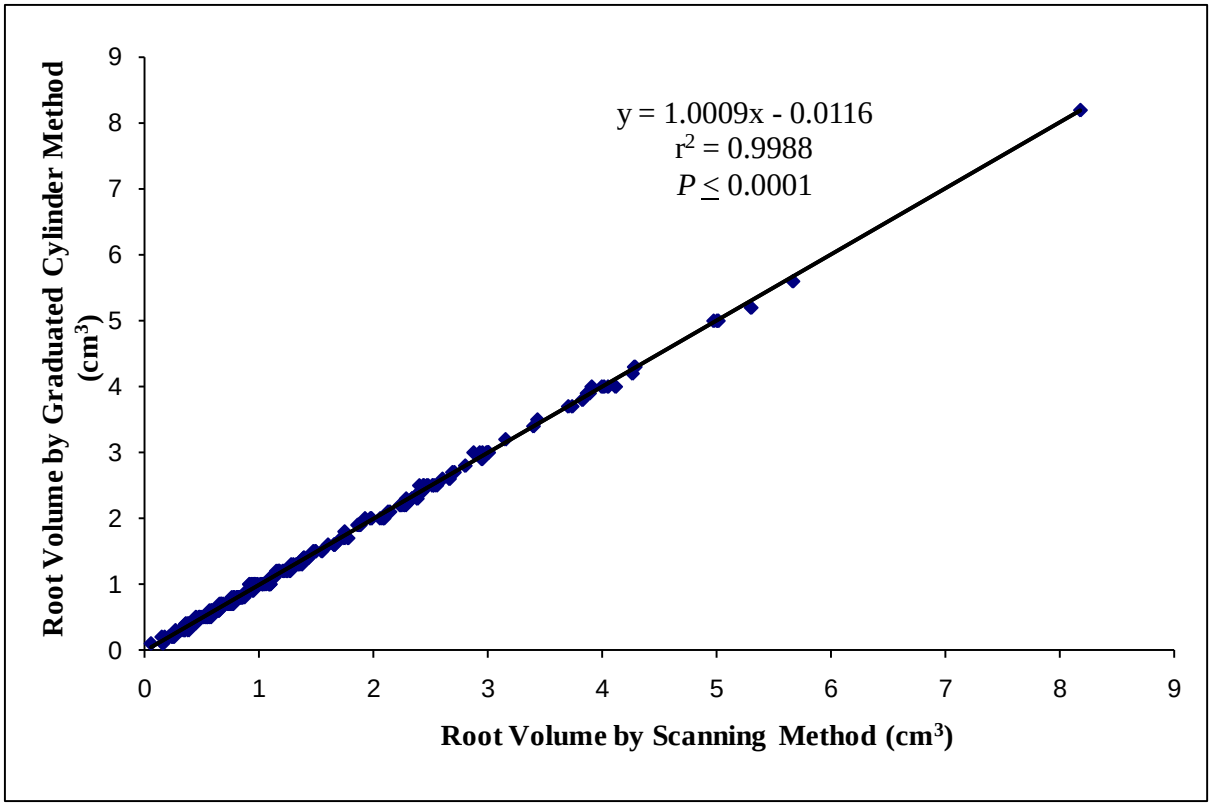

Figure 3-2. Regression relationship between the root volumes of bermudagrass measured by scanning method and graduated cylinder method in test two.

CHAPTER 4
TOLERANCE AND RESISTANCE OF SEASHORE PASPALUM CULTIVARS TO STING AND SPIRAL NEMATODES

Introduction

Seashore paspalum (*Paspalum vaginatum* Swartz) is a widely used warm-season turfgrass on golf courses. It is well adapted to the sandy soil and humid climate of Florida. Compared with bermudagrass (*Cynodon* spp.), seashore paspalum is highly tolerant of different environmental stresses. It can form a high quality turf in waterlogged soils, high salinity soils, and lower fertility soils (Duncan and Carrow, 2000). Several commercial cultivars have recently been developed and released for golf course fairways and putting greens. A survey of golf courses in Florida found that 87% of Florida golf courses were under risk of nematode damage (Crow, 2005a), two of the common nematode genera reported in that survey were *Belonolaimus* spp. and *Helicotylenchus* spp.

Belonolaimus longicaudatus Rau is frequently found in the sandy coastal soil of the southeastern areas of the United States, and was reported as the most damaging plant-parasitic nematode on turfgrasses (Crow, 2005a; Luc *et al*., 2007). *Belonolaimus longicaudatus* was reported on 50% of seashore paspalum golf courses and 40% of seashore paspalum lawns sampled in Florida (Hixson and Crow, 2004). Roots of 'SeaIsle 1' seashore paspalum were damaged by *B. longicaudatus* in greenhouse trials, but reproduction of the nematode on seashore paspalum was less than on bermudagrass (Hixson *et al*., 2004).

Helicotylenchus spp. are found throughout the southeastern United States on turfgrasses but cause more damage on sandy soils (O'Bannon and Inserra, 1989). Unlike *B. longicaudatus*, *Helicotylenchus* spp. are generally considered only minor pests on most turfgrasses, despite being commonly associated with them. Hixson and Crow (2004) reported that *Helicotylenchus* spp. were found on 88% of seashore paspalum golf courses and 85% of seashore paspalum home

lawns in Florida. Similarly, Crow (2005a) reported that *Helicotylenchus* spp. were found on over 85% of bermudagrass golf courses. However, high population densities (more than 500 *Helicotylenchus* spp./100 cm^3 soil) were common on seashore paspalum (Hixson and Crow, 2004), but high numbers were rare on bermudagrass (Crow, 2005a) in Florida. It has been hypothesized that seashore paspalum might be more susceptible to *Helicotylenchus* spp. than is bermudagrass (W. T. Crow, personal communication). *Helicotylenchus pseudorobustus* (Steiner, 1914) Golden, 1956 is the most commonly encountered spiral nematode on turfgrasses in Florida (W. T. Crow, personal communication). *Helicotylenchus pseudorobustus* is a semi-endoparasitic nematode and its feeding causes shortened and discolored roots with necrotic lesions (O'Bannon and Inserra, 1989). The aboveground symptoms of *H. pseudorobustus* damage on turf include a slow green-up in the spring, chlorosis and dieback of the leaf blades, stunting, reduction in vigor, and gradual thinning of the turf (UOIE, 2000).

Seashore paspalum is primarily used in coastal areas (Duncan and Carrow, 2000). However, improved cultivars are a recent development, and information about their resistance and tolerance to *B. longicaudatus* or *H. pseudorobustus* is limited. Most commercial cultivars have not been tested for nematode responses. Therefore, the objectives of this study were to evaluate the tolerance and resistance of commonly used seashore paspalum cultivars in Florida to *B. longicaudatus* and *H. pseudorobustus*, respectively.

Materials and Methods

Plant Materials

Seven commercial cultivars of *Paspalum vaginatum* ('Salam', 'SeaIsle 2000', 'SeaIsle 1', 'SeaIsle Supreme', 'SeaDwarf', 'SeaSpray' and 'Aloha') were tested in two sequential experimental trials in 2009.

Inoculum Preparation

Helicotylenchus pseudorobustus was maintained on three seashore paspalum cultivars Aloha, SeaDwarf, and SeaIsle 1, and *B. longicaudatus* was maintained on 'FX-313' St. Augustinegrass (*Stenotaphrum secundatum* Kuntze) grown in clay pots filled with pure sand under greenhouse conditions. Nematodes were extracted from soil using Cobb's decanting and sieving technique (Cobb, 1918; Flegg, 1967). All stages of nematodes including the juveniles and adults were collected. The nematode suspensions were concentrated using a 25-µm sieve. The average number of juveniles and adults from five 1-ml aliquots were extrapolated to the total volume of the suspension. Suspensions were kept in a refrigerator until used.

Nematode Species Identification

Morphometric and molecular analysis were conducted to identify *Helicotylenchus* to species used in this study. Twenty-five *H. pseudorobustus* females were randomly hand picked and measured at × 400 magnification under a compound light microscope with the aid of a drawing tube. The key and description of *Helicotylenchus* (Sher, 1966) were used to match with the measurements of the nematode body characters in this study for species identification. The methods for molecular analysis were the same as that of *Belonolaimus* as described in Chapter 3. Specimens of *Helicotylenchus* were also sent to Division of Plant Industry Florida, Department of Agriculture and Consumer Services, Gainesville, FL for further identification confirmation.

Greenhouse Studies

Nematode free aerial stolons of each cultivar were vegetatively propagated into (3.8 cm diameter × 21 cm deep, volume = 150 cm^3) UV stabilized Ray Leach "Cone-tainers"™ (SC10, Stuewe & Sons, Inc., Tangent, OR) filled with 100% USGA specification greens sand. The bottom of conetainers were lined with Poly-fil (Fairfield Processing Corporation, Danbury, CT) to prevent sand from escaping through the drainage holes. Two pieces of terminal aerial stolons

with one node each were planted into each conetainer. Two minutes of overhead irrigation mist was applied six times daily for two weeks to allow the sprigs to establish. From the third week, the irrigation was reduced to once a day in the morning for six minutes, and three minutes a day at the beginning of the fifth week. Six weeks after establishment, the grass was inoculated with no nematodes, 50 *B. longicaudatus* or 500 *H. pseudorobustus* per conetainer. Before inoculation, nematode suspensions were taken out of the refrigerator, concentrated to 10 or 25 nematodes/ml, respectively for *B. longicaudatus* and *H. pseudorobustus*, which were then set at room temperature for three hours. Five milliliter of *B. longicaudatus* suspension or 20 ml of the *H. pseudorobustus* suspension was evenly distributed into three 3-cm deep holes made 1 cm from the surface center of the conetainer for the treatment inoculated with *B. longicaudatus*, or with *H. pseudorobustus*, respectively. The holes were covered with a light layer of sand, and moistened with a light mist. No holes were made or solutions were inoculated to the uninoculated controls. The experiment was arranged as a randomized complete block design with five replications for each cultivar. To provide insulation, conetainers were placed in (60 × 35 × 15 cm) Beaver Plastics Styroblock™ (Stuewe & Sons, Inc., Tangent, OR). The experiments were set under a temperature range of 24 to 34^{o}C with natural daylight in an Envirotron greenhouse at University of Florida, Gainesville, FL. Grasses were fertilized once a week using 24-8-16 (N-P_2O_5-K_2O) at a rate of 0.5 kg N/100 m^2 per growing month. Turfgrass was clipped once a week at the mowing height of 2.5 cm.

Experiments were harvested 90 days after the inoculation of nematodes. Root and soil samples were collected from each conetainer. Roots were collected by removing the shoots and Poly-fil from each conetainer. Roots were then washed free of soil on an 853-μm (20-mesh) sieve and placed into a 50-ml plastic tube submerged with water. Finer roots were separated from

soil and collected into the plastic tube by submerging and shaking the 853-µm sieve in tap water. Roots were scanned into the computer and root lengths measured using WinRhizo root scanning equipment and software (Regent Instruments Inc., Ottawa, Canada). The percent root length reduction that indicates the difference between the nematode inoculated and non-inoculated treatments was calculated by the following formula: root length % reduction = [(root length of non-inoculated control – root length of inoculated plant) / root length of non-inoculated control] * 100.

Nematodes were extracted from the total soil in the conetainer by using the modified centrifugal flotation technique (Jenkins, 1964). The final nematode population densities (Pf) were counted under a microscope. The reproductive factor (Rf), which is an indicator of resistance (Oostenbrink, 1966), was calculated by the following formula: Rf = final nematode density (Pf) / initial inoculum density (Pi).

Data Analysis

Conetainers inoculated with *B. longicaudatus* or with *H. pseudorobustus* were compared to a common set of uninoculated control conetainers. The two sets of nematode data were not directly comparable to each other because the inoculation densities were different for each nematode. Therefore, the data were analyzed as two separate experiments, a *B. longicaudatus* experiment with seven cultivars and two treatments, and a *H. pseudorobustus* experiment with seven cultivars and two treatments. Total root lengths of the inoculated treatment and the non-inoculated control of each cultivar were compared by a linear single degree of freedom contrast at $P \leq 0.10$. Tolerance was determined by the difference in root length between the two treatments. A turfgrass cultivar was considered tolerant if there was no difference in total root length between the two treatments; otherwise, the cultivar was intolerant. The root lengths of

inoculated treatments were subjected to analysis of variance (ANOVA), and when significant, the differences among cultivars were compared by the Fishers protected least significant difference test at $P \leq 0.05$. Resistance was determined by the nematode reproductive factor (Rf) at harvest. A cultivar was considered resistant if Rf $\leq$ 1 and susceptible if Rf > 1. Final nematode population densities were subjected to analysis of variance (ANOVA), and the differences among cultivars were compared by the Fishers protected least significant difference test at $P \leq 0.05$. Statistical analysis was conducted by using the SAS program (SAS Institute, Cary, NC).

Results

Responses to *Belonolaimus longicaudatus*

Interactions between trials and cultivars were found, so results of the two trials were presented separately. The root lengths of four seashore paspalum cultivars were reduced by *B. longicaudatus* in at least one trial (Table 4-1). No differences were found for the inoculated root lengths or for the percent reductions between the cultivars in both trials (Table 4-1). Root reductions were less in trial two than in trial one. In trial one, four cultivars (SeaSpray, SeaIsle 1, Aloha, and SeaIsle 2000) had significant reductions in root length ($P \leq 0.10$), while only SeaSpray had significant damage from *B. longicaudatus* in trial two. Consistent for both trials, SeaIsle Supreme exhibited the least reduction in root length (5% average), while SeaSpray had the greatest reduction (24% average). Aloha, Salam, SeaDwarf, and SeaIsle Supreme had variable responses.

Nematode population densities at harvest increased for all cultivars during both trials (Table 4-2). The final nematode population densities of *B. longicaudatus* were higher in trial one than trial two. All cultivars were excellent hosts, since the lowest Rf value obtained for both trials was 2.7. The highest population densities were for SeaIsle 2000 with a Rf of 9.5 and 5.6,

respectively in trials one and trial two. Aloha had the lowest nematode Rf values, 2.7 and 2.8 for the two trials.

Responses to *Helicotylenchus pseudorobustus*

Morphometric analysis indicated that the spiral nematode species in this study was *H. pseudorobustus*. However, molecular sequences obtained were not consistent with those for *H. pseudorobustus* in Genbank. Therefore, the nematode in question will be concidered *H. pseudorobustus* sensu lato. Significant interactions between trials and cultivars also were found for *H. pseudorobustus* data, so the data for each trial was analyzed separately Root lengths were reduced only in trial one for Aloha and SeaDwarf; however, both trials resulted in significant root reductions for Salam, SeaIsle I and SeaIsle Supreme (Table 4-3). Similar to results with *B. longicaudatus*, less reduction in root length was observed in trial two than in trial one. Differences among cultivars in root length of the inoculated treatment were found in trial two. The root length of the inoculated treatment was the highest for SeaIsle 2000, which was considered tolerant to *H. pseudorobustus* infection. Root length reductions were greatest for SeaIsle 1 and SeaIsle Supreme. In trial two, the root length of SeaIsle Supreme was significantly lower than SeaSpray and SeaIsle 2000 for the inoculated treatments. Based on both trials, SeaSpray and SeaIsle 2000 were consistently tolerant to *H. pseudorobustus*.

The Rf values for *H. pseudorobustus* were <1 on all cultivars, indicating that these were poor hosts (Table 4-4). Final population densities of *H. pseudorobustus* were the highest in SeaIsle Supreme and lowest in SeaSpray and SeaIsle 2000; therefore, these latter two would be considered resistant.

Discussion

Damage of *B. longicaudatus* in seashore paspalum SeaIsle 1 has been previously reported. Hixson *et al*. (2004) found that *B. longicaudatus* caused significant reductions in root length of

SeaIsle 1 at 60 and 120 days after the inoculation of nematodes, and likewise in trial one of our study we also found significant reductions in root length of this cultivar 90 days after the inoculation of *B. longicaudatus*. Hixson *et al*. (2004) found that population densities of *B. longicaudatus* increased on SeaIsle 1 at 120 days after inoculation, which is corroborated by our studies showing that SeaIsle 1 was a good host to *B. longicaudatus* (Rf values averaged 5.3 and 2.9 in the two trials).

In comparison of these two trials, lower population densities of *B. longicaudatus* as well as less root damage were observed in trial two than in trial one, suggesting that less root damage was associated with the lower nematode population densities. For *B. longicaudatus*, the Rf was >1 for all seashore paspalum cultivars, but this was not the case for bermudagrass cultivars (Chapter 3). One hypothesis is that the relatively dense and robust root systems of seashore paspalum can support higher population densities of *B. longicaudatus*. This would indicate that the carrying capacity for *B. longicaudatus* on seashore paspalum is higher than for bermudagrass and, therefore, seashore paspalum may be more susceptible to this nematode. Although population densities of *Helicotylenchus* spp. >500/100 cm^3 of soil were found on seashore paspalum in the field (Hixson and Crow, 2004), the nematode population densities were much lower in the conetainers. The smaller soil and root volumes from the conetainers may limit the total nematode population densities that can be supported. Furthermore, when the nematodes were inoculated, the grass root systems were not fully established, and the carrying capacity on young developing roots is likely lower than for established roots in the field. The initial inoculum level of *H. pseudorobustus* of 500 nematodes/conetainer might have been too high. For future reproduction studies, inoculum densities could be reduced to 50 nematodes/conetainer. However,

the fact that root reductions occurred in one or both trials for several cultivars inoculated with *H. pseudorobustus* indicates that this nematode is a potential pathogen of seashore paspalum.

Salam, SeaDwarf, and SeaIsle Supreme were tolerant to *B. longicaudatus*. SeaSpray and SeaIsle 2000 were tolerant to *H. pseudorobustus*. No seashore paspalum cultivar was tolerant to both *B. longicaudatus* and *H. pseudorobustus*. Therefore it is unlikely that any one cultivar will be free of the threat of nematode problems in the field, especially considering that there are dozens of nematode species that are parasites of turfgrasses. Future field studies should be conducted to verify these findings. For future screening and development of nematode resistance in seashore paspalum, SeaIsle 2000 and Aloha can be used as standards for *B. longicaudatus*, and SeaIsle Supreme and SeaSpray could be used for *H. pseudorobustus*. Furthermore, interaction studies between *B. longicaudatus* and *H. pseudorobustus* could be conducted under greenhouse conditions since they often coexist in the field.

Table 4-1. Mean total root length of seven seashore paspalum cultivars 90 days after inoculation with *Belonolaimus longicaudatus* in two experiment trials. U = uninoculated and I = inoculated with 50 *B. longicaudatus*/conetainer.

	Total root length (cm)					
	Trial 1			Trial 2		
Cultivar	U	I	% reduction	U	I	% reduction
SeaSpray	2609*	1668[a]	36	2070*	1818	12
SeaIsle 1	2299*	1647	28	1792	1791	0
Aloha	1790[#]	1450	19	1837	1696	8
SeaIsle 2000	2016*	1645	18	1816	1738	4
Salam	1807	1515	16	1884	1643	13
SeaDwarf	1898	1666	12	1838	1711	7
SeaIsle Supreme	1806	1635	9	1801	1695	6

[#], *Uninoculated treatments significantly different from inoculated treatments at $P \leq 0.10$, and $P \leq 0.05$, respectively, according to the linear contrast analysis.
[a]Data are means of five replications, and data within a column followed by no letter are not statistically different ($P \leq 0.05$).

Table 4-2. Final mean population density (Pf) and reproduction factor (Rf) of *Belonolaimus longicaudatus* on seven seashore paspalum cultivars 90 days after inoculation with 50 *B. longicaudatus*/conetainer in two experimental trials.

	Final population density (Pf)[a]		Reproductive factor (Rf)	
Cultivar	Trial 1	Trial 2	Trial 1	Trial 2
SeaSpray	169 de[bc]	143 c	3.4 de	2.9 c
SeaIsle 1	266 bc	145 bc	5.3 bc	2.9 bc
Aloha	137 e	141 c	2.7 e	2.8 c
SeaIsle 2000	477 a	281 a	9.5 a	5.6 a
Salam	271 bc	197 abc	5.4 bc	3.9 abc
SeaDwarf	229 cd	231 ab	4.6 cd	4.6 ab
SeaIsle Supreme	335 b	213 abc	6.7 b	4.3 abc

[a]Numbers represent numbers of nematodes recovered from the whole conetainer.
[b]Data are means of five replications.
[c]Means within a column followed by the same letter are not different ($P \leq 0.05$), by Fishers protected least significant difference test.

Table 4-3. Mean total root length of seven seashore paspalum cultivars 90 days after inoculation with *Helicotylenchus pseudorobustus* in two experiment trials. U = uninoculated and I = inoculated with 500 *H. pseudorobustus*/conetainer.

	Total root length (cm)					
	Trial 1			Trial 2		
Cultivar	U	I	% reduction	U	I	% reduction
SeaSpray	2090	1903[a]	9	2364	2350 ab	1
SeaIsle 1	2543*	2010	21	2222[#]	1755 c	21
Aloha	2296[#]	1842	20	2391	2145 abc	10
SeaIsle 2000	2309	1967	15	2598	2592 a	0
Salam	2509*	1740	31	2425*	2024 bc	17
SeaDwarf	2527[#]	2074	18	2475	2441 ab	1
SeaIsle Supreme	2426*	1918	21	2256*	1864 c	17

[#], *Uninoculated treatments significantly different from inoculated treatments at $P \leq 0.10$, and $P \leq 0.05$, respectively, according to the linear contrast analysis.

[a]Data are means of five replications, and means within a column followed by the same letter or no letter are not different ($P \leq 0.05$), by Fishers protected least significant difference test.

Table 4-4. Mean population density (Pf) and reproduction factor (Rf) of *Helicotylenchus pseudorobustus* on seven seashore paspalum cultivars 90 days after inoculation with 500 *H. pseudorobustus*/conetainer in two experimental trials.

	Final population density (Pf)[a]		Reproductive factor (Rf)	
Cultivar	Trial 1	Trial 2	Trial 1	Trial 2
SeaSpray	72 b[bc]	94 cd	0.1 b	0.2 d
SeaIsle 1	237 a	291 b	0.5 a	0.6 b
Aloha	209 a	147 cd	0.4 a	0.3 cd
SeaIsle 2000	99 b	85 d	0.2 b	0.2 d
Salam	250 a	270 b	0.5 a	0.5 b
SeaDwarf	221 a	210 bc	0.4 a	0.4 bc
SeaIsle Supreme	265 a	422 a	0.5 a	0.8 a

[a]Numbers represent numbers of nematodes recovered from the whole conetainer.

[b]Data are means of five replications.

[c]Means within a column followed by the same letter are not different ($P \leq 0.05$), by Fishers protected least significant difference test.

CHAPTER 5
FIELD RESPONSES OF BERMUDAGRASS AND SEASHORE PASPALUM CULTIVARS TO STING AND SPIRAL NEMATODES

Introduction

Bermudagrass (*Cynodon* spp.) is a warm-season turfgrass widely used on golf courses, sports fields, and home lawns in tropical and subtropical regions. Use of seashore paspalum (*Paspalum vaginatum* Swartz) has increased primarily in coastal areas with the development of cultivars having finer leaf texture, high turf quality and excellent salinity tolerance (Dudeck and Peacock, 1985; Duncan, 1999a). However, a major limitation of planting turfgrasses in the sandy soils of the southeastern United States is the destruction of roots by plant-parasitic nematodes (Perry and Rhoades, 1982, Hixson *et al*., 2004). *Belonolaimus longicaudatus* Rau and *Helicotylenchus* spp. are found in the southeastern United States on turfgrasses and are prevalent in sandy soils (Holdeman, 1955; Christie, 1959; Robbins and Barker, 1974). *Belonolaimus longicaudatus* is the most damaging nematode species on turfgrasses in Florida (Crow, 2005a). The feeding of *B. longicaudatus* can cause stunted root growth, decreased plant water and nutrient uptake, and decreased rates of plant evapotranspiration (Johnson, 1970; Perry and Rhoades, 1982; Busey *et al*., 1991, 1993; Giblin-Davis *et al*., 1992a; Luc *et al*., 2006). *Helicotylenchus* spp. have been found in turfgrasses on golf courses and home lawns in Canada and the United States (Yu, *et al*., 1998; Hixson and Crow, 2004). Jordan and Mitkowski (2006) found that *Helicotylenchus* spp. occurred in all 38 golf courses and 110 putting greens (98.2%) surveyed in New England. A survey of seashore paspalum golf courses and lawns found that 50% of golf courses and 40% of home lawns in Florida were infested with *Belonolaimus longicaudatus*; while 88% of golf courses and 85% of home lawns were infested with *Helicotylenchus* spp. (Hixson and Crow, 2004). High densities of *Helicotylenchus* spp. (>500 nematodes/100 cm^3 soil) were often found associated with seashore paspalum in Florida (Hixson

and Crow, 2004). *Belonolaimus longicaudatus* was the most common nematode found at damaging numbers in a survey of bermudagrass golf courses in Florida, present on 84% of golf courses, 60% of fairways, and 52% of greens in the golf courses surveyed by Crow (2005a). High population densities of *B. longicaudatus* that could cause damage to turfgrass were present on 60% of the golf courses, 25% of individual fairways, and 21% of individual greens surveyed (Crow, 2005a). The feeding by *Helicotylenchus* spp. causes necrotic lesions and dieback of roots, which could further lead to the decline of the entire host plant (O'Bannon and Inserra, 1989).

Although several commercial bermudagrass cultivars and germplasm have been tested for their responses to *B. longicaudatus* or *Helicotylenchus* spp. under greenhouse conditions (Nign 1963; Johnson, 1970; Giblin-Davis *et al*., 1992b), information about their performance under field conditions is lacking. Davis *et al*. (2004) evaluated the host status of 15 commonly used forage grass species to *H. pseudorobustus* (Steiner, 1914) Golden, 1956. They found that tall fescue (*Festuca arundinacea* Schreb.), annual bluegrass (*Poa annua* L.), paspalum (*Paspalum* spp.), and perennial ryegrass (*Lolium perenne* L.) were all good hosts to *H. pseudorobustus* under controlled conditions. Mixed populations of *B. longicaudatus* and *Helicotylenchus* spp. in the field have been reported (Sasser *et al*., 1975; Lewis *et al*., 1993; Sikora *et al*., 2001); however, little information is available about the responses of bermudagrass or seashore paspalum to both nematode species under field conditions. Information also is lacking for individual cultivar responses when multiple nematode species coexist in the field. Therefore, the objectives of this study were: to evaluate the responses of several commercial bermudagrass cultivars to *B. longicaudatus* under field conditions; to evaluate the responses of several commercial seashore paspalum cultivars to *B. longicaudatus* and *H. pseudorobustus* under field conditions; to determine if there is a correlation between the population densities of different

nematode species in field; and to evaluate turf health of the different grasses while growing in a nematode-infested environment.

Materials and Methods

Two-year Field Study

A field experiment was conducted from May 2008 to June 2010 at the IFAS Agronomy Forage Research Unit (29°48.0' North, 82°25.1' West) in Hague, Florida. The soil type was a flatwood soil (2% clay, 6% slit, and 92% sand) with 5% organic matter and a pH value of 5.9. A field was selected for this experiment that was naturally infested with *B. longicaudatus* and *H. pseudorobustus*, along with small numbers of *Meloidogyne* sp., *Paratrichodorus* sp., *Mesocriconema ornatum*, *Hemicycliophora* sp., *Hemicriconemoides* sp., *Pratylenchus* sp., and *Tylenchorhynchus* sp. Fifty-five 1.5-m × 1.5-m square plots were laid out, with 0.3-m-wide non-planted border areas. Treatments consisted of 11 turfgrass cultivars: five dwarf bermudagrass cultivars 'Tifgreen', 'Champion', 'MiniVerde', 'TifEagle', and 'Floradwarf'; three non-dwarf cultivars 'Tifway', 'Celebration', and 'TifSport', and three seashore paspalum cultivars 'Aloha', 'Sea Isle 1', and 'SeaDwarf'. The experiment was a randomized complete block design with five replications.

Grasses were propagated by planting nematode free aerial stolons into 15-cm diameter clay plots in a glasshouse at the University of Florida under a temperature range of 24 to 34°C with natural daylight. Grasses were fertilized once a week using 24-8-16 (N-P_2O_5-K_2O) at a rate of 0.5 kg N/100 m^2 per growing month. Turfgrass was watered by an overhead irrigation for 6 min everyday and clipped once a week. Ten pots were maintained for each cultivar. Well-established nematode-free grasses were transplanted from the greenhouse to the field plots on May 26, 2008. For planting each pot was divided in half that provided four plugs for each plot. Plugs were planted into the corners of 0.6-m × 0.6-m squares in the plot centers. Grasses were watered for

15 min three times a day for the first 20 days, and irrigated as needed thereafter. Fertilizer 10-10-10 (N-P_2O_5-K_2O) was applied biweekly at a rate of 0.5 kg N/100 m^2 per growing month from May to October.

Nematode population densities in each plot were assayed just prior to planting. Nine soil cores (2.5-cm-diam. × 10-cm-deep) were randomly collected using a 2.5-cm-diam. cone-shaped sampler to form a representative sample for each plot. Sample holes were then refilled with air-dried natural field soil from the same field. The soil in each sample was well mixed and nematodes were extracted from 100-cm^3 subsample of soil using a modified centrifugal-flotation technique (Jenkins, 1964). The plant-parasitic nematodes were identified to genus and counted under an inverted light microscope at × 32 magnification. Nematode population densities were assayed similarly every three months throughout the study. Grasses were mowed at 2.5 cm by a reel mower, and gramineous weeds were hand picked when needed. Pesticide applications included 2,4-D and dicamba mixture (Outlaw™, Helena Chemical Company, Collierville, TN) at a rate of 1.8 L/ha for control of weeds, azoxystrobin (Heritage®, Syngenta, Wilmington, DE) at a rate of 0.6 kg/ha to prevent fungal disease, *Bacillus thuringiensis*, subsp. *kurstaki* (DiPel®, Valent BioSciences, Libertyville, IL) at a rate of 1.1 kg/ha and fipronil (TopChoice®, Bayer CropScience, Research Triangle Park, NC) at a rate of 5.6 kg/ha to control caterpillar (order Lepidoptera) and mole crickets (*Scapteriscus* spp.) were applied when needed. The rates are product rates.

Turfgrass health was determined by evaluating root lengths and percent green cover every three months throughout the growing season. Digital images of each plot were analyzed using the SigmaScan Pro software (SPSS, Inc., Chicago, IL) to determine the percentage of green pixels in the image (PGC) (Karcher and Richardson, 2005). Turfgrasses were dormant during

March of both years; therefore, these images were not included in the analysis. Root samples were collected from two soil cores (4-cm-diam. × 15-cm-deep) taken randomly in each plot to form a representative sample, and the holes were then filled with air-dried natural field soil from the same field. Samples were stored in a cooler until processing. Roots were collected by removing the shoots and thatch, and washing free of soil on an 853-µm sieve. The roots were then placed into a 50-ml plastic tube and submerged with water. Finer roots were further separated from soil and collected into the plastic tube by submerging and shaking the 853-µm sieve in tap water. Roots were digitally scanned using WinRhizo root scanning equipment and software (Regent Instruments, Ottawa, Ontario, CA). Total root length of each sample was quantified.

One-year Field Study

A separate field experiment was conducted from April 2009 to July 2010 in a nearby field at the same facility (Agronomy Forage Research Unit). The soil type was the same as the previous study. This field was naturally infested with low numbers of *B. longicaudatus*, *H. pseudorobustus*, *Meloidogyne* sp., *Paratrichodorus* sp., *Mesocriconema* sp., *Hemicycliophora* sp., *Hemicriconemoides* sp., and *Tylenchorhynchus* sp. One hundred plots were laid out with the same dimensions as those of the 2008 study. Four dwarf bermudagrass cultivars TifEagle, 'Jones Dwarf', 'Tifdwarf', and 'Emerald Dwarf'; nine non-dwarf cultivars Tifway, TifSport, Celebration, 'Riviera', 'Patriot', 'TifGrand', 'Midiron', 'Princess 77', and 'Tifton10'; and seven seashore paspalum cultivars 'Salam', 'SeaIsle 2000', SeaIsle 1, 'SeaIsle Supreme', SeaDwarf, 'SeaSpray' and Aloha were tested from 2009 to 2010. Cultivars were propagated and maintained using the same protocol as the two-year field study. Well-established nematode-free grasses were transplanted from the greenhouse to the field plots on April 24, 2009. Two pieces of grass for each cultivar were planted into two corners in the center of each plot. Turfgrasses were

maintained and cultivated in the same way as the field studies in 2008, except that the fertilizer rate was half of that applied to the 2008 plots. Nematode populations were assayed at the beginning of this study in April, as well as July and October of 2009 and January, April, and July of 2010. Percent green cover was evaluated October 2009, April, and July 2010. Nematode population densities in the plots and percent green cover were recorded accordingly. In spring 2010 it became evident that some plots were contaminated with different grass cultivars. Also, a number of the dwarf bermudagrasses did not fill in. The contaminated plots were killed with glyphosate (Roundup, Monsanto, St Louis, MO) in April 2010 and subsequently these plots, along with those that had not filled in, were replanted. Data from these plots were not included in the analysis. Grasses were watered for 15 min three times a day after transplanting for 30 days, and then fertilizer was applied as the field studies in 2008.

Statistical Analysis

Percent green cover, total root length, and nematode population densities at each sample date were subjected to analysis of variance (ANOVA), and the differences among cultivars were compared using Fishers protected least significant difference test at $P \leq 0.05$. Data for bermudagrass dwarf cultivars, non-dwarf cultivars, and seashore paspalum cultivars were analyzed separately. Means of all bermudagrass cultivars as a group were compared with that of all seashore paspalum cultivars. A logarithmic regression analysis was conducted to determine the relationship between the soil population densities of *B. longicaudatus* and *H. pseudorobustus* in each individual cultivar; separate linear regression analyses were conducted to determine the relationship between 1) the soil population densities of *B. longicaudatus* or *H. pseudorobustus* and turfgrass total root length; 2) and the population densities of *B. longicaudatus* or *H. pseudorobustus* and percent green cover in each individual cultivar. Statistical analysis was conducted by using the SAS software (SAS Institute, Cary, NC).

Results

Two-year Field Study

Bermudagrass cultivars

There was no difference in the population densities of *B. longicaudatus* among all dwarf cultivars throughout the two years (Table 5-1). By the end of the study, densities of *B. longicaudatus* increased for Champion and MiniVerde, and decreased for Floradwarf, Tifgreen, TifEagle compared to their respective initial population densities in May 2008. In December 2008, the population density of *B. longicaudatus* was the highest for Tifway and lowest for TifSport, and in June 2009, the nematode population density associated with TifSport was significantly lower than densities observed Tifway and Celebration (Table 5-1). Mean population densities of *B. longicaudatus* were not different between the dwarf and non-dwarf cultivars.

Population densities of *H. pseudorobustus* were not different among the dwarf cultivars for the duration of the study (Table 5-2). Population densities of *H. pseudorobustus* increased in non-dwarf bermudagrass cultivars from 6 to 270 nematodes/100 cm^3 of soil during the experiment. In June and December 2009, and June 2010, population densities of *H. pseudorobustus* were significantly higher in TifSport than the other two cultivars. Over the course of this experiment, population density increased from 6 to 744 *H. pseudorobustus*/100 cm^3 (124 fold) on TifSport. A negative regression relationship between the population densities of *H. pseudorobustus* and *B. longicaudatus* was observed for Celebration (Figure 5-1 A) and TifSport bermudagrass (Figure 5-1 B). Mean population densities of *H. pseudorobustus* were not different between the dwarf and non-dwarf cultivars.

Total Root Lengths were not different for the dwarf bermudagrass cultivars in this study (Table 5-3). In September 2009, the root length of non-dwarf cultivar Celebration was significantly greater than both Tifway and TifSport root lengths. Differences were not identified

among the non-dwarf cultivars for the remaining root length sampling dates. There was no difference in percent green cover among the dwarf cultivars from June 2009 to June 2010 except for December 2009 (Table 5-4). In December 2009, the PGC of Tifgreen was significantly higher than that of Floradwarf, TifEagle and MiniVerde; and Champion had greater PGC than MiniVerde. No other dates provided significant differences for PGC among the dwarf cultivars nor were differences found (at any dates) between the non-dwarf cultivars. The mean root lengths or turf densities of dwarf cultivars were not different from those of the non-dwarf cultivars throughout the study.

A negative linear relationship was found between the total root length and the population density of *B. longicaudatus* (Figure 5-2) as well as PGC (Figure 5-3 B) in Celebration bermudagrass. Regression showed that for each *B. longicaudatus* in 100 cm^3 of soil, there was a corresponding reduction of 5 cm in total root length and 0.14% in PGC of Celebration. A negative linear relationship between the population densities of *H. pseudorobustus* and the total root length (Figure 5-4 A) in Floradwarf bermudagrass was observed. A negative linear relationship between the population densities of *H. pseudorobustus* and PGC was observed in Floradwarf (Figure 5-5 A). For each *H. pseudorobustus* in 100 cm^3 of soil, there was a corresponding reduction of 1 cm in total root length and 0.20% in PGC.

Results from the regression analysis also showed that *H. pseudorobustus* reduced the root length of Tifgreen bermudagrass. For each *H. pseudorobustus* in 100 cm^3 of soil, there was a corresponding reduction of 0.6 cm in total root length (Figure 5-4 B). In addition, *H. pseudorobustus* affected TifEagle. Figure 5-5 B indicates that the PGC of TifEagle was reduced by 0.26% for each *H. pseudorobustus*/100 cm^3 of soil.

Seashore paspalum cultivars

Population densities of *B. longicaudatus* declined in all seashore paspalum cultivars at the end of the study compared with the initial (May 2008) population densities (Table 5-5). Population densities of *B. longicaudatus* were the highest in SeaIsle 1 (130 nematodes/100 cm^3 of soil) and lowest in Sea Dwarf (75 nematodes/100 cm^3 of soil) in December of 2008 and June 2009, respectively. However, there were no treatment differences at other dates. All the seashore paspalum cultivars were good hosts to *H. pseudorobustus*. Population densities of *H. pseudorobustus* increased from less than 13 to as high as 1414 nematodes/100 cm^3 of soil during the study (Table 5-6). However, there was no difference among the three cultivars throughout the two years.

The total root lengths of the three cultivars varied from 373 to 993 cm, but were not different throughout the study (Table 5-7). No association was found between the population densities of *B. longicaudatus* or *H. pseudorobustus* and the root length of seashore paspalum. Percent green cover ranged from 44 to 80%. In June, September 2009, and June 2010, PGC of SeaDwarf was significantly higher than Aloha and SeaIsle 1 (Table 5-8). A negative linear relationship was found between the PGC of Aloha and the population density of *B. longicaudatus* (Figure 5-3 A). The PGC of Aloha was reduced by 0.94% for each *B. longicaudatus* in 100 cm^3 of soil (Figure 5-3 A). As with bermudagrass, associations between *B. longicaudatus* and *H. pseudorobustus* were found in seashore paspalum. A negative logarithmic relationship was found between the population densities of *H. pseudorobustus* and *B. longicaudatus* in Aloha (Figure 5-6 A), Sea Dwarf (Figure 5-6 B), and Sea Isle 1 (Figure 5-6 C).

When all bermudagrass cultivars were compared as a group to the seashore paspalum cultivars, population densities of *B. longicaudatus* were significantly higher in bermudagrass than seashore paspalum on several sampling dates (Table 5-9). The root lengths of bermudagrass

and seashore paspalum were not different throughout the study (Table 5-10). However, PGC was different between the two species for two of four sampling dates (Table 5-10).

One-year Field Study

Bermudagrass cultivars

Although 13 bermudagrass cultivars were planted in the field, many were not included in the analysis due to contamination and poor establishment. Therefore, only Celebration and Tifway were included. Aboveground PGC was measured in October 2009, April and July of 2010, respectively. These turf densities ranged from 17 to 60%, but no difference was detected between the two cultivars at any time (Table 5-11).

From April 2009 to April 2010, the population densities of *B. longicaudatus* increased continuously in both cultivars through April of 2010. However, in July 2010, the nematode population densities dropped below 10 *B. longicaudatus*/100 cm^3 of soil (Table 5-12). No difference in population densities of *B. longicaudatus* or *H. pseudorobustus* was detected between these two cultivars throughout the study (Table 5-12).

Seashore paspalum cultivars

No differences in PGC among the seven cultivars of seashore paspalum were detected (Table 5-13). Population densities of *B. longicaudatus* were <10 nematodes/100 cm^3 of soil in all months except for January 2010 (Table 5-14). Population densities of *H. pseudorobustus* increased greatly from <6 to 495 nematodes/100 cm^3 of soil in seashore paspalum cultivars from April 2009 to July 2010 (Table 5-15).

Discussion

All bermudagrass cultivars except TifSport were good hosts to *B. longicaudatus*. During the two year study, population densities of *B. longicaudatus* increased on Champion and MiniVerde, but slightly dropped in Tifgreen, TifEagle, and Celebration, and dropped by 32%,

33%, and 93% respectively in Floradwarf, Tifway and TifSport. Population densities of *B. longicaudatus* on TifSport continuously declined and were the lowest among the cultivars evaluated (Table 5-1), indicating that TifSport might have some level of resistance to *B. longicaudatus*. Conversely, TifSport was a good host for *H. pseudorobustus*, and supported a 124- fold increase in reproduction. Therefore, TifSport was the most resistant to *B. longicaudatus,* and the most susceptible to *H. pseudorobustus*. This difference in response of TifSport to two nematode species might be of great significance to consider when selecting a turfgrass for use or when designing a breeding program for improving nematode responses.

The population densities of *B. longicaudatus* were two to five and a half times higher in bermudagrass than in seashore paspalum, which might indicate that bermudagrass is a better host to *B. longicaudatus* than seashore paspalum (Table 5-9). Contrastingly, population densities of *H. pseudorobustus* were significantly higher in seashore paspalum than in bermudagrass from September 2008 to June 2010 (Table 5-9). Population densities of *H. pseudorobustus* in seashore paspalum varied from two to 13 times higher than in bermudagrass, which indicated that seashore paspalum could be a better host to *H. pseudorobustus* than bermudagrass. Different from most bermudagrass cultivars, the population densities of *B. longicaudatus* in seashore paspalum declined from near 109 to 13 nematodes/100 cm^3 soil. Potential explanations for this decline include: 1) Seashore paspalum might be a non-host to *B. longicaudatus* under field conditions, although this would contradict the results of greenhouse experiments (Chapter 4; Hixson *et al*., 2005) and general field observations (W. T. Crow, personal communication); 2) Seashore paspalum might be intolerant to *B. longicaudatus* and have a low carrying capacity, agreeing with observations by Hixson *et al*. (2005); or 3) Field interactions between *B. longicaudatus* and *H. pseudorobustus* may have limited reproduction of *B. longicaudatus*.

Seashore paspalum was a better host for *H. pseudorobustus* than for *B. longicaudatus*. Population densities of *H. pseudorobustus* increased 100 to 200-fold while those of *B. longicaudatus* decreased by 70 to 96% in the seashore paspalum cultivars Aloha, Sea Dwarf, and Sea Isle 1. This observation and the inverse regression equations suggest that competition or interactions exist between *B. longicaudatus* and *H. pseudorobustus*, and that *H. pseudorobustus* might suppress the reproduction of *B. longicaudatus* in seashore paspalum and for TifSport and Celebration bermudagrass (Figure 5-1, Figure 5-6). However, the suppressive effect was not obvious in other bermudagrass cultivars. Therefore, the interaction between the two nematode species was host dependent, and could vary between genotypes and species.

Although in April and July of 2010 the population densities of *H. pseudorobustus* in Celebration and Tifway were numerically higher than those in Tifway, no statistical difference was observed (Table 5-12), which could be due to the high variability of the nematode densities among the plots (91 to 237 and 2 to 17 nematodes/100 cm^3 of soil in April 2010 in Celebration and Tifway, and 23 to 485 and 0 to 10 nematodes/100 cm^3 of soil in July 2010).

Although *B. longicaudatus* and *Helicotylenchus* spp. were reported to coexist in the field (Sasser *et al*., 1975; Lewis *et al*., 1993; Sikora *et al*., 2001), very few studies have been conducted to test the interaction or competition between them. Only Sasser *et al*. (1975) reported that there was a significant positive correlation between the population densities of *H. dihystera* and *B. longicaudatus* 60 days ($r = 0.37$, $P < 0.01$), 90 ($r = 0.28$, $P < 0.01$) and 120 days ($r = 0.38$, $P < 0.01$) after planting peanut in the field. This does not conflict with our results since their sampling dates were relatively soon after planting the crop, when both the crop and nematode population densities were growing, which could result in a positive correlation. Johnson (1970) tested the pathogenicity and interaction of *Criconemoides ornatus* (syn. *Mesocriconema*

ornatum), *Tylenchorhynchus martini* (syn. *T. annulatus*), and *B. longicaudatus* on six bermudagrass cultivars. He found that all bermudagrass cultivars supported high population densities of the three nematode species but *B. longicaudatus* was a better competitor than *C. ornatus* and *T. martini*. The population densities of *C. ornatus* and *T. martini* were suppressed more than *B. longicaudatus* by other nematode species. Similar to our study, we also found that *B. longicaudatus* was a better competitor than *H. pseudorobustus* in most (seven) bermudagrass cultivars. In the future, greenhouse studies could be conducted to study the interactions and pathogenicity between *B. longicaudatus* and *H. pseudorobustus* on different turfgrass hosts.

A negative linear relationship between the population densities of *B. longicaudatus* and total root length or aboveground PGC were observed for Celebration. Greenhouse studies (Chapter 3) had shown that *B. longicaudatus* caused significant root length reductions in Celebration, confirming that Celebration was not tolerant to *B. longicaudatus*. The negative relationships between nematode population density and turfgrass growth parameters could indicate that Floradwarf, Tifgreen, and TifEagle may not be tolerant to *H. pseudorobustus*. A negative linear relationship between the population densities of *B. longicaudatus* and aboveground PGC of seashore paspalum Aloha was observed. Considering these field results and that root length was reduced in a controlled greenhouse trial (Chapter 4), it appears that Aloha may be intolerant to *B. longicaudatus* infestation. Based on the ability to maintain greater root lengths and PGC in nematode infested soil, SeaDwarf might be a tolerant cultivar (Table 5-7, Table 5-8). A previous greenhouse study also indicated that SeaDwarf was tolerant to *B. longicaudatus* damage (Chapter 4). Compared with greenhouse studies, field studies more closely approximated a real-life situation where multiple nematode species or pathogens coexist.

Furthermore, these cultivars could be tested under different populations or isolates of *B. longicaudatus* and *H. pseudorobustus* in the future.

Generally speaking, bermudagrass is a better host to *B. longicaudatus* than *H. pseudorobustus*, and *B. longicaudatus* is more damaging than *H. pseudorobustus* to bermudagrass. The exception was TifSport, which was resistant to *B. longicaudatus*, but an excellent host to *H. pseudorobustus*. The seashore paspalum cultivars evaluated were better hosts to *H. pseudorobustus* than *B. longicaudatus*, and *H. pseudorobustus* appeared more damaging than *B. longicaudatus* to seashore paspalum. Seashore paspalum had higher PGC than bermudagrass in the nematode-infested field studies. TifSport bermudagrass might be a good choice for tees and fairways infested with *B. longicaudatus*. SeaDwarf might be a good seashore paspalum cultivar to use to use on greens infested with *B. longicaudatus*.

Table 5-1. Population density of *Belonolaimus longicaudatus* on eight bermudagrass cultivars for the two-year field study at Hague, FL.

Cultivar	Nematode population density[a]								
	May 2008	Sep 2008	Dec 2008	Mar 2009	Jun 2009	Sep 2009	Dec 2009	Mar 2010	Jun 2010
	Dwarf cultivars								
Champion	86[b]	66	276	355	178	20	40	116	118
Floradwarf	98	49	327	310	170	14	31	101	67
Tifgreen	113	92	301	273	162	33	85	225	109
MiniVerde	94	78	327	255	137	13	68	70	132
TifEagle	96	76	248	248	186	7	30	84	79
Mean	97	72	296	288	167	17	51	119	101
	Non-dwarf cultivars								
Tifway	80	43	206 a	197	61 a	8	21	44	54
Celebration	96	26	144 ab	73	69 a	5	26	25	70
TifSport	102	46	71 b	40	26 b	3	20	17	7
Mean	93	38	140	103	52	5	22	29	44

[a]Numbers represent numbers of nematodes recovered from 100 cm^3 of soil.
[b]Data are means of five replications; and for each cultivar type, means within a column followed by the same letter or no letter are not different ($P \leq 0.05$), Fishers protected least significant difference test.

Table 5-2. Population density of *Helicotylenchus pseudorobustus* on eight bermudagrass cultivars for the two-year field study at Hague, FL.

Cultivar	Nematode population density[a]								
	May 2008	Sep 2008	Dec 2008	Mar 2009	Jun 2009	Sep 2009	Dec 2009	Mar 2010	Jun 2010
	Dwarf cultivars								
Champion	5[b]	5	13	6	20	13	76	74	100
Floradwarf	11	7	15	5	11	61	65	84	117
Tifgreen	11	16	20	25	30	84	175	137	187
MiniVerde	4	6	4	4	10	22	14	25	48
TifEagle	22	17	17	31	19	7	66	64	120
Mean	11	10	14	14	18	37	79	77	114
	Non-dwarf cultivars								
Tifway	9	4	5	3	3 b	5	8 b	10	47 b
Celebration	2	4	2	2	3 b	40	23 b	114	20 b
TifSport	6	16	130	227	262 a	198	592 a	333	744 a
Mean	6	8	46	77	89	81	208	152	270

[a]Numbers represent numbers of nematodes recovered from 100 cm^3 of soil.
[b]Data are means of five replications; and for each cultivar type, means within a column followed by the same letter or no letter are not different ($P \leq 0.05$), Fishers protected least significant difference test.

Table 5-3. Total root length of eight bermudagrass cultivars in nematode infested field plots for the two-year field study at Hague, FL.

Cultivar	Total root length (cm)					
	Mar 2009	Jun 2009	Sep 2009	Dec 2009	Mar 2010	Jun 2010
	Dwarf cultivars					
Champion	540[a]	405	473	539	436	337
Floradwarf	498	486	447	732	402	438
Tifgreen	634	707	801	842	671	583
MiniVerde	553	328	357	370	540	273
TifEagle	619	539	611	710	493	465
Mean	569	493	538	639	508	419
	Non-dwarf cultivars					
Tifway	659	723	566 b	831	808	739
Celebration	572	558	1054 a	1042	895	1003
TifSport	528	620	605 b	748	809	848
Mean	586	634	742	874	837	863

[a]Data are means of five replications; and for each cultivar type, means within a column followed by the same letter or no letter are not different ($P \leq 0.05$), Fishers protected least significant difference test.

Table 5-4. Aboveground percent green cover of eight bermudagrass cultivars in nematode infested field plots for the two-year field study at Hague, FL.

Cultivar	Percent green cover (%)			
	Jun 2009	Sep 2009	Dec 2009	Jun 2010
	Dwarf cultivars			
Champion	61[a]	76	34 ab	57
Floradwarf	70	73	28 bc	39
Tifgreen	65	75	44 a	59
MiniVerde	72	68	22 c	33
TifEagle	73	55	26 bc	28
Mean	68	69	31	43
	Non-dwarf cultivars			
Tifway	69	79	47	64
Celebration	71	80	54	63
TifSport	72	76	57	61
Mean	71	78	53	63

[a]Data are means of five replications; and for each cultivar type, means within a column followed by the same letter or no letter are not different ($P \leq 0.05$), Fishers protected least significant difference test.

Table 5-5. Population density of *Belonolaimus longicaudatus* on three seashore paspalum cultivars for the two-year field study at Hague, FL.

Cultivar	Nematode population density[a]								
	May 2008	Sep 2008	Dec 2008	Mar 2009	Jun 2009	Sep 2009	Dec 2009	Mar 2010	Jun 2010
Aloha	87[b]	54	104 ab	116	28 ab	7	24	23	17
SeaDwarf	134	33	75 b	115	25 b	6	21	10	6
SeaIsle 1	105	51	130 a	128	39 a	8	16	26	15

[a]Numbers represent numbers of nematodes recovered from 100 cm^3 of soil.
[b]Data are means of five replications; and means within a column followed by the same letter or no letter are not different ($P \leq 0.05$), Fishers protected least significant difference test.

Table 5-6. Population density of *Helicotylenchus pseudorobustus* on three seashore paspalum cultivars for the two-year field study at Hague, FL.

Cultivar	Nematode population density[a]								
	May 2008	Sep 2008	Dec 2008	Mar 2009	Jun 2009	Sep 2009	Dec 2009	Mar 2010	Jun 2010
Aloha	8[b]	10	162	282	611	1029	1066	1045	1414
SeaDwarf	13	33	161	254	451	585	939	515	1377
SeaIsle 1	5	15	84	226	482	564	1282	894	1070

[a]Numbers represent numbers of nematodes recovered from 100 cm^3 of soil.
[b]Data are means of five replications, and data within a column followed by no letter are not statistically different ($P \leq 0.05$).

Table 5-7. Total root length of three seashore paspalum cultivars in nematode infested field plots for the two-year field study at Hague, FL.

Cultivar	Total root length (cm)					
	Mar 2009	Jun 2009	Sep 2009	Dec 2009	Mar 2010	Jun 2010
Aloha	506[a]	438	413	647	457	373
SeaDwarf	622	651	558	807	760	768
SeaIsle 1	552	365	577	993	514	572

[a]Data are means of five replications, and data within a column followed by no letter are not statistically different ($P \leq 0.05$).

Table 5-8. Aboveground percent green cover of three seashore paspalum cultivars in nematode infested field plots for the two-year field study at Hague, FL.

Cultivar	Percent green cover (%)			
	Jun 2009	Sep 2009	Dec 2009	Jun 2010
Aloha	69 b	92 b	76[a]	44 b
SeaDwarf	71 a	96 a	82	64 a
SeaIsle 1	64 b	91 b	75	44 b

[a]Data are means of five replications; and means within a column followed by the same letter or no letter are not different ($P \leq 0.05$), Fishers protected least significant difference test.

Table 5-9. Population density of *Belonolaimus longicaudatus* and *Helicotylenchus pseudorobustus* on bermudagrass and seashore paspalum for the two-year field study at Hague, FL.

Cultivar	May 2008	Sep 2008	Dec 2008	Mar 2009	Jun 2009	Sep 2009	Dec 2009	Mar 2010	Jun 2010
	Population density of *Belonolaimus longicaudatus*[a]								
Bermudagrass	96[b]	60	237 a	219 a	124 a	13	40	85	72 a
Seashore paspalum	109	46	103 b	119 b	31 b	7	20	20	13 b
	Population density of *Helicotylenchus pseudorobustus*[a]								
Bermudagrass	9	9 b	26 b	38 b	45 b	54 b	127 b	105 b	173 b
Seashore paspalum	9	20 a	136 a	254 a	515 a	726 a	1096 a	818 a	1287 a

[a]Numbers represent numbers of nematodes recovered from 100 cm^3 of soil.
[b]Data are means of five replications; and means within a column followed by the same letter or no letter are not different ($P \leq 0.05$), Fishers protected least significant difference test.

Table 5-10. Total root length and aboveground percent green cover of bermudagrass and seashore paspalum in nematode infested field plots for the two-year field study at Hague, FL.

Cultivar	Mar 2009	Jun 2009	Sep 2009	Dec 2009	Mar 2010	Jun 2010
	Total root length (cm)					
Bermudagrass	576[a]	546	614	727	632	586
Seashore paspalum	560	484	516	815	577	571
	Percent green cover (%)					
Bermudagrass	----	69	73	39 b	51	----
Seashore paspalum	----	65	93	78 a	51	----

[a]Data are means of five replications; and means within a column followed by the same letter or no letter are not different ($P \leq 0.05$), Fishers protected least significant difference test.
----Data were not collected.

Table 5-11. Aboveground percent green cover of two bermudagrass cultivars in nematode infested field plots for the one-year field study at Hague, FL.

Cultivar	Percent green cover (%)		
	Oct 2009	Apr 2010	Jul 2010
Celebration	54[a]	51	55
Tifway	34	60	17

[a]Data are means of five replications, and data within a column followed by no letter are not statistically different ($P \leq 0.05$).

Table 5-12. Nematode population density of *Belonolaimus longicaudatus* and *Helicotylenchus pseudorobustus* on two bermudagrass cultivars for the one-year field study at Hague, FL.

Cultivar	Apr 2009	Jul 2009	Oct 2009	Jan 2010	Apr 2010	Jul 2010
	Population density of *Belonolaimus longicaudatus*[a]					
Celebration	3[b]	10	17	74	93	10
Tifway	2	5	3	41	103	5
	Population density of *Helicotylenchus pseudorobustus*[a]					
Celebration	4	1	7	35	165	251
Tifway	5	12	3	18	7	3

[a]Numbers represent numbers of nematodes recovered from 100 cm^3 of soil.
[b]Data are means of five replications, and data within a column followed by no letter are not statistically different ($P \leq 0.05$).

Table 5-13. Aboveground percent green cover of seven seashore paspalum cultivars in nematode infested field plots for the one-year field study at Hague, FL.

Cultivar	Percent green cover (%)		
	Oct 2009	Apr 2010	Jul 2010
SeaSpray	59[a]	77	34
SeaIsle 1	52	70	41
Aloha	57	67	48
SeaIsle 2000	53	77	54
Salam	48	61	31
SeaDwarf	56	75	61
SeaIsle Supreme	56	62	53

[a]Data are means of five replications, and data within a column followed by no letter are not statistically different ($P \leq 0.05$).

Table 5-14. Nematode population density of *Belonolaimus longicaudatus* on seven seashore paspalum cultivars for the one-year field study at Hague, FL.

Cultivar	Nematode population density[a]					
	Apr 2009	Jul 2009	Oct 2009	Jan 2010	Apr 2010	Jul 2010
SeaSpray	10[b]	2	2	4	10	2
SeaIsle 1	1	2	1	16	9	2
Aloha	1	3	3	16	8	4
SeaIsle 2000	2	4	2	15	8	1
Salam	1	3	6	16	8	4
SeaDwarf	1	2	1	8	5	1
SeaIsle Supreme	2	5	6	30	10	5

[a]Numbers represent numbers of nematodes recovered from 100 cm^3 of soil.
[b]Data are means of five replications, and data within a column followed by no letter are not statistically different ($P \leq 0.05$).

Table 5-15. Nematode population density of *Helicotylenchus pseudorobustus* on seven seashore paspalum cultivars for the one-year field study at Hague, FL.

Cultivar	Nematode population density[a]					
	Apr 2009	Jul 2009	Oct 2009	Jan 2010	Apr 2010	Jul 2010
SeaSpray	10[b]	89	40	340	368	443
SeaIsle 1	16	59	79	279	346	362
Aloha	9	19	96	199	286	495
SeaIsle 2000	11	21	107	183	163	163
Salam	12	59	77	195	339	240
SeaDwarf	9	71	134	315	241	428
SeaIsle Supreme	5	17	122	339	179	324

[a]Numbers represent numbers of nematodes recovered from 100 cm^3 of soil.
[b]Data are means of five replications, and data within a column followed by no letter are not statistically different ($P \leq 0.05$).

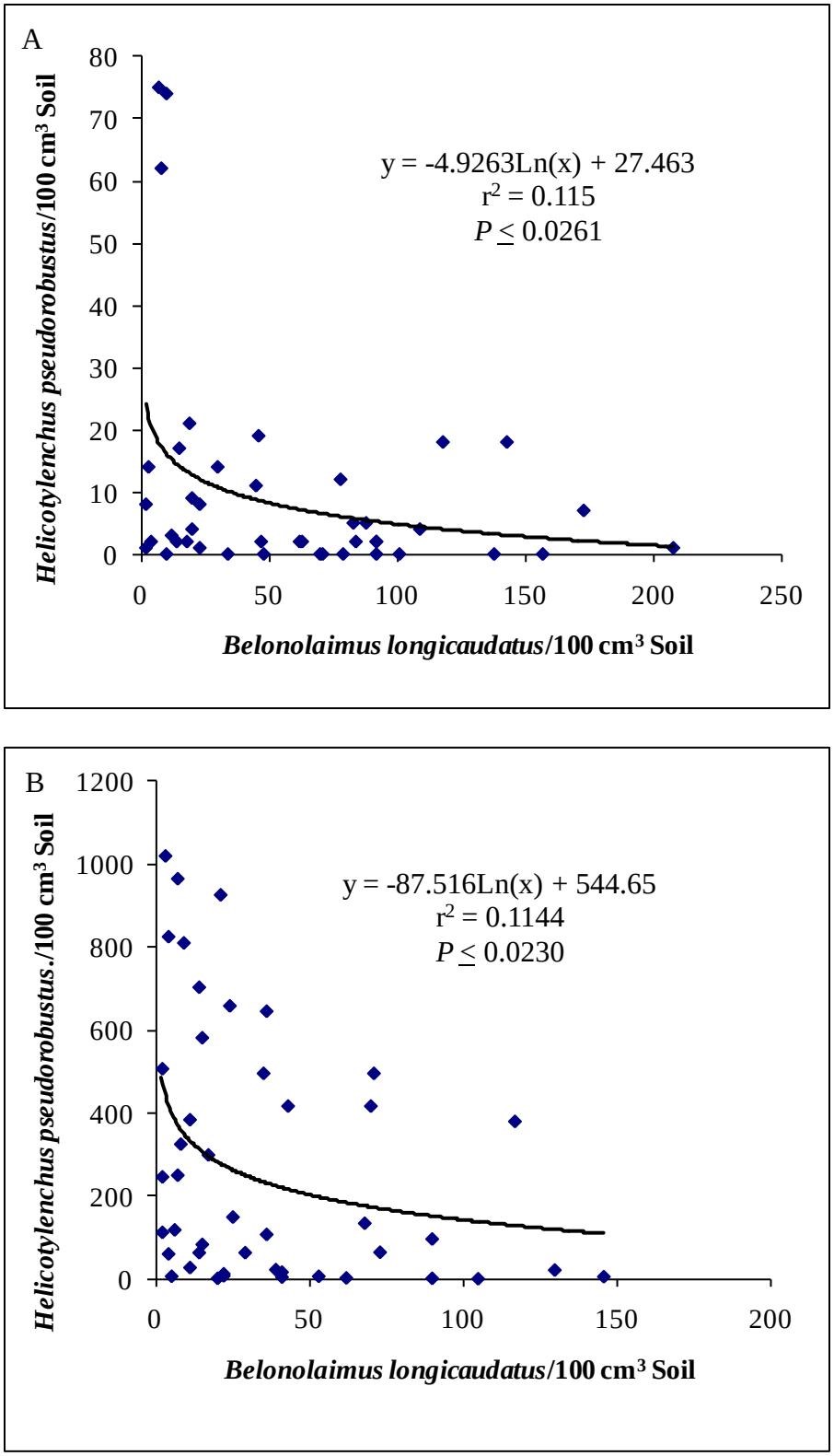

Figure 5-1. Regression relationship between population density of *Helicotylenchus pseudorobustus* and *Belonolaimus longicaudatus* in 'Celebration' and 'TifSport' bermudagrass for the two-year field study at Hague, Florida. A: 'Celebration', B: 'TifSport'.

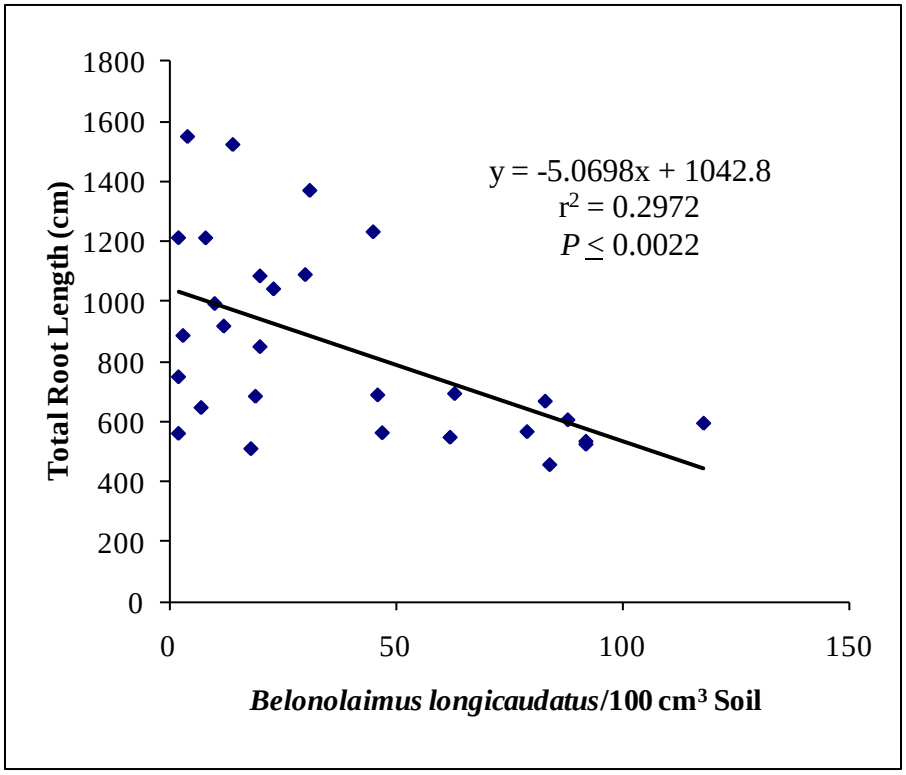

Figure 5-2. Regression relationship between the total root length of 'Celebration' bermudagrass and population density of *Belonolaimus longicaudatus* for the two-year field study at Hague, Florida.

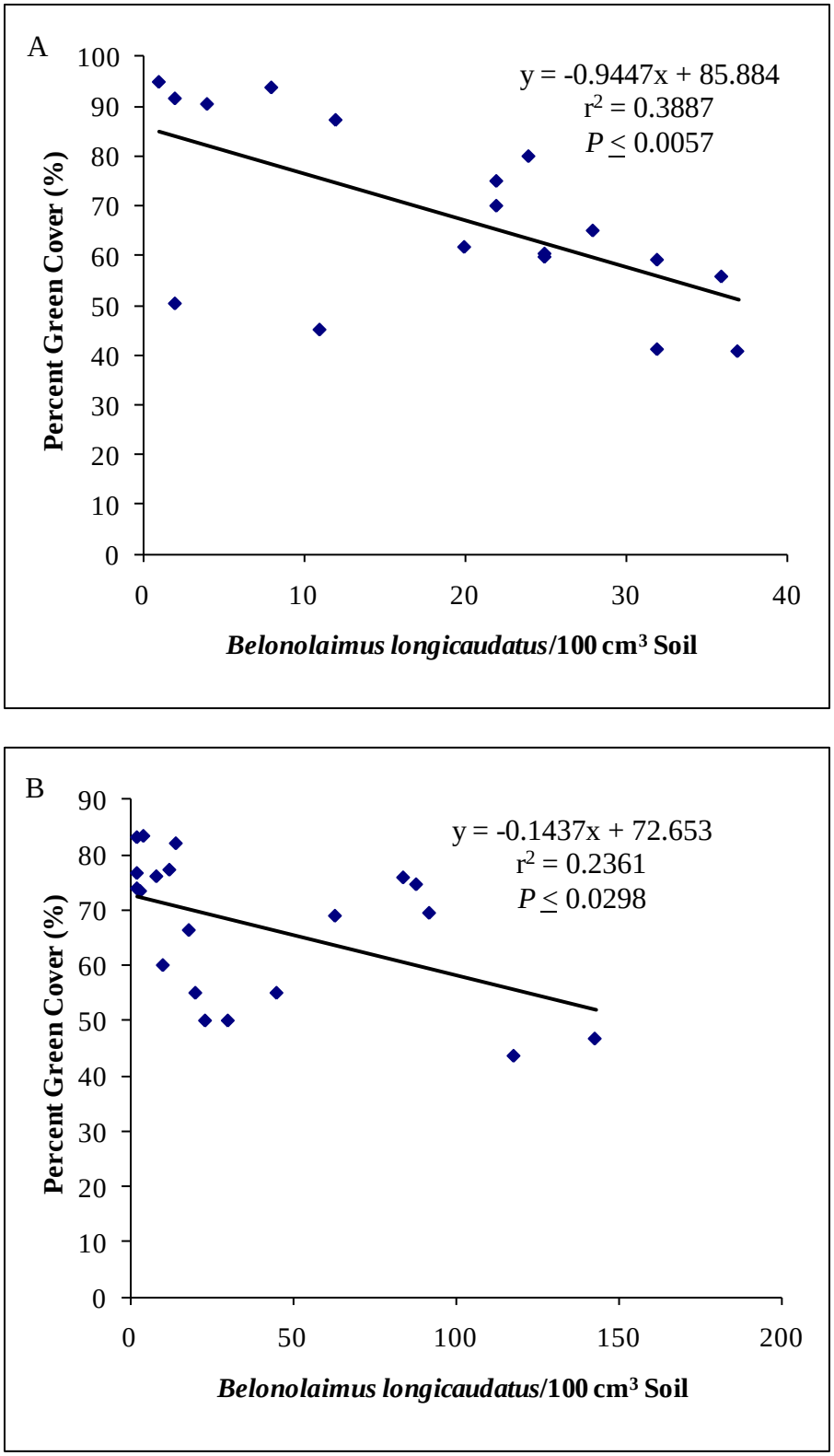

Figure 5-3. Regression relationship between the percent green cover in 'Aloha' seashore paspalum and 'Celebration' bermudagrass and the population densities of *Belonolaimus longicaudatus*, respectively for the two-year field study at Hague, Florida. A: 'Aloha', B: 'Celebration'.

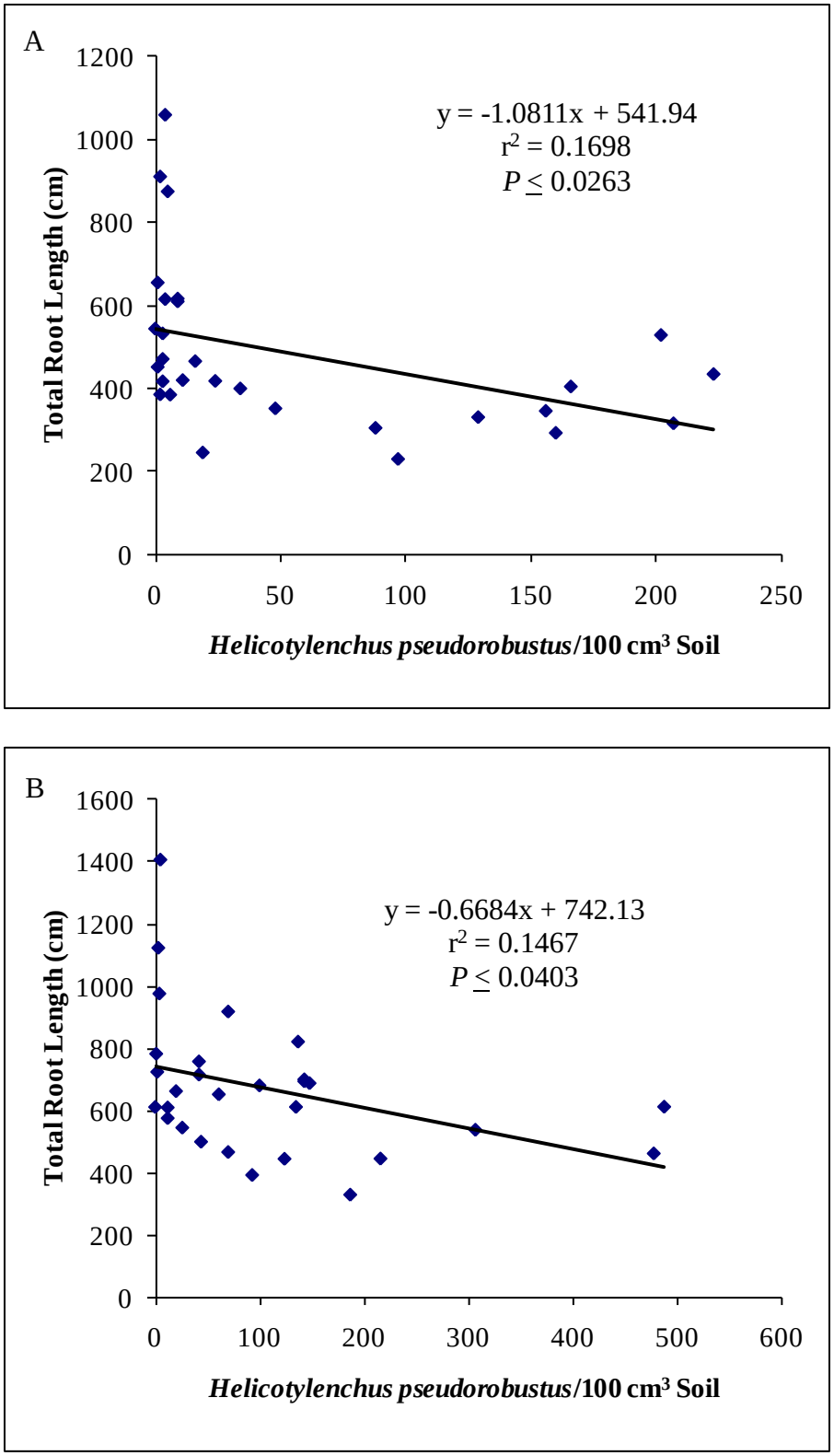

Figure 5-4. Regression relationship between the total root length of 'Floradwarf' and 'Tifgreen' bermudagrass and the population densities of *Helicotylenchus pseudorobustus* for the two-year field study at Hague, Florida. A: 'Floradwarf', B: 'Tifgreen'.

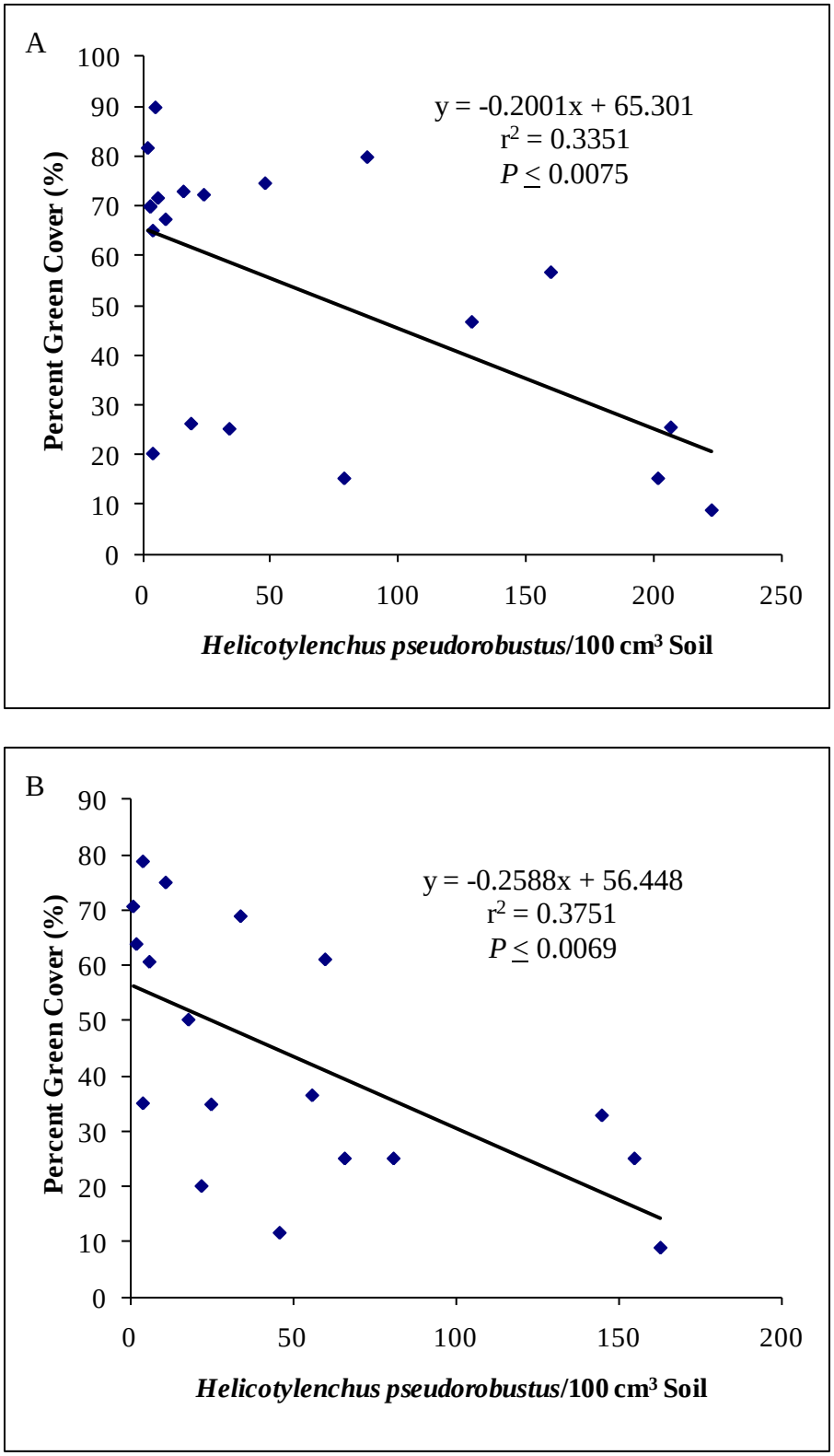

Figure 5-5. Regression relationship between percent green cover in 'Floradwarf' and 'TifEagle' bermudagrass and the population density of *Helicotylenchus pseudorobustus* for the two-year field study at Hague, Florida. A: 'Floradwarf', B: 'TifEagle'.

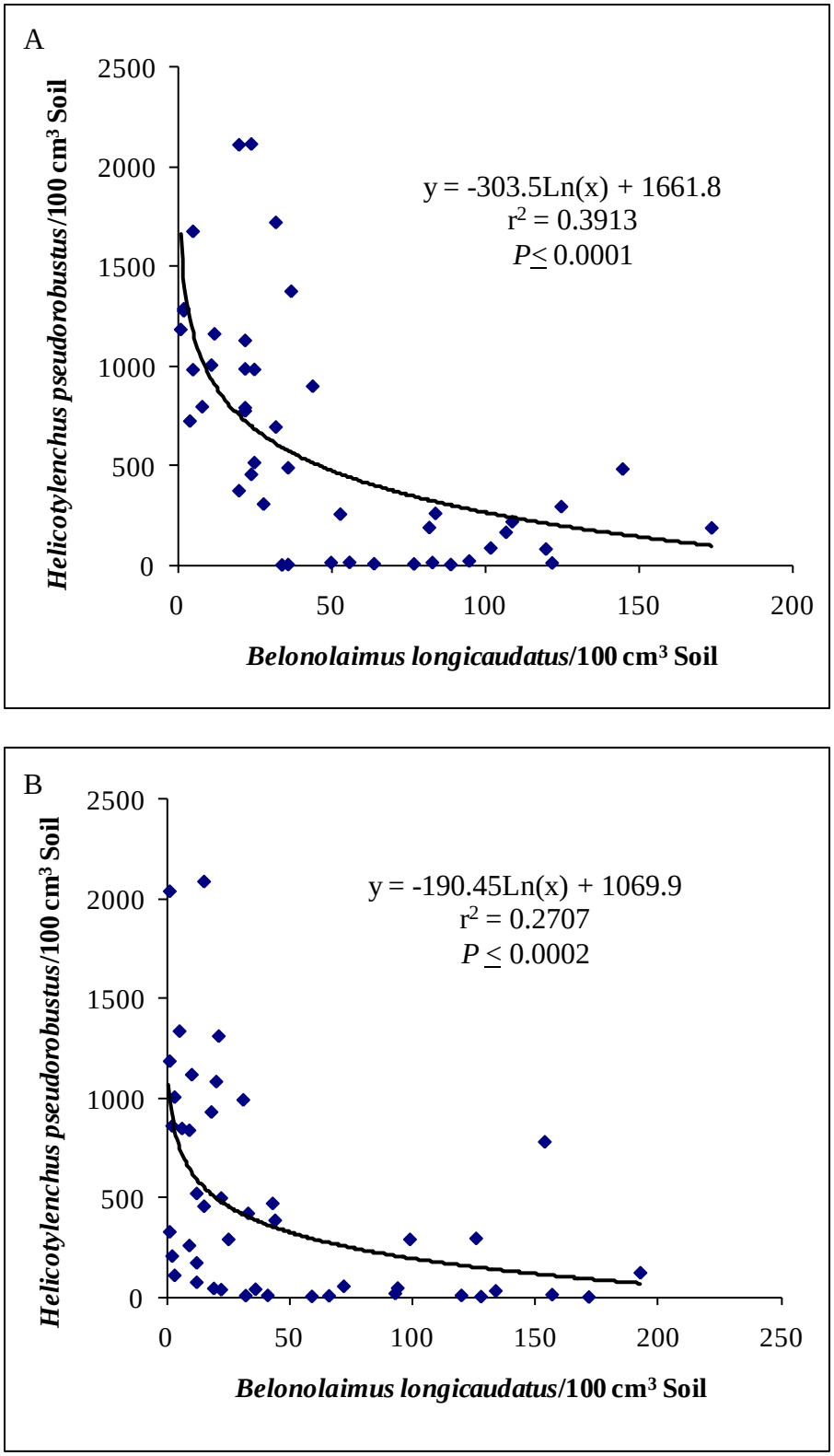

Figure 5-6. Regression relationship between population density of *Helicotylenchus pseudorobustus* and *Belonolaimus longicaudatus* in 'Aloha', 'SeaDwarf', and 'SeaIsle 1' seashore paspalum for the two-year field study at Hague, Florida. A: 'Aloha', B: 'SeaDwarf', C: 'SeaIsle 1'.

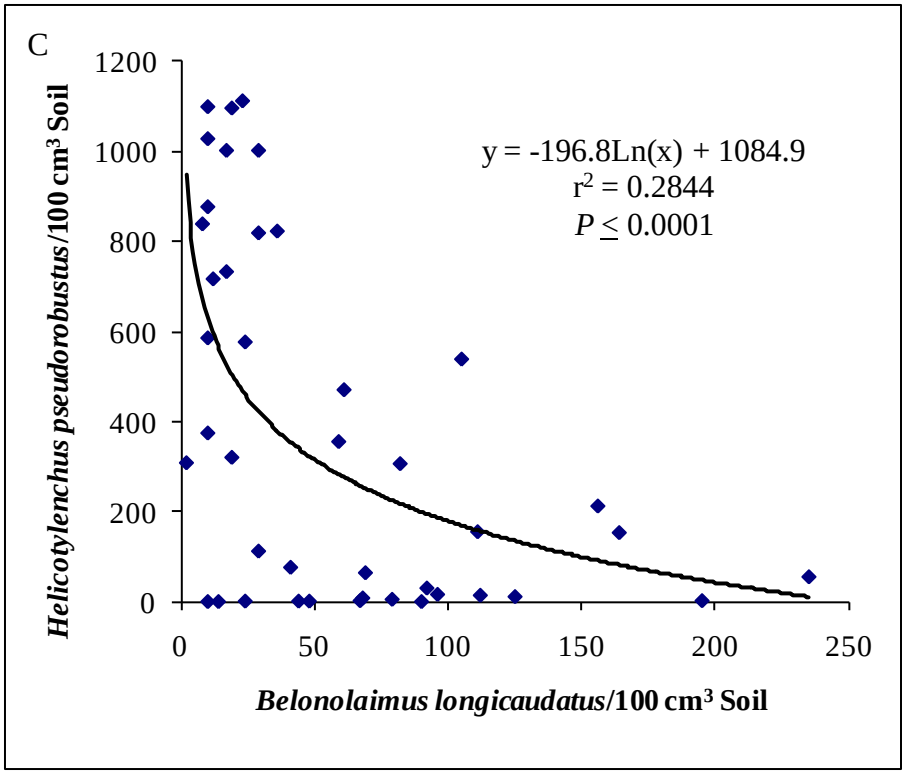

Figure 5-6. Continued.

CHAPTER 6
DNA CONTENT OF BERMUDAGRASS ACCESSIONS IN FLORIDA

Introduction

Bermudagrass (*Cynodon* spp.) is widely distributed in China, India, Africa, Australia, South America and the southern region of the United States (Abulaiti and Yang, 1998; Wu *et al*., 2006). In the United States, it is distributed throughout the warmer regions: from Florida northward to Maryland and New Jersey along the east coast, and westward along the southern border to California (Harlan *et al*., 1970; Abulaiti and Yang, 1998). In Florida, bermudagrass is one of the most widely used warm-season grasses. Improved fine-textured cultivars produce a vigorous and dense turf that are widely used on golf courses, sports fields, lawns and parks (Trenholm *et al*., 2003). There are nine species in the genus *Cynodon*, and the basic chromosome number is nine (Harlan and de Wet, 1969; Wu *et al*., 2006). Tetraploid *Cynodon dactylon* L. (Pers.) var *dactylon* (2n = 4x = 36), known as common bermudagrass, is characterized as having medium to coarse leaf blades, an aggressive growth rate and its wide adaptability to differing climates and soil conditions. *Cynodon transvaalensis* Burtt-Davy (2n = 2x = 18), African bermudagrass, is a diploid species (Forbes and Burton, 1963; Wu *et al*., 2006) described as having a fine leaf texture and overall poor color. It is not widely adapted and is only found in a small geographic region in the countries of South Africa and Lesotho. Common bermudagrass is used extensively as a turfgrass, while African bermudagrass has had only very limited turfgrass use as a stand-alone species. Triploid (2n = 3x = 27) hybrids (*C. dactylon* var. *dactylon* × *C. transvaalensis*) from these two species have become some of the most widely used turfgrasses in the world and are standards for use on golf courses where warm-season turfgrasses are utilized. Pentaploid (2n = 5x = 45) and hexaploid (2n = 6x = 54) plants have been previously reported (Johnston, 1975; Hanna *et al*., 1990; Burton *et al*., 1993; Wu *et al*., 2006; Kang *et al*., 2007).

‘Tifton 10’, released as a hexaploid cultivar, has been used on golf courses, athletic fields, and home lawns (Hanna *et al*., 1990).

Flow cytometry (FCM) was originally utilized for analyzing animal cells, and was subsequently adapted for analysis of plant cells (Galbraith, 1990). FCM has provided a rapid and accurate DNA content analysis and ploidy level determination for plant breeding programs (Dolezel *et al*., 1989; Arumuganathan and Earle, 1991; Schwartz *et al*., 2010b). FCM also has been used in plant cell cycles analysis (Galbraith *et al*., 1983) and sex identification for dioecious plants (Costich *et al*., 1991). FCM has been used in genome analysis for cool season grass species such as Kentucky bluegrass (*Poa pratensis* L.) (Barcaccia *et al*., 1997), fine fescue (*Festuca* spp.) (Huff and Palazzo, 1998), ryegrass (*Lolium* spp.) (Barker *et al*., 2001), and bentgrass (*Agrostis* spp.) (Bonos *et al*., 2002). Further, FCM has been used to determine genome sizes and ploidy levels for warm-season grass species such as buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.], *Paspalum* spp., *Zoysia* spp., and *Cynodon* spp. (Jarret *et al*., 1995; Taliaferro *et al*., 1997; Johnson *et al*., 1998; Vaio *et al*., 2007; Schwartz *et al*., 2010b). Arumuganathan *et al*. (1999) reported the nuclear genome sizes of diploid, triploid, and tetraploid bermudagrass genotypes. Triploid, tetraploid, pentaploid, and hexaploid genotypes were identified in Chinese and Korean bermudagrass species, respectively by Wu *et al*. (2006) and Kang *et al*. (2007).

Information is lacking regarding the DNA content or ploidy level of bermudagrass accessions collected in Florida. Bermudagrass is the most widely used species on golf courses in Florida (Trenholm *et al*., 2003). Existing cultivars are susceptible to several pests that result in the use of pesticides for maintenance of high quality turf required for golf courses and sports fields. Therefore, the screening and breeding of bermudagrass germplasm is warranted to develop pest resistant cultivars for use in Florida and lower latitudes. Prior to making crosses it is

essential to determine the ploidy level of identified superior performing lines that might be utilized in a breeding program. Ploidy level identification of Florida adapted germplasm would allow for the correct pairing of elite lines as parents to develop superior cultivars well adapted to Florida with improved levels of tolerance/resistance to common pest problems that currently require pesticide applications. The objective of this study was to determine the nuclear DNA content and ploidy level of selected superior (Florida adapted) UF bermudagrass germplasm accessions.

Materials and Methods

Plant Materials

Forty-seven *Cynodon* accessions selected for having superior turfgrass performance in Gainesville, FL and three commercial cultivars ('Tifway', 'Tifgreen', and 'Tifton 10') with known ploidy levels or nuclear DNA contents were included in this test (Table 6-1). A known diploid African bermudagrass accession was also included as a reference. Each genotype was vegetatively propagated into 15-cm-diam pots filled with 100% USGA specification greens sand (USGA, 1993) and grown at the University of Florida Envirotron greenhouse facility. The accessions were maintained at a temperature range of 24 to 34^{o}C under natural daylight, watered for six min a day by an overhead automatic irrigation system and fertilized once every other week using 24-8-16 (N-P_2O_5-K_2O) at a rate of 0.5 kg N/100 m^2 per growing month. Top growth was clipped once a week leaving aerial stolons growing over the edge of the pots to be used for FCM.

Flow Cytometry

Flow cytometry analyses were conducted in the forage evaluation support laboratory (FESL) at the University of Florida on a Partec PA, one-parameter flow cytometer (Partec GmbH, Otto-Hahn-Str. 32, D-48161 Munter, Germany) with a 100-watt HBO short arc lamp

emitting UV light at 420 nm to excite fluorescence. Nuclear DNA content was measured by procedures modified from Arumuganathan and Earle (1991). When at least 10 aerial stolons were present in each pot, flow cytometry analysis was initiated.

A CyStain® PI Absolute P (05-5002, Partec North America, Inc., Mt. Laurel, NJ) nuclei extraction and DNA staining buffer kit was used to prepare samples. Triploid trout erythrocyte nuclei (BioSure® Inc., Grass Valley, CA) with a nuclear DNA content of 7.2 pg/2C nucleus^{-1} (Hardie and Hebert, 2003; 2004) were used as an internal standard. Approximately 50 mg of fresh nodal or stolon tip tissue from each accession was chopped with a razor blade on a petri dish, into which 400 µL of nuclear extraction solution was added. After incubation for one minute, the solution was transferred into a 5-mL test tube through a 50-µm Partec CellTrics® monofil nylon filter. After adding 1.6 mL DNA staining solution and incubating for another 10 minutes at room temperature, five drops of triploid trout erythrocyte nuclei were added into the test tube and well mixed with the solution. The test tube with the solution was put into the flow cytometer and DNA content of each plant sample was measured based on at least 10,000 scanned nuclei per sample. For each accession, three replications were measured on three different days. Sample DNA content was calculated by the following formula: sample nuclear DNA content = [(mean position of sample peak) / (mean position of the control peak)] × DNA content of the control (Arumuganathan *et al*., 1999). The mean and standard deviation of the genome size were calculated for each genotype. The ploidy levels of the genotypes were then determined by the relative comparisons with genome size ranges of previously bermudagrass ploidy levels (Taliaferro *et al*., 1997; Arumuganathan *et al*., 1999; Wu *et al*., 2006; Kang *et al*., 2007).

Results and Discussion

Mean nuclear DNA contents and ploidy levels for the 47 bermudagrass accessions and three reference cultivars are presented in Table 6-1. Representative histograms of the flow

cytometry peaks are shown in Figures 6-1 to 6-7. The peaks of the trout erythrocyte nuclei were relatively smaller than those of plant samples, because there were fewer cells in the triploid trout erythrocyte nuclei than the plant samples, but this did not affect the results. Clear and consistent peaks were obtained for all genotypes, and the cells in the G2 phase were also observed. Nuclear DNA content of UF bermudagrass accessions ranged from 1.38 to 2.75 pg/2C nucleus^{-1} (Table 6-1), which was lower in value than those reported for Chinese and Korean accessions (Wu *et al*., 2006; Kang *et al*., 2007). Using previously reported ploidy levels associated with bermudagrass nuclear DNA content (Taliaferro *et al*., 1997; Arumuganathan *et al*., 1999; Wu *et al*., 2006; Kang *et al*., 2007), the ploidy levels of the Florida adapted bermudagrass accessions were determined. Ploidy levels and genome size ranges included: 19 (41%) triploid accessions with a genome size range of 1.38 to 1.61 pg/2C nucleus^{-1}, 24 (51%) tetraploid accessions with a genome size of 1.94 to 2.24 pg/2C nucleus^{-1}, one (2%) pentaploid accession with the genome size of 2.47 pg/2C nucleus^{-1}, and three (6%) hexaploid accessions with the genome size of 2.64 to 2.75 pg/2C nucleus^{-1}. The genome sizes of all accessions were inside the previously reported ploidy ranges (Table 6-1).

The nuclear DNA contents of cultivars Tifway, Tifgreen, and Tifton 10 in this study were very close to the values previously reported (Arumuganathan *et al*., 1999; Wu *et al*., 2006), which corroborated the accuracy of this assay. Compared with the Korean and Chinese accessions (Wu *et al*., 2006; Kang *et al*., 2007), a lower percentage of tetraploid genotypes were identified among these superior UF accessions. Relatively more triploid accessions (42%) were identified, which are likely mutants of commercial triploid cultivars. The 47 bermudagrass accessions evaluated were selected, based on their mult-year performance, from a larger germplasm collection of 180 accessions. The 180 accessions were collected from primarily

managed turf sites in the state of Florida and many from golf courses that were likely planted with a triploid bermudagrass cultivar. Because triploids are known for having superior turfgrass performance it is very probable that inadvertently collected triploids would have been selected as part of the group of 47 accessions that represent those genotypes with the best overall multi-year performance. If the entire collection of 180 genotypes had been evaluated it is that the percentage of triploids would have likely reduced and the percentage of tetraploids increased.

Plant leaves were used for nuclear extraction and staining, but the results were not as consistent as those of terminal nodes or stolon tips. The same problems were observed in *Zoysia* spp. (Schwartz *et al*., 2010b). Plant tissues under stress or with disease were also used; however, they yielded variable results. Therefore, healthy, non-stressed nodal plant tissue should be used for flow cytometry studies. Plants have been used as internal controls for grasses in previous FCM studies. Diploid barley (*Hordeum vulgare* L.), hexaploid wheat (*Triticum aestivum* L.), and tobacco (*Nicotiana tabacum* L.) were used to test nuclear DNA contents of 13 turfgrass species (Arumuganathan *et al*., 1999). Tetraploid 'Savannah' bermudagrass was used as an internal standard for bermudagrass in FCM studies because its nuclear content was similar to other genotypes tested (Kang *et al*., 2007). In this study, triploid Tifway, with known DNA nuclear content (Arumuganathan *et al*., 1999; Wu *et al*., 2006), was used as an internal control. However, clear and repeatable peaks were not obtained for all genotypes, especially for those with a nuclear DNA content similar to Tifway. Interactions could occur between plant samples and the control, which might counteract the peaks of the samples. Plant control with a DNA content overlapping those of the samples was not a good internal standard in this study. However, with a larger nuclear DNA content (7.2 pg/2C nucleus^{-1}), no interaction was found between trout erythrocyte nuclei and bermudagrass cells. Clear, consistent, and repeatable peaks were obtained

for all genotypes. Therefore, trout erythrocyte nuclei were a very good internal standard for bermudagrass FCM analysis. Other animal blood cells such as those of channel catfish (*Ictalurus punctatus* Rafinesque) also have been reported as good standard for bermudagrass nuclear DNA content measurement (Wu *et al*., 2006). Using this modified method, the CVs for the peaks of all genotypes were less than 6.0%. The standard deviations of nuclear DNA content ranged from 0.01 to 0.16 pg/2C nucleus^{-1}, which agreed with the previous studies by Arumuganathan *et al*. (1999), Wu *et al*. (2006) and Kang *et al*. (2007), in that flow cytometry was a very precise method for bermudagrass genome size measurement.

All tetraploid and hexaploid accessions could be used to further the University of Florida bermudagrass breeding program. Superior collected triploid accessions should be compared with current commercial triploid standards for biotic and abiotic stress responses. A triploid with improved pest responses could be considered for release with no further breeding required.

Table 6-1. Nuclear DNA content and ploidy level of 47 University of Florida bermudagrass accessions and three commercial cultivars.

Accession	DNA content mean ± SD (pg/2C)	Inferred ploidy (2n)	Accession	DNA content mean ± SD (pg/2C)	Inferred ploidy (2n)
102	1.61 ± 0.06[a]	3x	355	2.24 ± 0.12	4x
131	2.02 ± 0.12	4x	445	2.05 ± 0.05	4x
132	2.20 ± 0.12	4x	481	1.94 ± 0.08	4x
157	2.00 ± 0.03	4x	489	1.38 ± 0.16	3x
171	2.09 ± 0.09	4x	490	1.50 ± 0.15	3x
173	1.45 ± 0.08	3x	525	1.54 ± 0.07	3x
227	2.20 ± 0.14	4x	528	2.08 ± 0.16	4x
282	1.49 ± 0.06	3x	PI 289922	2.47 ± 0.07	5x
283	1.54 ± 0.08	3x	PI 290868	2.08 ± 0.06	4x
285	1.48 ± 0.09	3x	PI 290872	2.00 ± 0.03	4x
286	1.49 ± 0.08	3x	PI 290895	1.51 ± 0.02	3x
291	1.47 ± 0.08	3x	PI 291590	1.94 ± 0.09	4x
293	1.52 ± 0.08	3x	UFC03	2.08 ± 0.15	4x
295	2.70 ± 0.01	6x	UFC06	2.19 ± 0.06	4x
296	1.48 ± 0.11	3x	UFC07	2.75 ± 0.09	6x
297	1.63 ± 0.03	3x	UFC11	2.16 ± 0.06	4x
299	1.98 ± 0.05	4x	UFC12	2.06 ± 0.01	4x
301	1.98 ± 0.10	4x	UFC25	1.59 ± 0.05	3x
304	2.16 ± 0.03	4x	UFC26	1.56 ± 0.03	3x
319	1.44 ± 0.02	3x	UFC29	2.00 ± 0.11	4x
320	1.60 ± 0.10	3x	UFC30	1.97 ± 0.06	4x
334	2.03 ± 0.02	4x	Tifway	1.53 ± 0.05	3x
343	2.10 ± 0.06	4x	Tifgreen	1.58 ± 0.05	3x
344	2.64 ± 0.06	6x	Tifton 10	3.06 ± 0.01	6x
347	2.06 ± 0.09	4x	AB[b]	1.17 ± 0.07	2x
352	1.50 ± 0.01	3x			

[a]Means and standard deviations of three replications.
[b]AB is a African bermudagrass accession.

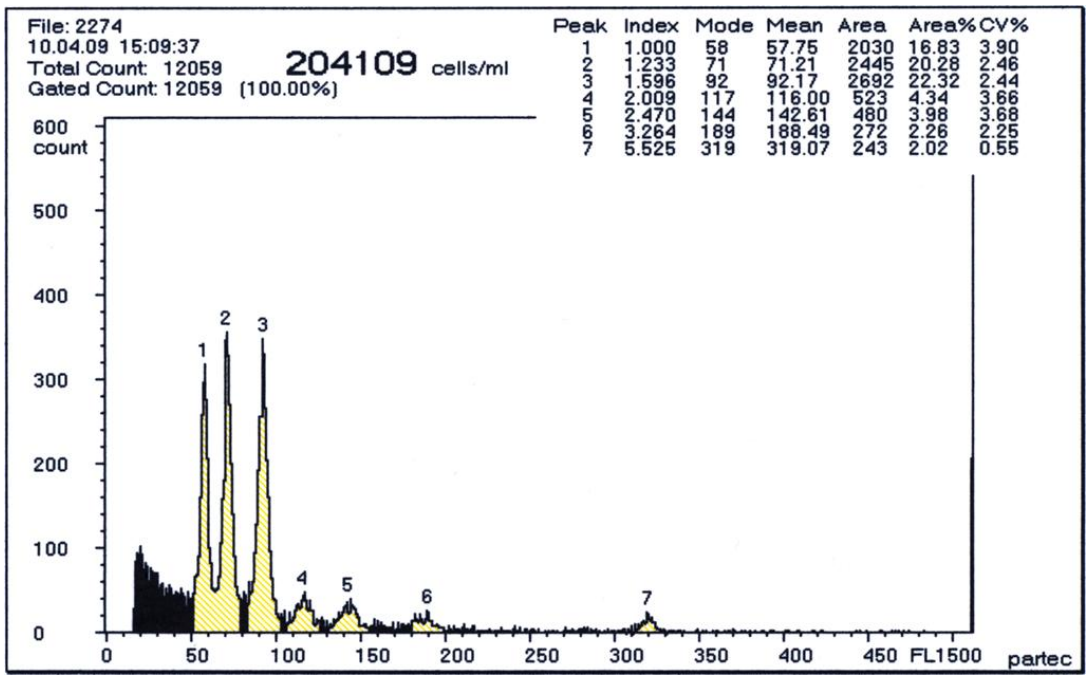

Figure 6-1. Flow cytometric histogram of diploid, triploid, and tetraploid bermudagrass and trout erythrocyte nuclei. Peak 1 = diploid accession, Peak 2 = triploid cultivar (Tifgreen) control, Peak 3 = tetraploid accession, Peak 4 = G2 phase of diploid accession, Peak 5 = G2 phase of triploid cultivar control, Peak 6 = G2 phase of tetraploid accession, Peak 7 = trout erythrocyte nuclei (control).

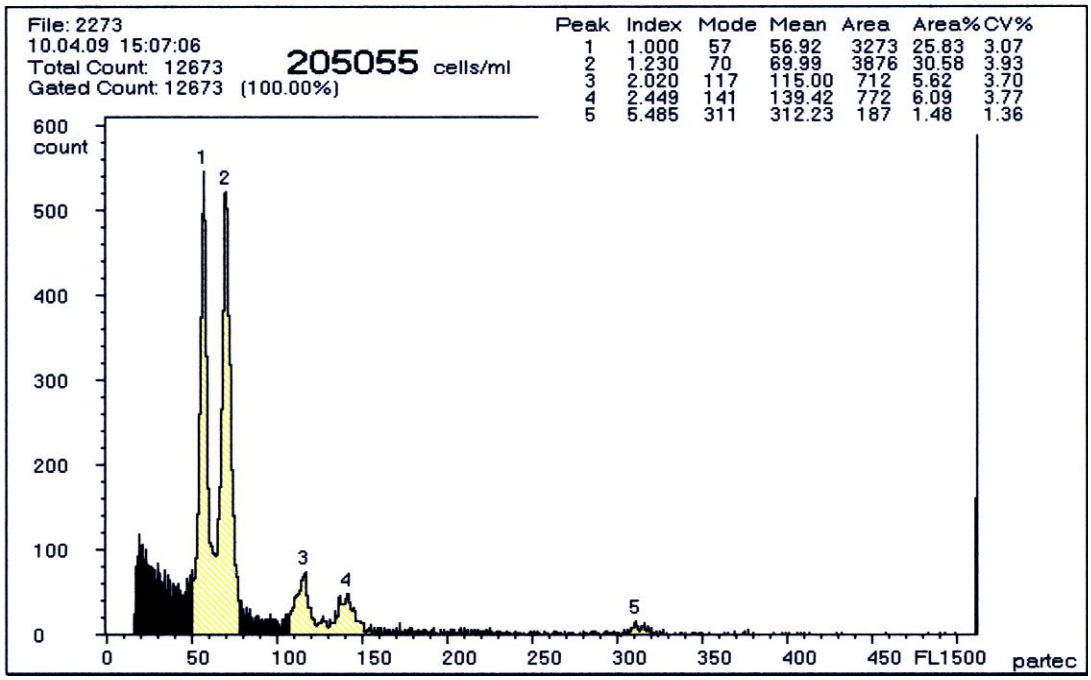

Figure 6-2. Flow cytometric histogram of diploid and triploid bermudagrass and trout erythrocyte nuclei. Peak 1 = diploid accession, Peak 2 = triploid cultivar (Tifgreen) control, Peak 3 = G2 phase of diploid accession, Peak 4 = G2 phase of triploid cultivar control, Peak 5 = trout erythrocyte nuclei (control).

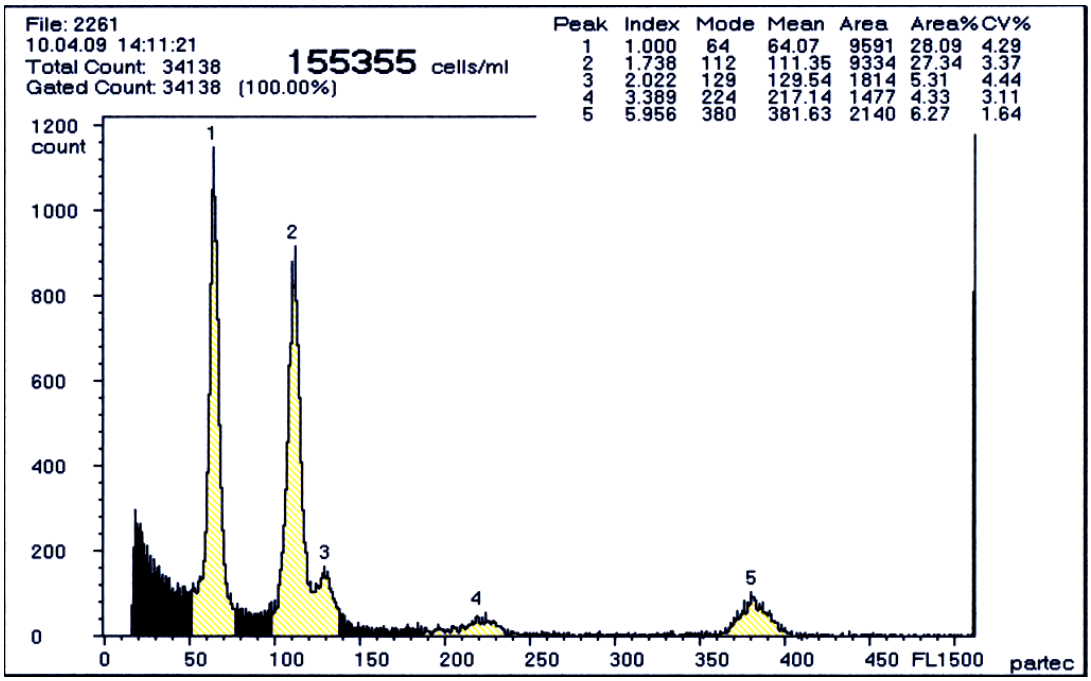

Figure 6-3. Flow cytometric histogram of diploid and tetraploid bermudagrass and trout erythrocyte nuclei. Peak 1 = diploid accession, Peak 2 = tetraploid accession, Peak 3 = G2 phase of diploid accession, Peak 4 = G2 phase of tetraploid accession, Peak 5 = trout erythrocyte nuclei (control).

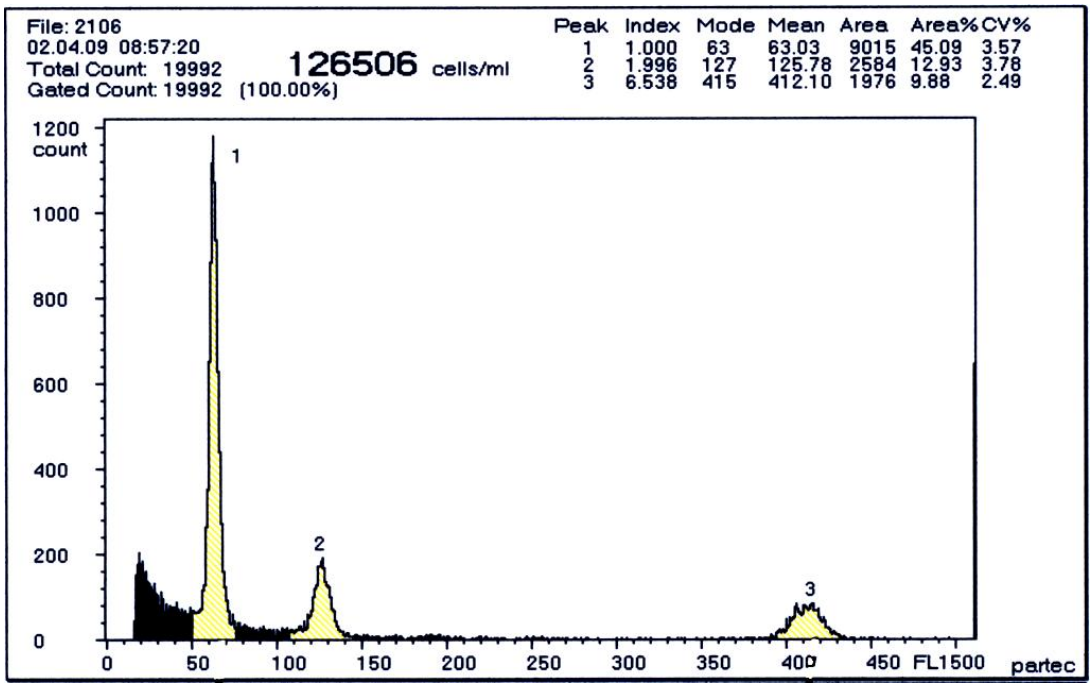

Figure 6-4. Flow cytometric histogram of diploid bermudagrass accession and trout erythrocyte nuclei. Peak 1 = diploid accession, Peak 2 = G2 phase of diploid accession, Peak 3 = trout erythrocyte nuclei (control).

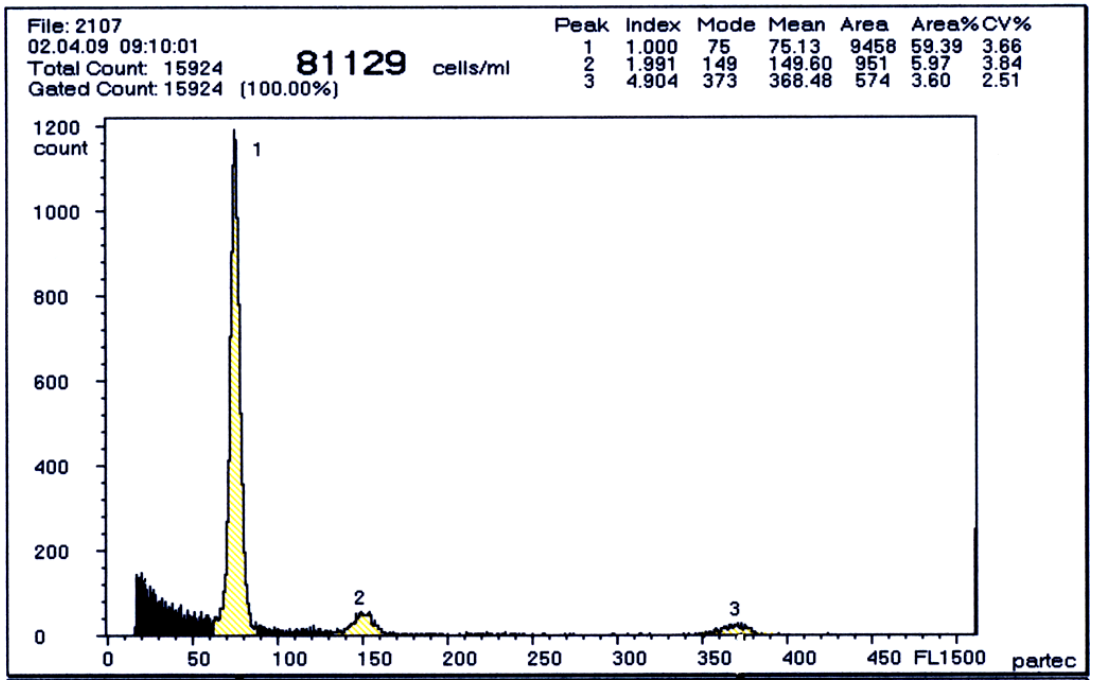

Figure 6-5. Flow cytometric histogram of triploid bermudagrass cultivar control and trout erythrocyte nuclei. Peak 1 = triploid cultivar (Tifway) control, Peak 2 = G2 phase of triploid cultivar control, Peak 3 = trout erythrocyte nuclei (control).

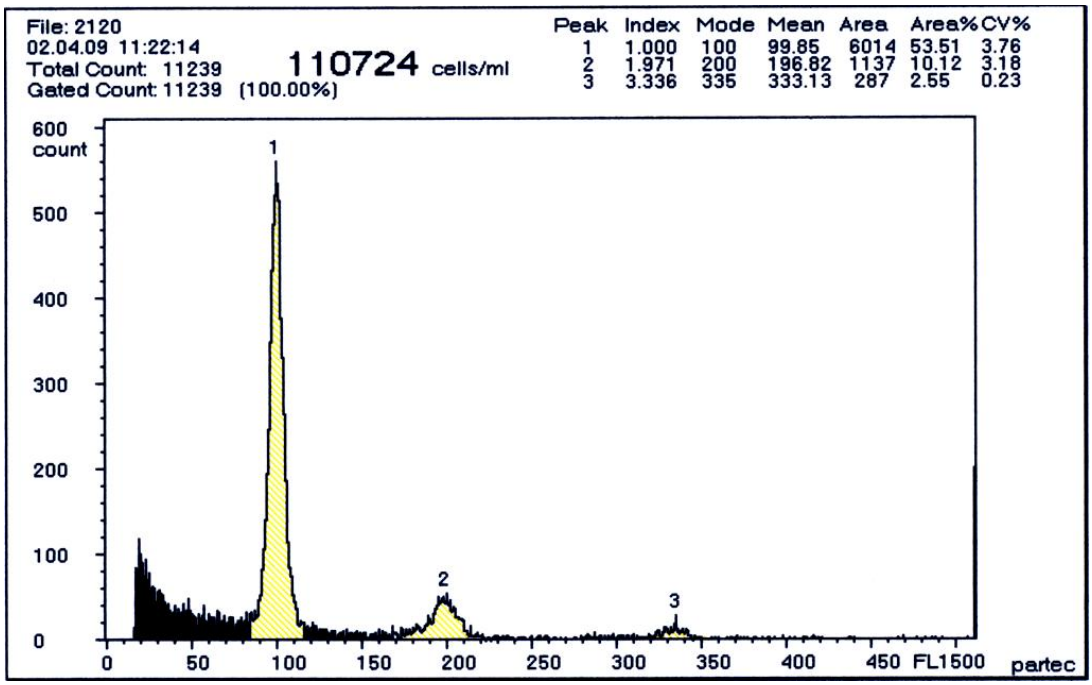

Figure 6-6. Flow cytometric histogram of tetraploid bermudagrass and trout erythrocyte nuclei. Peak 1 = tetraploid accession, Peak 2 = G2 phase of tetraploid accession, Peak 3 = trout erythrocyte nuclei (control).

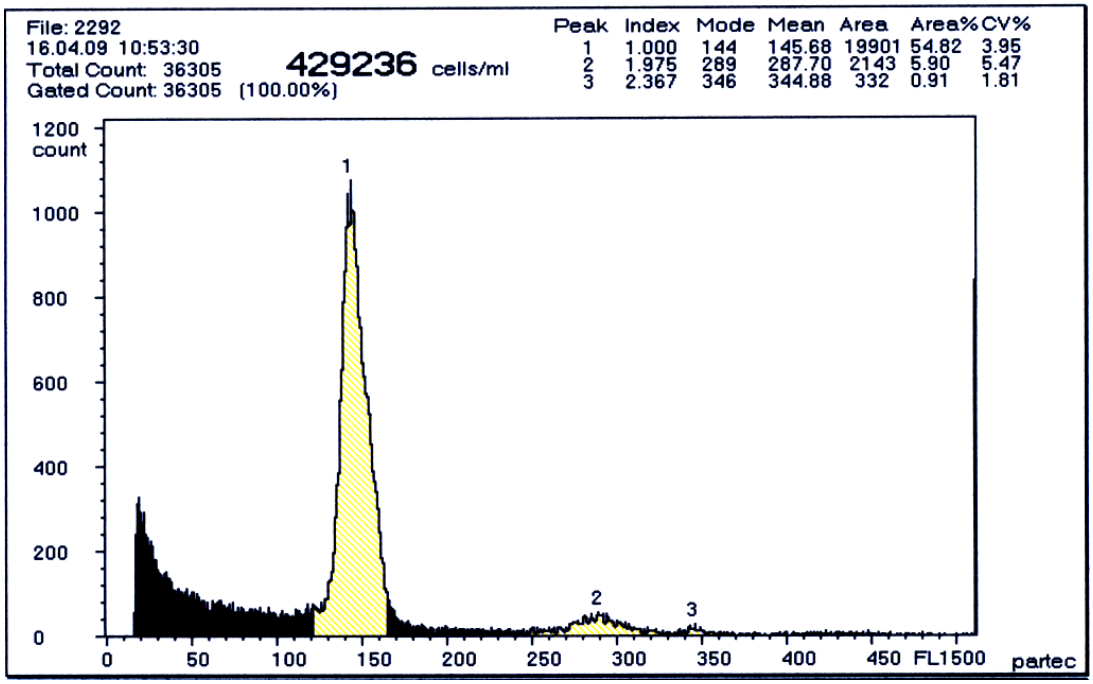

Figure 6-7. Flow cytometric histogram of hexaploid bermudagrass cultivar control and trout erythrocyte nuclei. Peak 1 = hexaploid cultivar (Tifton 10) control, Peak 2 = G2 phase of hexaploid cultivar control, Peak 3 = trout erythrocyte nuclei (control).

CHAPTER 7
SCREENING BERMUDAGRASS GERMPLASM ACCESSIONS FOR RESPONES TO STING NEMATODES

Introduction

Bermudagrass (*Cynodon* spp.) is the predominant turfgrass used in the southern United States and other warm regions in the world. A limitation for the utilization of bermudagrass in the southeastern United States is the sting nematode (*Belonolaimus longicaudatus* Rau), which is frequently found in sandy coastal soils. It has been considered the most damaging plant-parasitic nematode on bermudagrass in Florida (Crow, 2005a; Luc *et al*., 2007). Sting nematode causes damage to greens, fairways, and rough areas on golf courses (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. Utilization 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 nematode tolerance and resistance of seven commercial bermudagrass cultivars and 30 germplasm accessions. They found that 26 accessions showed a significant reduction in root dry weights compared with the uninoculated controls and that 25 of them supported the reproduction of *B. longicaudatus*. Currently, there are few known sting nematode-resistant or -tolerant bermudagrass genotypes available. Except for ‘TifEagle’, all bermudagrass cultivars used on greens are related to each other (K. E. Kenworthy, personal communication); 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. *dactylo*n × *C. transvaalensis* Burtt-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 due, 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 has through multi-year evaluations identified superior, Florida adapted, experimental accessions of common and African bermudagrass to utilize in crosses to develop new sterile bermudagrass hybrids for use on golf courses (K. E. Kenworthy, personal communication). 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 range of damage caused by sting nematodes on superior UF accessions of common and African bermudagrass, and to select germplasm accessions with superior nematode 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 tested are listed in Table 7-1.

Inoculum Preparation

Belonolaimus longicaudatus was maintained on 'FX-313' St. Augustinegrass (*Stenotaphrum secundatum* Kuntze) grown in clay pots filled with pure sand under greenhouse conditions (Giblin-Davis *et al*., 1992a). Nematodes were extracted from soil by using Cobb's decanting and sieving technique (Cobb, 1918; Flegg, 1967). All stages of nematodes including the juveniles and adults were collected. The nematode suspensions were concentrated using a 25-µm (500-mesh) sieve. The average number of juveniles and adults were counted from five 1-ml aliquots and extrapolated to the total volume of the suspension. Suspensions were kept in a refrigerator until used.

Nematode Responses of Germplasm Accessions

Nematode free aerial stolons of each cultivar/accession was vegetatively propagated into (3.8 cm diameter × 21 cm deep, volume = 150 cm^3) UV stabilized Ray Leach "Cone-tainers"™ (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 two weeks to allow the sprigs to establish. From the third week, the irrigation was reduced to once a day in the morning for six minutes, and three minutes a day from the beginning of the fifth week. Six weeks after establishment, grass was inoculated with no nematodes or 50 *B. longicaudatus* per conetainer. Before inoculation, suspensions of *B. longicaudatus* were taken out of the refrigerator, concentrated to 10 nematodes/ml, and set at room temperature for three hours. None or a total of 5 ml of the suspensions were inoculated into two 3-cm deep holes made 1 cm from the base of the grass near the root zone in 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 set under a temperature range of 24 to 34^{o}C with natural daylight in a greenhouse at the Envirotron at the University of Florida, Gainesville, FL. Grasses were fertilized once a week using 24-8-16 (N-P_2O_5-K_2O) at a rate of 0.5 kg N/100 m^2 per growing month. Turfgrass was mowed once a week at a mowing height of 2.5 cm.

Experiments were harvested 90 days 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. Finer roots were separated from soil and collected into the plastic tube by submerging and shaking the 853-µm sieve in tap 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. Percent reduction in the root length of inoculated plants compared with the non-inoculated control was calculated by the following formula: [(root length of non-inoculated control – root length of inoculated plant) / root length of non-inoculated control] * 100.

Nematodes were extracted from the whole soil in the conetainer by using the modified centrifugal flotation technique (Jenkins, 1964). The final nematode population densities (Pf) were counted under a microscope. The reproductive factor (Rf), which is an indicator of resistance (Oostenbrink, 1966), was calculated by the following formula: Rf = final nematode density (Pf) / initial inoculum density (Pi), where Pi = 50 nematodes per conetainer.

Data Analysis

Data from the two trials were analyzed separately. Total root lengths of the inoculated treatment and the non-inoculated control of each cultivar were compared by a linear contrast with the P values at $P \leq 0.1$. Tolerance was determined by the difference in root length between the two treatments. A turfgrass cultivar/accession was tolerant if there was no difference in total root length between the two treatments; otherwise, the cultivar/accession was intolerant. Resistance was determined by the nematode reproductive factor (Rf) at harvest. A cultivar/accession was considered resistant if Rf $\leq$ 1 and susceptible if Rf > 1. Final nematode population densities were subjected to analysis of variance (ANOVA), and the differences among cultivar/accessions were compared by the Fishers protected least significant difference test at $P \leq 0.05$. Statistical analysis was conducted by using the SAS program (SAS Institute, Cary, NC).

Results

In trial one, differences in root length reductions caused by sting nematodes were observed among accessions (Table 7-2). Among the accessions, the root lengths varied from 689 to 1625 cm for the uninoculated controls, and 423 to 1453 cm for the inoculated treatments. The root length reductions of the inoculated treatment were ranged from 1 to 55%. Minimum reductions (<15%) in root length were observed for 17 accessions (Table 7-2). For these 17 accessions, there was no difference in root length between the two treatments; therefore, these accessions were labeled as tolerant to *B. longicaudatus* in trial one. Cultivars Celebration, TifGrand, Tifway and 17 accessions had a root length reduction of 16 to 30% (Table 7-2). Among them, Celebration and nine accessions had significant reductions in root length by *B. longicaudatus*, which was an indication of susceptible response. Reductions in the range of 30 to 40% were found for TifSport and eight bermudagrass accessions, and only ‘131’, ‘334’, ‘PI 291590’, and

‘AB42’ were intolerant to *B. longicaudatus* infection (Table 7-2). The rest of the tested accessions AB4, UFC12, UFC25, and UFC29 all had a root length reduction of more than 40%, and were considered intolerant to *B. longicaudatus* (Table 7-2).

Results were not consistent between the two trials for all accessions. Roots were relatively longer in trial two than trial one (Table 7-2). In trial two, the total root length of the non-inoculated controls and inoculated treatments varied from 920 to 2358 cm, and 426 to 1817 cm, respectively (Table 7-2). Compared with the uninoculated treatments, the root length reductions ranged from 2 to 55%. In trial two, less than 15% reduction in root length occurred in 13 bermudagrass accessions, all of which were considered tolerant to *B. longicaudatus* (Table 7-2). The accessions ‘PI 289922’, ‘PI 209872’, ‘PI 290895’, and ‘UFC30’ all had a root length reduction of more than 40%, and were classified as susceptible to *B. longicaudatus* (Table 7-2). Reductions in the range of 30 to 40% were found for TifGrand and eight accessions, and all of them were susceptible to *B. longicaudatus* infection (Table 7-2). The rest of the accessions had moderate damage of 16 to 30% reductions in root length, which was significant for cultivars Celebration and TifEagle and nine accessions (Table 7-2).

Accessions that were consistently tolerant to *B. longicaudatus* in both tests were considered useful for future breeding. These accessions were: ‘132’, ‘171’, ‘173’, ‘295’, ‘296’, ‘343’, ‘355’, ‘PI 290868’, ‘AB1’, ‘AB3’, ‘AB33’, ‘AB37’, ‘UFC11’ and ‘UFC26’.

Accessions also showed differences in host status to *B. longicaudatus* (Table 7-3). Nematode population densities increased in Celebration, TifGrand, ‘157’, ‘445’, ‘AB21’, ‘AB39’ in trial one, with the maximum increase of 2.3 fold in ‘AB39’. In trial two, TifGrand, Tifway, ‘157’, ‘347’, 355, ‘445’, ‘481’, ‘AB21’, AB3, AB42, and ‘UFC03’ supported the reproduction of *B. longicaudatus*. The highest Rf of 2.4 was in 355. We considered accessions

that supported the reproduction of *B. longicaudatus* even in one trial as susceptible accessions, and those that suppressed its reproduction in both trials as resistant accessions. Based on both trials, all accessions listed above were susceptible, while the rest were considered resistant to *B. longicaudatus*.

Based on both trials, accessions that were both tolerant and resistant to *B. longicaudatus* included: 132, 171, 173, 295, 296, 343, PI 290868, UFC11 UFC26, AB1, AB33, and AB37. Among them, 132, 171, 173, 343, PI 290868, and UFC11 are common bermudagrass accessions (4x), 296 and UFC26 are triploid accessions (3x), 295 is a hexaploid bermudagrass (6x), and AB1, AB33, as well as AB37 were African bermudagrass accessions (2x). Resistance and tolerance to *B. longicaudatus* were identified in accessions with diverse genetic backgrounds (diploid, triploid, tetraploid, and hexaploid), which should provide valuable material for future cultivar breeding to increase the variability of the cultivars.

Discussion

This study was consistent with the previous cultivar tests under greenhouse conditions (Chapter 3) in that Celebration and TifEagle were consistently intolerant to *B. longicaudatus* infection in both trials. This study agreed with the previous greenhouse studies (Chapter 3) in that TifEagle did not support the reproduction of *B. longicaudatus*. In the previous greenhouse study (Chapter 3), we found that TifSport was consistently tolerant to *B. longicaudatus* damage, and the same results were obtained in this study. However, one of the trials in the previous studies (Chapter 3) showed that Tifway suffered significant root length reductions, but in this study, no significant damage in roots was observed in either trial. Generally, these four cultivars performed consistently during these trials, and they should be useful as good standards for bermudagrass germplasm screening.

TifGrand is a newly released triploid bermudagrass cultivar with good shade tolerance (equal to Celebration). It was reported that this cultivar could tolerate up to high shade levels of 60% to 70% (USGA, 2009) and it has excellent mole cricket non-preference resistance (USGA, 2009). However, this cultivar did not have good nematode resistance or tolerance as shown in this study. In both trials, TifGrand supported reproduction of *B. longicaudatus* and had significant reductions in root length in one trial. Therefore, this cultivar was considered as both susceptible and intolerant to *B. longicaudatus* damage. The results of this study should not be considered definitive since 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 susceptibility to *B. longicaudatus* and that the selection of genotypes with improved responses to commercial cultivars is possible. This study showed that sting nematodes did not reduce the total root length in four African bermudagrass accessions and seven common bermudagrass accessions ($P > 0.1$). These genotypes showed a tolerant response to sting nematode damage, which could be useful for future cultivar breeding for nematode tolerance. On the other hand, several genotypes did not support the reproduction of sting nematodes (Rf <1), a good indicator of resistance. Eight African bermudagrass accessions and 22 common bermudagrass accessions were considered resistant to *B. longicaudatus*, which might be potentially useful for future cultivar breeding for nematode resistance. Accessions that are both resistant and tolerant to *B. longicaudatus* were identified with variable ploidy levels. This is the first reporting 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 the future improvement of bermudagrass and serve to reduce the dependence on nematicides.

Table 7-1. Mean total root length of five cultivars and 46 germplasm accessions of bermudagrass 90 days after inoculation with *Belonolaimus longicaudatus* in two experiment trials. U = uninoculated and I = inoculated with 50 *B. longicaudatus*/conetainer.

		Total Root Length (cm)					
		Trial 1			Trial 2		
Genotype	Ploidy (2n)	U	I	% reduction	U	I	% reduction
Celebration	3x	1535*	1143	23 abcd[ab]	2358[#]	1810	22 abcd
TifGrand	3x	1561	1197	17 abcd	1635*	1046	33 abcd
TifEagle	3x	1219[#]	670	31 abcd	1641[#]	1007	24 bcd
TifSport	3x	1311	901	37 abcd	1614	1354	16 abcd
Tifway	3x	1101	776	27 abcd	1624	1221	26 abcd
131	4x	1146*	716	36 abcd	1906	1683	8 d
132	4x	1367	1133	16 bcd	2042	1817	12 bcd
157	4x	1405	1268	8 cd	2115[#]	1649	21 abcd
171	4x	1198	1086	6 cd	1650	1546	6 d
173	3x	1126	991	6 cd	1572	1444	8 d
227	4x	1258	1070	15 d	1739*	1145	32 abcd
295	6x	1322	1026	22 abcd	1594	1525	2 d
296	3x	1062	805	32 abcd	1482	1187	20 abcd
299	4x	912	782	10 bcd	1862*	1028	37 abcd
301	4x	689[#]	463	27 abcd	1168	1042	7 d
304	4x	1049[#]	651	28 abcd	1832*	1237	31 abcd
334	4x	1435*	950	32 abcd	1640	1369	17 abcd
343	4x	1255	993	6 cd	1486	1403	11 bcd
344	6x	792	587	22 abcd	1339*	927	28 abcd
347	4x	1292	839	21 abcd	1952[#]	1305	30 abcd
355	4x	1443	1167	33 abcd	1823	1247	26 abcd
445	4x	1263[#]	946	22 abcd	2175*	1560	28 abcd
481	4x	1577*	1064	29 abcd	2078	1664	17 abcd
525	3x	777	745	3 d	1787[#]	1307	27 abcd
528	4x	1392[#]	1142	17 bcd	1483*	930	23 abcd
PI 289922	5x	650	528	17 abcd	1406*	618	55 a
PI 290868	4x	757	706	18 abcd	920	717	18 abcd
PI 290872	4x	872	747	6 cd	1336*	643	49 abc
PI 290895	3x	798	691	35 abcd	1210[#]	426	43 abcd
PI 291590	4x	1599*	1061	33 abcd	2182[#]	1701	22 abcd
AB1	2x	1124	910	31 abcd	1580	1167	14 abcd
AB2	2x	1043[#]	776	24 abcd	1008	927	10 cd
AB21	2x	1605	1453	7 cd	2220*	1325	39 abcd
AB3	2x	1563	1172	23 abcd	1844	1714	6 d
AB33	2x	1106	1063	2 d	1880	1607	16 abcd
AB37	2x	1327	1184	10 bcd	1550	1501	13 bcd
AB38	2x	1051	751	24 abcd	1420[#]	1035	22 abcd
AB39	2x	1625	1434	6 cd	2014*	1312	34 abcd
AB4	2x	1034*	578	42 abc	1231	1029	18 abcd

Table 7-1. Continued.

		Total Root Length (cm)					
		Trial 1			Trial 2		
Genotype	Ploidy (2n)	U	I	% reduction	U	I	% reduction
AB42	2x	1553*	1052	31 abcd	2506*	1561	37 abcd
AB43	2x	1126[#]	744	27 abcd	1353	1347	26 abcd
AB7	2x	1464*	1040	27 abcd	1789	1465	16 abcd
UFC03	4x	1010[#]	730	28 abcd	1232	801	25 abcd
UFC06	4x	1279	1126	15 bcd	1867*	1306	29 abcd
UFC07	6x	1277	1201	2 d	1450[#]	1091	25 abcd
UFC11	4x	841	669	8 cd	1093	780	30 abcd
UFC12	4x	1001*	423	55 a	1115*	725	31 abcd
UFC25	3x	1104*	579	47 ab	1306	1143	2 d
UFC26	3x	863	781	13 bcd	1214	1080	5 d
UFC29	4x	1023*	539	43 abc	1428*	870	37 abcd
UFC30	4x	939	821	11 bcd	1071*	484	52 ab

[#], *Uninoculated treatments significantly different from inoculated treatments at $P \leq 0.1$, and $P \leq 0.05$, respectively, according to the linear contrast analysis.

[a]Data are means of six replications.

[b]Means within a column followed by the same letter are not different ($P \leq 0.05$), Fishers protected least significant difference test.

Table 7-2. Final mean population density (Pf) and reproductive factor (Rf) of *Belonolaimus longicaudatus* on five cultivars and 46 germplasm accessions of bermudagrass, 90 days after inoculation with 50 *B. longicaudatus*/conetainer in two experimental trials.

Genotype	Ploidy (2n)	Final Population Density (Pf)[a]		Reproductive Factor (Rf)	
		Trial 1	Trial 2	Trial 1	Trial 2
Celebration	3x	61 bcd[bc]	8 j	1.2 bcd	0.2 j
TifGrand	3x	111 a	80 b	2.2 a	1.6 b
TifEagle	3x	21 de	25 defghij	0.4 de	0.5 defghij
TifSport	3x	25 cde	30 cdefghij	0.5 cde	0.6 cdefghij
Tifway	3x	15 de	53 bcdefghi	0.3 de	1.1 bcdefghi
131	4x	40 bcde	14 j	0.8 bcde	0.3 j
132	4x	29 cde	16 ij	0.6 cde	0.3 ij
157	4x	72 abc	62 bcde	1.4 abc	1.2 bcde
171	4x	21 de	7 j	0.4 de	0.1 j
173	3x	32 cde	13 j	0.6 cde	0.3 j
227	4x	35 cde	19 hij	0.7 cde	0.4 hij
295	6x	23 cde	25 defghij	0.5 cde	0.5 defghij
296	3x	49 bcde	27 defghij	1.0 bcde	0.5 defghij
299	4x	39 bcde	10 j	0.8 bcde	0.2 j
301	4x	22 de	21 hij	0.4 de	0.4 hij
304	4x	21 de	9 j	0.4 de	0.2 j
334	4x	8 e	5 j	0.2 e	0.1 j
343	4x	14 de	24 efghij	0.3 de	0.5 efghij
344	6x	29 cde	35 cdefghij	0.6 cde	0.7 cdefghij
347	4x	49 bcde	60 bcdefg	1.0 bcde	1.2 bcdefg
355	4x	40 bcde	120 a	0.8 bcde	2.4 a
445	4x	86 ab	53 cdefghi	1.7 ab	1.1 cdefghi
481	4x	50 bcde	60 bcdef	1.0 bcde	1.2 bcdef
525	3x	4 e	38 cdefghij	0.1 e	0.8 cdefghij
528	4x	43 bcde	23 fghij	0.9 bcde	0.5 fghij
PI 289922	5x	15 de	14 j	0.3 de	0.3 j
PI 290868	4x	6 e	17 ij	0.1 e	0.3 ij
PI 290872	4x	5 e	19 hij	0.1 e	0.4 hij
PI 290895	3x	16 de	2 j	0.3 de	0.0 cdefghij
PI 291590	4x	36 cde	32 cdefghij	0.7 cde	0.6 ij
AB1	2x	17 de	15 ij	0.3 de	0.3 ij
AB2	2x	2 e	17 ij	0.0 e	0.3 ij
AB21	2x	112 a	68 bc	2.2 a	1.4 bc
AB3	2x	31 cde	63 bcd	0.6 cde	1.3 bcd
AB33	2x	11 e	15 ij	0.2 e	0.3 ij
AB37	2x	26 cde	17 ij	0.5 cde	0.3 ij
AB38	2x	33 cde	6 j	0.7 cde	0.1 j
AB39	2x	114 a	17 ij	2.3 a	0.3 ij
AB4	2x	9 e	19 hij	0.2 e	0.4 hij
AB42	2x	43 bcde	82 ab	0.9 bcde	1.6 ab
AB43	2x	25 cde	30 bcdefghij	0.5 cde	0.6 bcdefghij

Table 7-2. Continued.

Genotype	Ploidy (2n)	Final Population Density (Pf)		Reproductive Factor (Rf)	
		Trial 1	Trial 2	Trial 1	Trial 2
AB7	2x	24 cde	16 ij	0.5 cde	0.3 ij
UFC03	4x	10 e	55 bcdefgh	0.2 e	1.1 bcdefgh
UFC06	4x	20 de	24 defghij	0.4 de	0.5 defghij
UFC07	6x	8 e	8 j	0.2 e	0.2 j
UFC11	4x	12 de	17 ij	0.2 de	0.3 ij
UFC12	4x	14 de	17 ij	0.3 de	0.3 ij
UFC25	3x	7 e	18 hij	0.1 e	0.4 hij
UFC26	3x	21 de	22 ghij	0.4 de	0.4 ghij
UFC29	4x	43 bcde	37 cdefghij	0.9 bcde	0.7 cdefghij
UFC30	4x	30 cde	17 ij	0.6 cde	0.3 ij

[a]Numbers represent numbers of nematodes recovered from the whole conetainer.
[b]Data are means of six replications.
[c]Means within a column followed by the same letter are not different ($P \leq 0.05$), Fishers protected least significant difference test.

CHAPTER 8
SUMMARY

In recent years, management of nematodes in turf has become more and more challenging due to environmental concerns and resulting cancellation of nematicides. Utilization of nematode resistant and tolerant turfgrass cultivars was tested in this study as an environmentally safe nematode management practice. Resistant and tolerant bermudagrass (*Cynodon* spp.) and seashore paspalum (*Paspalum vaginatum* Swartz) cultivars were identified in greenhouse and field studies to sting nematode (*Belonolaimus longicaudatus* Rau) and spiral nematode (*Helicotylenchus pseudorobustus* (Steiner, 1914) Golden 1945).

The greenhouse studies indicated that bermudagrass dwarf cultivars 'Tifdwarf' and 'Emerald Dwarf' were tolerant but susceptible to *B. longicaudatus*, and 'Champion', 'TifEagle', and 'Floradwarf' were intolerant to *B. longicaudatus*. No dwarf cultivars were both tolerant and resistant to *B. longicaudatus*. Non-dwarf cultivars 'TifSport', 'Patriot', and 'Riviera' were tolerant to *B. longicaudatus*, while 'Princess 77', 'Midlawn', 'Celebration', and 'Midiron' were damaged by *B. longicaudatus* in both trials. TifSport and Riviera might be both resistant and tolerant to *B. longicaudatus*. Non-dwarf cultivar Princess 77 was the best bermudagrass host to *B. longicaudatus* with population increases of 6.9-fold and 4.4-fold in two trials.

Under greenhouse conditions, all seashore paspalum cultivars supported the reproduction of *B. longicaudatus*, Rfs ranged from 2.7 to 9.5 in the two trials. However, population densities of *H. pseudorobustus* declined in all seashore paspalum cultivars due to the high initial inoculum density of 500 nematodes/conetainer. Seashore paspalum cultivars showed differences in root length reductions caused by nematodes. 'Salam', 'SeaDwarf', and 'SeaIsle Supreme' were tolerant to *B. longicaudatus*, and 'SeaSpray' and 'SeaIsle 2000' were tolerant to *H. pseudorobustus* in both trials. No cultivar was tolerant to both nematode species.

The field study showed that bermudagrass was a better host for *B. longicaudatus*, whereas seashore paspalum was a better host for *H. pseudorobustus*. A negative logarithmic relationship was found between the population densities of *B. longicaudatus* and *H. pseudorobustus* in bermudagrass Celebration and TifSport and seashore paspalum 'Aloha' SeaDwarf, and 'SeaIsle 1'. Densities of *B. longicaudatus* remained above 54 nematodes/100 cm^3 soil by the end of the study in all bermudagrass cultivars except for TifSport, in which the nematode population density declined to 7 *B. longicaudatus*/100 cm^3 of soil, a 93% reduction compared with the beginning of the study. Population densities of *B. longicaudatus* and *H. pseudorobustus* decreased by 88% and increased 14300%, respectively, in seashore paspalum. TifSport and Sea Dwarf were the best bermudagrass and seashore paspalum cultivars, respectively, to use in this site infested with *B. longicaudatus*. All seashore paspalum cultivars evaluated were good hosts of *H. pseudorobustus* in the field. A negative linear relationship was found between the population density of *B. longicaudatus* and the root length or percent green cover of the bermudagrass Celebration and the seashore paspalum Aloha, which might indicate their intolerance to *B. longicaudatus* damage. The regression analysis showed that the total root length or percent green cover of bermudagrass Floradwarf, TifEagle, and 'Tifgreen' was decreased with increasing population densities of *H. pseudorobustus* in the soil, which may indicate their intolerance to *H. pseudorobustus*.

The nuclear DNA content and ploidy level of 48 University of Florida bermudagrass germplasm accessions that had good turfgrass characters under field conditions were determined by the flow cytometry method. Twenty triploid, 24 tetraploid, one pentaploid, and three hexaploid accessions were identified. All tetraploid, pentaploid, hexaploid, some triploid accessions, as well as 12 African accessions were further screened for nematode responses under

greenhouse conditions. We found that four African and seven common bermudagrass accessions were tolerant to *B. longicaudatus*, and eight African and 22 common bermudagrass accessions were resistant to *B. longicaudatus*. Three diploid, two triploid, six tetraploid, and one hexaploid bermudagrass accessions were found to be both resistant and tolerant to *B. longicaudatus*. Nematode resistance and tolerance were identified in different ploidy levels, which could aid in the turfgrass breeding program by increasing the genetic diversity for breeding future bermudagrass cultivars for golf course cultivation.

Greenhouse studies should be conducted in the future to test the interaction and competition between *B. longicaudatus* and *H. pseudorobustus* in turfgrass. Bermudagrass and seashore paspalum could be inoculated with *B. longicaudatus* alone, *H. pseudorobustus* alone, or both nematode species together, with non-inoculated controls as a comparison. The interaction relationship could be tested under different hosts or turfgrass cultivars. This screening of nematode resistant and tolerant bermudagrass germplasm accessions under greenhouse conditions was preliminary, and they should be further tested under field conditions with multiple nematode species or other pathogens coexisting in soil. Furthermore, the grass cultivars and germplasm accessions should eventually be tested with different populations and geographical isolates of *B. longicaudatus* or *H. pseudorobustus* to more broadly select for resistance.

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BIOGRAPHICAL SKETCH

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