

*Belonolaimus longicaudatus* AND *Hoplolaimus galeatus* ON SEASHORE PASPALUM  
(*Paspalum vaginatum*)

By

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Abstract of Thesis Presented to the Graduate School  
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*Belonolaimus longicaudatus* AND *Hoplolaimus galeatus* ON SEASHORE PASPALUM  
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Seashore paspalum (*Paspalum vaginatum*) has great potential for use in salt-affected turfgrass sites. Seashore paspalum has a natural tolerance to drought and high salinity irrigation. Therefore, use of this grass on golf courses, athletic fields, and lawns in subtropical coastal areas may aid in conservation of fresh water resources. Plant-parasitic nematodes are damaging pathogens of turfgrasses in Florida, with *Belonolaimus longicaudatus* and *Hoplolaimus galeatus* considered among the most damaging. Glasshouse experiments were performed to compare the susceptibility of 'SeaIsle 1' seashore paspalum to *B. longicaudatus* and *H. galeatus* with 'Tifdwarf' bermudagrass (*Cynodon dactylon* × *C. transvaalensis*), and to examine the effects of increasing irrigation salinity levels on *B. longicaudatus* and *H. galeatus*. 'SeaIsle 1' seashore paspalum and 'Tifdwarf' bermudagrass grown in 1,500-cm<sup>3</sup> clay pots filled with 100% United States Golf Association (USGA) specification sand and inoculated with either nematode served as experimental units. Both nematode species reproduced well on either

grass, but only *B. longicaudatus* consistently stunted root growth. *Belonolaimus longicaudatus* reduced root growth by 35 to 45% at 120 days after inoculation on both grasses, but these reductions did not indicate a difference ( $P > 0.05$ ) in susceptibility between grasses. These data suggest that 'SeaIsle 1' seashore paspalum is not less susceptible to *B. longicaudatus* or *H. galeatus* than is 'Tifdwarf' bermudagrass. Final population densities of *H. galeatus* followed a negative linear regression ( $P \leq 0.01$ ) with increasing salinity levels. Final population densities of *B. longicaudatus* were quadratically ( $P \leq 0.01$ ) related to increasing salinity levels from 0 to 25 dS/m. An increase in population densities of *B. longicaudatus* was observed at moderate salinity levels (10 and 15 dS/m) compared to 0 dS/m ( $P \leq 0.05$ ). Reproduction and feeding of *B. longicaudatus* and *H. galeatus* decreased at salinity levels of 25 dS/m and above. Root length comparisons revealed that *B. longicaudatus* caused root stunting at low salinity levels, 0 to 10 dS/m ( $P \leq 0.05$ ). However, at higher salinity irrigation treatments, 15 to 25 dS/m, root lengths were not different ( $P > 0.05$ ) between inoculated and uninoculated plants. These results indicate that the ability of *B. longicaudatus* to feed and stunt root growth was negatively affected at salinity levels of 15 dS/m and above.

## CHAPTER 1 INTRODUCTION AND LITERATURE REVIEW

### Introduction

Coastal areas in the southeastern United States, including a majority of Florida, are ideal habitats for *Belonolaimus longicaudatus* Rau, the sting nematode (Christie, 1959; Holdeman, 1955), and *Hoplolaimus galeatus* (Cobb, 1913) Thorne 1935, the lance nematode (Ahmad and Chen, 1980; Williams, 1973). Both are important pathogens of turfgrass (Perry et al., 1970). New turfgrass cultivars or ecotypes are commonly introduced into the turfgrass industry, but in rare cases, a new species of turfgrass begins to become more widely utilized. Seashore paspalum (*Paspalum vaginatum* Swartz) is a new turfgrass species quickly growing in popularity due to the breeding and development of cultivars with a fine leaf texture and tolerance to drought and high salinity irrigation (Duncan, 1999a; Morton, 1973).

*Belonolaimus longicaudatus* and *H. galeatus* have been reported as pathogens of many bermudagrass (*Cynodon dactylon* (L.) Pers and *Cynodon* spp. hybrids) (Di Edwardo, 1963; Giblin-Davis et al., 1992b; Johnson, 1970; Rivera-Camarena, 1963; Winchester and Burt, 1964), and St. Augustinegrass (*Stenotaphrum secundatum*) (Busey et al., 1991; Giblin-Davis et al., 1992a; Henn and Dunn, 1989; Kelsheimer and Overman, 1953) cultivars. However, no published data currently exist on the susceptibility of seashore paspalum to *B. longicaudatus* or *H. galeatus*. Knowledge is also lacking on the response of *B. longicaudatus* and *H. galeatus* to high salinity irrigation. After personal communication with two turfgrass managers in south Florida, Morton (1973)

hypothesized that low nematode counts may be associated with seashore paspalum irrigated with saline water. Further investigation is necessary to confirm this hypothesis.

Typically, susceptibility of a plant to a pathogen is based on yield reduction caused by the pathogen, but yield is difficult to determine in turfgrass. Therefore, root length data and the reproductive capability of the nematode are major determining factors of susceptibility in turfgrasses. When levels of susceptibility are determined, soil and root nematode assays may be useful in predicting a need for treatment. This information will help avoid unnecessary post-plant nematicide treatments or failure to apply a needed one.

### *Belonolaimus longicaudatus*

#### Taxonomy

The first reported nematode in the genus *Belonolaimus* was the species *B. gracilis* collected from the rhizosphere of a pine tree (*Pinus* spp.) near Ocala, FL (Steiner, 1949). Subsequently, *B. gracilis* was reported to damage peanut (*Arachis hypogaea*) in Virginia (Owens, 1951), strawberry (*Trifolium fragiferum*), celery (*Apium graveolens*), and corn (*Zea mays*) in Florida (Christie et al., 1952), and cotton (*Gossypium* spp.), soybean (*Glycine max*), and cowpea (*Vigna unguiculata*) in South Carolina (Graham and Holdeman, 1953). Later, Rau (1958) described *B. longicaudatus*, which soon became accepted as the more common sting nematode damaging agricultural crops and turfgrasses in the southeastern United States (Perry and Rhoades, 1982; Smart and Nguyen, 1991). No published data exist on *B. gracilis* except for the original description in 1949. Tail and stylet lengths can separate the two species. Specimens of *B. longicaudatus* have a shorter tail and a longer stylet than those of *B. gracilis* (Rau 1958). Rau (1963) continued his work with the genus *Belonolaimus*, describing three more species: *B. euthychilus*, *B. maritimus*, and *B. nortoni*. Presently, the genus includes 9

species, the five mentioned previously, and *B. anama*, *B. jara*, *B. lineatus*, and *B. lolii* (Fortuner and Luc, 1987).

#### Distribution and Biology

*Belonolaimus longicaudatus* is found primarily in deep sandy soils in the lower coastal plains of the southeastern United States from Virginia to Florida (Christie, 1959; Holdeman, 1955), but has been reported in New Jersey (Myers, 1979), Kansas (Dickerson et al., 1972), Arkansas (Riggs, 1961), Oklahoma (Russell and Sturgeon, 1969), Texas (Christie, 1959; Wheeler and Starr, 1987), and recently in California (Mundo-Ocampo et al., 1994). Holdeman (1955) reported confirmed findings of *B. longicaudatus* in Florida, Georgia, South Carolina, North Carolina, and Virginia, with possible findings in Alabama, Mississippi, and Louisiana. *Belonolaimus longicaudatus* is most commonly found in Florida because it is well adapted to soils with > 80% sand content and < 10% clay content (Miller, 1972; Robbins and Barker, 1974). Populations of *B. longicaudatus* have been discovered at golf courses located outside the native habitat of the nematode. These areas in the Bahamas, Bermuda, Puerto Rico, Costa Rico, and California are ideal for *B. longicaudatus* reproduction because the soil profiles are at least 90% sand (Lopez, 1978; Mundo-Ocampo et al., 1994; Perry and Rhoades, 1982). Imported sod from Florida and Georgia appear to be the cause of the infestations in the Bahamas, Bermuda, and Puerto Rico (Perry and Rhoades, 1982).

*Belonolaimus longicaudatus* is recognized as the most pathogenic ectoparasitic plant nematode to turfgrasses in Florida. Acceptable visual quality often is not possible without treatment of the nematode infestation. Root injury typically consists of greatly reduced root systems, with short stubby roots exhibiting dark lesions along the root axis

and along the tip. Aboveground symptoms usually consist of severe stunting, incipient wilting, leaf chlorosis, or death (Perry and Rhoades, 1982).

Reproduction of *B. longicaudatus* is exclusively accomplished through amphimixis, requiring both males and females to be present (Huang and Becker, 1999). Females lay eggs as long as food is readily available (Huang and Becker, 1999). Eggs are laid two at a time with one coming from each opposed ovary of the amphidelphic reproductive system (Han, 2001). The entire lifecycle was achieved in 24 days at 28 °C on excised corn roots grown in vitro (Huang and Becker, 1997; 1999).

*Belonolaimus longicaudatus* is a migratory ectoparasitic nematode that inserts its stylet deep into the root tip and withdraws the cellular contents. The presence of a host is necessary for survival and reproduction, because there is no known survival mechanism such as anabiosis or diapause (Huang and Becker, 1997).

Temperature can have a dramatic effect on the survivability and reproduction of *B. longicaudatus* (Boyd and Perry, 1971; Robbins and Barker, 1974). Seasonal temperature variation greatly influenced the population levels and distribution of the sting nematode. April and May temperatures in Gainesville, FL are typically favorable for high populations, while June and July are least favorable (Boyd and Perry, 1971). Reproduction of *B. longicaudatus* was greatest at 25 to 30 °C, with one population achieving some reproduction at 35 °C while the other population declined. Both populations had minimal reproduction at 20 °C (Robbins and Barker, 1974).

#### Host Range

*Belonolaimus longicaudatus* has an extremely wide host range, including many small grains, forage crops, fruits, vegetables, ornamentals, trees, and turfgrasses (Bekal

and Becker, 2000; Holdeman and Graham, 1953; Owens, 1951; Perry and Rhoades, 1982; Robbins and Barker, 1973). Holdeman (1955) reported watermelon (*Citrullus lanatus*) and tobacco (*Nicotiana tabacum*) to be non-hosts. Additionally, asparagus (*Asparagus officinalis*), buckhorn plantain (*Plantago lanceolata*), okra (*Abelmoschus esculentus*), and pokeweed (*Phytolacca americana*) were reported to be non-hosts (Robbins and Barker, 1973). Later, a California population also was found to be unable to reproduce on okra, watermelon, and tobacco (Bekal and Becker, 2000). Physiological races have been suggested numerous times by many researchers due to host preferences and reproductive isolation, but none are widely recognized (Abu-Gharbieh and Perry, 1970; Owens, 1951; Robbins and Barker, 1973; Robbins and Hirschmann, 1974). Robbins and Barker (1973) reported that some poor or non-hosts for three populations of *B. longicaudatus* from North Carolina were actually good to excellent hosts for one population from Georgia. Also, when populations with different morphological characteristics and host preferences were allowed to mate with each other, infertile individuals were produced (Robbins and Hirschmann, 1974). Morphological differences and reproductive potential suggest that the populations from North Carolina and Georgia may be different species (Robbins and Hirschmann, 1974).

### *Hoplolaimus galeatus*

#### Taxonomy

Lance nematodes belong to the extremely diverse subfamily of Hoplolaiminae that includes hundreds of species of plant-parasitic nematodes. *Hoplolaimus galeatus* was originally described as *Nemonchus galeatus* (Cobb, 1913), but later Thorne (1935) determined this nematode to be identical in general size and shape to *Hoplolaimus coronatus*. *Hoplolaimus galeatus* and *H. coronatus* were later found to be conspecific,

with *H. galeatus* having date priority (Sher, 1961). The nematode was thereafter referred to as *Hoplolaimus galeatus*. Identifying morphological characteristics on the female include an offset cephalic region, usually with 5 annules, 2 phasmids (one anterior to the vulva and the other posterior), and a rounded tail with 10 to 16 annules. *Hoplolaimus galeatus* reproduces by amphimixis with an equal ratio of females to males (Hirschmann, 1959; Smart, 1965; Wen and Chen, 1972).

#### Distribution and Biology

*Hoplolaimus galeatus* has a wider distribution than *B. longicaudatus*, but is most numerous in the southeastern United States. Woody or graminaceous plants appear to be especially favorable hosts (Williams, 1973). *Hoplolaimus galeatus* is usually a seemingly unmanageable problem because of its widespread distribution and the frequent failure of nematicides to reduce their numbers while controlling other plant parasitic nematodes (Perry et al., 1970). Krusberg and Sasser (1956) found that cotton plants growing under drought conditions that were stunted, yellowing, and almost completely defoliated returned much higher numbers of *H. galeatus* in the soil and roots near the rhizosphere than did healthy plants. The nematodes primarily fed endoparasitically on the cotton roots, causing considerable damage to the root cortex during penetration. Disruption of the vascular tissue occurred during feeding due to the preference for phloem (Krusberg and Sasser, 1956). Slash (*Pinus elliottii*) and Loblolly pine (*Pinus taeda*) seedlings suffered 50% mortality in North Carolina due to infestation by *H. galeatus*. Glasshouse experiments confirmed these findings, showing roots with black lesions and most of the root cortex destroyed (Ruehle and Sasser, 1962). Experiments with bermudagrass determined that total plant weight was reduced by 50% over 6 months by *H. galeatus* (Di Edwardo, 1963).



### Host Range

Lance nematodes (*H. galeatus*) are pathogenic on a variety of agronomic crops and turfgrasses. The rate of reproduction of *H. galeatus* was higher on rye (*Secale cereale*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), oats (*Avena sativa*), soybean, corn, cabbage (*Brassica oleracea*), and bean (*Phaseolus vulgaris*) than on pepper (*Capsicum* spp.), eggplant (*Solanum melongena*), lettuce (*Lactuca sativa*), tobacco, and pea (Ahmad and Chen, 1980). *Hoplolaimus galeatus* can cause serious damage to cotton (Krusberg and Sasser, 1956), pine (Ruehle and Sasser, 1962), oak (Viggars and Tarjan, 1949), wheat (Ahmad and Chen, 1980), and turfgrasses (Di Edwardo, 1963; Perry et al., 1970). In a study involving *H. coronatus* on cotton, host symptoms were more prominent in dry conditions, but plants supplied with adequate moisture could tolerate high populations (Krusberg and Sasser, 1956). When cotton followed bahiagrass (*Paspalum notatum*), the *H. galeatus* population dropped to below damaging levels (Rodriguez-Kabana and Pearson, 1972). *Hoplolaimus galeatus* was also found to feed endo and ectoparasitically on alfalfa (*Medicago sativa*) and bermudagrass (Ng and Chen, 1980). *Hoplolaimus galeatus* caused reductions in magnesium and water uptake, shoot and root growth, and number of root hairs on tall fescue (*Festuca arundinacea*) (Rodriguez-Kabana et al., 1978).

Lance nematodes (*Hoplolaimus* spp.) were found to be associated with unhealthy St. Augustinegrass lawns in the Tampa, FL area (Kelsheimer and Overman, 1953). Other surveys specifically associated *H. galeatus* with damaged lawns in Florida (Good et al., 1956; Perry and Good, 1968; Whitton, 1956). *Hoplolaimus galeatus* reproduced well on seven different cultivars of St. Augustinegrass, indicating similar host suitability among these cultivars (Henn and Dunn, 1989). Time course experiments with 'Floritam' and

'FX-313' St. Augustinegrass indicated that *H. galeatus* had no effect on plant growth, even though soil counts of *H. galeatus* exceeded the proposed action threshold of 40 nematodes/100 cm<sup>3</sup> of soil (Crow et al., 2003) for 84 days during the course of the experiment (Giblin-Davis et al., 1995). Alyceclover (*Alysicarpus vaginalis*), corn, and some vegetables supported high populations of *H. galeatus*, but measurable yield suppression was not detected in any of these crops (Mashela et al., 1992b; Norton and Hinz, 1976; Rhoades, 1987).

#### Nematodes and Turfgrass

In 1951, plant-parasitic nematodes were reported to be associated with the 'yellow tuft' disease of bentgrass (*Agrostis palustris*) in Virginia (Tarjan and Ferguson, 1951). A subsequent survey in Rhode Island by Troll and Tarjan (1954) indicated that several species of nematodes might be causing serious damage on turfgrass as either primary or secondary plant pathogens. Nematodes were not recognized as pathogens of turfgrass in Florida until 1953, when V. G. Perry applied three nematicides on a thinning bermudagrass golf course green at Sanford, FL. One month after treatment, the turfgrass began to grow and later completely covered the putting surface. This indicated that the death of plant-parasitic nematodes allowed the grass to recover from root damage (Perry and Rhoades, 1982). Christie et al. (1954) stated that sting and lance nematodes were responsible for most of the nematode damage to turfgrass in Florida. In Florida, *B. longicaudatus* became recognized as a pathogen of significant importance to turfgrasses (Christie et al., 1954; Perry et al., 1970; Rhoades, 1962). Perry and Rhoades (1982) estimated that over 80% of some 700 Florida golf courses used nematicides routinely.

Many hybrid bermudagrasses (*Cynodon dactylon* (L.) Pers × *C. transvaalensis* Burt-Davis) have been determined to be suitable and susceptible hosts to *B. longicaudatus* (Giblin-Davis et al., 1992b). 'Ormond' bermudagrass was demonstrated to be extremely susceptible to *B. longicaudatus*, with severe yellowing, stunting of top growth, and stunting of the roots (Winchester and Burt, 1964). St. Augustinegrass also was found to be a suitable and susceptible host to *B. longicaudatus*, with root reductions occurring when compared to uninoculated plants (Giblin-Davis et al., 1992a; Rhoades, 1962). Rhoades (1962; 1965) also reported that *Trichodorus christiei* and *T. proximus* reduced St. Augustinegrass root weight when compared to uninoculated plants. Sting and lance nematodes were extracted from soil samples taken from declining areas of St. Augustinegrass in the Tampa Bay area (Kelsheimer and Overman, 1953). Sting and lance nematodes were both found to be pathogenic on 'Tifton 328' bermudagrass in a susceptibility experiment. Sting nematode populations increased from 250 to approximately 3,700 per pot, while lance nematode populations increased from 250 to approximately 2,100 per pot (Rivera-Camarena, 1963).

#### Salinity and Nematodes

High-salinity irrigation has been shown to negatively affect some root-knot nematodes. *Meloidogyne incognita* egg hatching decreased by 96% when exposed to a 28.8 dS/m solution (Lal and Yadav, 1975). Salinity has demonstrated negative effects on the hatching and infectivity of *M. javanica* and *M. arenaria* juveniles (Dropkin et al., 1958; Maqbool et al., 1987). Khan and Khan (1990) found that after only 7 days of salinity exposure, *M. incognita* and *M. javanica* had significant reductions in hatching and increases in mortality. Population levels of *Aphelenchus avenae*, *Pratylenchus*

*thornei*, *Helicotylenchus* spp., and *Rotylenchulus reniformis* were lower at increased salt concentrations over nonsaline treatments. No differences were observed in populations of *Heterodera avenae* and *Tylenchorhynchus mashhoodi* at increased salt concentrations when compared to controls (Lal and Yadav, 1976). Some species of root-knot nematodes (*M. spartinae*) and sting nematodes (*B. maritimus*) may be well adapted to high-salinity conditions (Rau, 1963; Rau and Fassuliotis, 1965).

### Seashore Paspalum

Seashore paspalum (*Paspalum vaginatum* Swartz) is a warm season, perennial grass, with both rhizomatous and stoloniferous growth, and a V-shaped inflorescence (Duncan and Carrow, 2000; Morton 1973). Seashore paspalum is indigenous to South Africa and South America, and has been found in Argentina, Brazil, Australia, the Caribbean Islands, and Pacific islands. Within North America, it has been found throughout the southern latitudes of the United States. Morton (1973) indicates that St. Simons Island, GA, is the origin of seashore paspalum in North America. The grass is best adapted to coastal areas (brackish areas, sand dunes, and beaches) with tropical and sub-tropical temperatures. Regions from 30 to 35° N and S latitudes are most suitable (Duncan and Carrow, 2000; Morton, 1973; Skerman and Riveros, 1990). Seashore paspalum encompasses a wide range of ecotypes from fine to coarse leaf textures, and can be used in a multitude of turfgrass applications (Duncan and Carrow, 2000).

Several seashore paspalum ecotypes have been researched for their multiple stress resistance characteristics. In particular, advantageous characteristics include salinity tolerance (Carrow and Duncan, 1998; Marcum and Murdoch, 1994; Peacock and Dudeck, 1985), drought tolerance, (Huang et al. 1997a; 1997b), wear and traffic tolerance

(Trenholm et al. 1999; 2001a; 2001b), water logging tolerance (Malcolm, 1977), and tolerance of other stresses including pH, diseases, insects, and weeds (Duncan, 1999b; Johnson and Duncan, 1997; 1998; Wiseman and Duncan, 1996). Fertility requirements are much lower for seashore paspalum than many similarly textured grasses (Beard et al., 1982; 1991). Suggested nitrogen rates for fairways, tees, and sports fields range from 0.98 to 2.94 kg/100 m<sup>2</sup> annually depending on geographical location (Duncan, 1999b). In Florida, recommended nitrogen rates are slightly higher, ranging from 1.96 to 5.88 kg/100 m<sup>2</sup> annually (Trenholm and Unruh, 2003). Seashore paspalum greens ecotypes were reported to have higher quality ratings at 3 to 4 mm mowing height when compared to 'Tifgreen' bermudagrass (Duncan, 1999b).

#### Current Research

The research reported hereafter was performed in an environmentally controlled glasshouse at the University of Florida Envirotron Turfgrass Research Laboratory in Gainesville, FL. Experiments were performed in consecutive summers in an optimal environment for both nematodes and turfgrasses. The objectives of the research were to:

1. Determine susceptibility of 'SeaIsle 1' seashore paspalum to a population of *B. longicaudatus* and a population of *H. galeatus*.
2. Compare 'SeaIsle 1' seashore paspalum to 'Tifdwarf' bermudagrass for differences in susceptibility to a population of *B. longicaudatus* and a population of *H. galeatus*.
3. Determine how high salinity irrigation can affect the reproduction of a population of *B. longicaudatus* and a population of *H. galeatus*.
4. Conduct a survey to determine which plant-parasitic nematodes are associated with established seashore paspalum golf courses and home lawns in Florida.

CHAPTER 2  
SUSCEPTIBILITY OF SEASHORE PASPALUM TO *Belonolaimus longicaudatus*  
AND *Hoplolaimus galeatus*

Introduction

Seashore paspalum (*Paspalum vaginatum* Swartz) is a warm-season turfgrass quickly expanding in popularity. Due to the fine leaf texture and natural tolerance to drought and high salinity, seashore paspalum has become more prevalent in coastal salt-affected turfgrass sites (Duncan, 1999a; Morton, 1973). One major limitation of cultivating turfgrasses in the sandy soils of the southeastern United States is the destruction of roots by phytoparasitic nematodes (Perry and Rhoades, 1982). The sting nematode (*Belonolaimus longicaudatus* Rau) and the lance nematode (*Hoplolaimus galeatus* (Cobb) Thorne) are destructive pathogens on a variety of agricultural crops, including turfgrasses (Ahmad and Chen, 1980; Holdeman and Graham, 1953; Perry and Rhoades, 1982; Smart and Nguyen, 1991). While *B. longicaudatus* is found primarily in the coastal plains of the southeastern United States (Christie, 1959; Holdeman, 1955), *H. galeatus* has a much wider distribution (Williams, 1973). *Belonolaimus longicaudatus* damages lateral roots as soon as they are formed, causing a stunting of root growth, decreased water and nutrient uptake, and decreased rates of evapotranspiration (Busey et al., 1991; Johnson, 1970; Perry and Rhoades, 1982). *Hoplolaimus galeatus* enters the root tissue as a migratory endoparasite and is thought to damage roots by feeding and physical tunneling through the root cortex cell walls (Henn and Dunn, 1989; Krusberg and Sasser, 1956; Lewis and Fassuliotis, 1982; Perry et al., 1970; Williams, 1973).

Both nematodes have been reported as economically important pathogens of turfgrasses in the southeastern United States (Christie et al., 1954; Kelsheimer and Overman, 1953; Perry and Rhoades, 1982). *Belonolaimus longicaudatus* has been reported as a pathogen of many bermudagrass (*Cynodon dactylon* (L.) Pers. and *Cynodon* spp. hybrids), and St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze) cultivars (Busey et al., 1991; Giblin-Davis et al., 1992a; 1992b; Johnson, 1970). Seven cultivars of St. Augustinegrass were discovered to have similar host suitability for *H. galeatus* in the glasshouse and in microplots (Henn and Dunn, 1989). However, root and shoot growth of 'FX-313' and 'Floritam' St. Augustinegrass were not affected by *H. galeatus* in a population dynamics and pathogenicity study (Giblin-Davis et al., 1995). Two populations of *B. longicaudatus* readily reproduced on 'Tifdwarf' bermudagrass (*Cynodon dactylon* × *C. transvaalensis* Burt-Davis), and caused extensive root damage (Giblin-Davis et al., 1992b; Johnson, 1970). A forage grass study determined that there was differential host suitability and susceptibility to *B. longicaudatus* in *Digitaria* spp., *Paspalum* spp., and *Chloris* spp. introductions (Boyd and Perry, 1969).

At present, no published data exist on the susceptibility of seashore paspalum to *H. galeatus* or *B. longicaudatus*. The objectives of this study were to: i) determine the susceptibility and host suitability of 'SeaIsle 1' seashore paspalum to a population of *B. longicaudatus* and a population of *H. galeatus*, and ii) compare 'SeaIsle 1' seashore paspalum to 'Tifdwarf' bermudagrass for differences in susceptibility to a population of *B. longicaudatus* and a population of *H. galeatus*.

### Materials and Methods

Experiments were performed to compare the relative susceptibility of 'SeaIsle 1' seashore paspalum and 'Tifdwarf' bermudagrass to *B. longicaudatus* and *H. galeatus*. Experiments were conducted during the spring and summer of 2002 and 2003 at the University of Florida Turfgrass Envirotron Glasshouse in Gainesville, FL.

Commercially available cultivars of *Paspalum vaginatum* and *Cynodon dactylon* × *C. transvaalensis* were evaluated in this experiment. A sod square of 'SeaIsle 1' seashore paspalum was obtained from R. R. Duncan at the University of Georgia and 'Tifdwarf' bermudagrass was available from previous experiments. Nematode-free plugs of each grass were obtained by rooting aerial cuttings of stolons from each grass in tapered RLC-7 (UV Stabilized) Super "Stubby" Cells (cell depth = 14 cm; diam. = 3.8 cm; volume = 115 ml) (Ray Leach Single Cell Cone-tainer, Stuewe & Sons, Inc., Corvallis, OR) filled with 140 g of uninfested soil. Soil used for growth media consisted of 100% United States Golf Association (USGA) specification sand. The soil texture was analyzed using the sieving method for testing a USGA root zone mix (USGA Green Section Staff, 1993) (Table 2-1). An absorbent cotton ball was placed at the bottom of each cell to prevent soil from escaping through the drain holes. The soil was then thoroughly wetted to allow for settling. A depression was made in each cell and two aerial stolons were planted on opposing sides of the depression. Stolons (5 to 8 cm long) were terminal cuttings with two or three nodes. During the fall and winter months, the cells were placed on a glasshouse bench 1.25 m below an enclosed high bay 1,000-watt metal halide growth lamp (Hi-Tek Series, Lithonia Lighting, Conyers, GA) set on a 12-hour cycle to simulate the longer day-lengths required for optimal growth. The grass was



fertilized biweekly using a fertilizer solution (20%-20%-20% (N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O) plus trace elements) (Peters Professional All-Purpose Plant Food, Spectrum Brands, St. Louis, MO) at a rate equivalent to 49.0 kg N/ha/month, 21.6 kg P/ha/month, and 40.7 kg K/ha/month. Both grasses were allowed to develop a substantial root system for four weeks.

Table 2-1. Composition of experimental soil compared to the United States Golf Association root zone mix specifications

Sand Type	Particle Size	Experimental Soil	USGA specifications
Fine Gravel	2.0 to 3.4 mm	0.1%	Not more than 10% of the total particles in this range, including a maximum of 3% fine gravel (preferably none)
Very Coarse Sand	1.0 to 2.0 mm	3.7%	
Coarse Sand	0.5 to 1.0 mm	30.8%	Minimum of 60% of the particles must fall in this range
Medium Sand	0.25 to 0.50 mm	53.4%	
Fine Sand	0.15 to 0.25 mm	10.3%	Not more than 20%
Very Fine Sand	0.05 to 0.15 mm	1.7%	Not more than 5%

Data are means of five replicates

Plugs of seashore paspalum and bermudagrass obtained from the cells were transferred into 14.5 × 16-cm-diam. clay pots (1,500 cm<sup>3</sup>) filled with 100% USGA specification sand (Table 2.1). Insect screening (7 lines × 5 lines/cm<sup>2</sup>) was cut into 5 × 5-cm squares and placed at the bottom of each pot to prevent soil from escaping through the drain hole. Roots were washed free of soil and trimmed to approximately 5 cm below the crown to promote fresh root growth. Two depressions were made in each pot on opposite sides and two plugs of turfgrass were planted per pot. These experimental units were placed on a screened bench in an environmentally controlled glasshouse and irrigated as needed for 14 days to allow for adjustment to the new environment.

A population of *B. longicaudatus* obtained from R. M. Giblin-Davis that originated from unmanaged field soil in the Sanford, FL area and were allowed to reproduce on 'FX 313' St. Augustinegrass was used in this experiment. A population of *H. galeatus* was obtained from a 'Floradwarf' bermudagrass putting green at the G. C. Horn Turfgrass Field Laboratory in Gainesville, FL. Inocula were extracted from soil using a modified Baermann funnel method (McSorley and Federick, 1991). In 2002, the source of the *H. galeatus* population was contaminated with other plant-parasitic nematodes, so 100 individual *H. galeatus* of mixed life stages were handpicked to inoculate into 16 pots of each grass. Since the *B. longicaudatus* population was not contaminated, handpicking was not necessary. A solution of *B. longicaudatus* at various life stages in tap water was calibrated by counting nematodes from 1-ml aliquots on a grided counting slide (Hawksley and Sons Limited, Lancing, Sussex, United Kingdom) replicated ten times. Approximate numbers of nematodes were measured with a pipette from water suspensions of inocula. *Belonolaimus longicaudatus* ( $107 \pm 8$ ) was inoculated into 16 pots of each grass. In 2003, *H. galeatus* and *B. longicaudatus* were obtained from the previous year's experiment and inocula for both species were obtained using a modified Baermann funnel method (McSorley and Federick, 1991). Suspensions were made for each nematode, and standardized to deliver approximately 200 nematodes per pot. *Hoplolaimus galeatus* ( $199 \pm 13$ ) and *B. longicaudatus* ( $211 \pm 10$ ) were inoculated into 20 pots of each grass, respectively. A higher level of nematodes was used in the second year to achieve higher reproduction. In both years, nematodes were suspended in 50 ml of tap water and equally distributed into four cavities formed in the soil near the base of the plant. Uninoculated plants received 50 ml of tap water. After

inoculation, the cavities were then pinched closed with surrounding soil and tap water was applied as needed to avoid wilting.

Experimental units were arranged in a randomized complete block design in the glasshouse on screened benches. In 2002, separate experiments were performed for each nematode consisting of a total of 64 pots each. Each experiment had eight treatments with eight replications. In 2003, the two nematode species were placed into the same experiment, allowing for fewer control pots and a higher number of replications. The second experiment had 12 treatments with 10 replications, for a total of 120 pots. For both experiments, at 60 days after inoculation, one pot of each treatment was removed from each block and analyzed, and 120 days after inoculation the remaining pots were analyzed. Treatments were seashore paspalum and bermudagrass inoculated with the specified number of *H. galeatus* or *B. longicaudatus* allowed to reproduce for 60 or 120 days, and uninoculated controls.

Throughout the course of the 2002 experiments, which lasted from 21 April 2002 to 10 September 2002, average monthly high and low temperatures in the glasshouse ranged from 31 to 34 °C, and 23 to 26 °C, respectively. In 2003, experiments began 28 February 2003 and ended 13 July 2003. Average monthly high and low temperatures ranged from 27 to 32 °C and 19 to 24 °C, respectively. Using a 1.18-liter pump sprayer, an insecticide/miticide (Mavrik Aquaflow, Wellmark International, Schaumburg, IL) was applied at the labeled rate twice during the course of 2003 experiments for control of bermudagrass mites (*Eriophyes cynodoniensis* Sayed) and rhodesgrass mealybugs (*Antonina graminis* Maskell). 'Tifdwarf' bermudagrass was fertilized on a biweekly basis with 40 ml of a solution consisting of 5,100 mg NH<sub>4</sub>NO<sub>3</sub> (34% N), 3,177 mg KCl, 252

mg  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ , 435 mg  $\text{CaSO}_4$ , 246 mg  $\text{MgSO}_4$ , 1.55 mg  $\text{H}_3\text{BO}_3$ , 0.34 mg  $\text{MnSO}_4$ , 0.58 mg  $\text{ZnSO}_4$ , 0.13 mg  $\text{CuSO}_4$ , and 3.5 mg  $\text{FeSO}_4$  per 1 liter of deionized water. A separate fertilizer solution was made for 'SeaIsle 1' seashore paspalum. The KCl was doubled to compensate for the different potassium requirements of seashore paspalum (Duncan and Carrow, 2000). Total N for both grass species was 624 mg/pot for 120 days and 346 mg/pot for 60 days. Using fabric scissors (Fiskars Brand Inc., Madison, WI), the grass was trimmed biweekly to approximately 2.5 cm above the soil surface. Tissue clippings were collected in 15 cm  $\times$  23 cm mailing envelopes (Quality Park Products, St. Paul, MN) using a spouted 2.84-liter sample pan (40.6 cm  $\times$  30.5 cm  $\times$  5 cm) (Seedburo, Chicago, IL), and dried at 75 °C for 48 hours to obtain cumulative shoot dry weight.

After 60 and 120 days, randomly-selected pots of each treatment from each block were brought into the laboratory for destructive analysis. This provided a total of 32 pots for each nematode at each sampling date in 2002 and a total of 60 pots at each sampling date in 2003. Shoots were trimmed as close to the soil as possible and saved for cumulative dry weight analysis. Then, using a stainless steel T-sampling tool, a root core (approximately 4-cm-diam.  $\times$  14 cm deep) was taken from the center of each pot to serve as a representative root sample. The remaining contents of each pot were emptied into their individually labeled plastic bags and thoroughly hand mixed. A 100-cm<sup>3</sup> soil sample was taken from each bag and nematodes were extracted using a modified centrifugal-sugar flotation technique (Jenkins, 1964). Nematodes in the entire soil sample were counted using an inverted light microscope at  $\times$  32 magnification.

Root cores were washed free of soil on a sieve with 1.7-mm pore openings nested within a sieve with 75- $\mu\text{m}$  pore openings. Roots were removed from any aboveground

growth and placed in a 50-ml disposable plastic centrifuge tube containing 30 ml of tap water. The 75- $\mu$ m-pore sieve was then submerged in 5 cm of tap water to allow the finer roots to float out and separate from the soil. These fine roots were collected using laboratory forceps and placed into the 50-ml plastic centrifuge tubes. Five drops (0.25 ml) of a 1% methylene blue mixture was added to the 30 ml of tap water to stain the roots. After a minimum of 24 hours in the solution, the roots were removed, placed on a 75- $\mu$ m-pore sieve, and washed free of excess dye. Stained roots were placed into a glass-bottom tray and scanned using a HP ScanJet 2cx desktop scanner (Hewlett Packard, Boise, ID) to create a black and white bitmap image of the roots (Kaspar and Ewing, 1997; Pan and Bolton, 1991). The GSRoot (Louisiana State University, Baton Rouge, LA) software program was used to analyze the bitmap images. This program measures root lengths and surface areas from scanned images. Root length data were recorded for seven diameter ranges (< 0.05 mm, 0.05 to 0.10 mm, 0.10 to 0.20 mm, 0.20 to 0.30 mm, 0.30 to 0.40 mm, 0.40 to 0.50 mm, and > 0.50 mm). The resulting values were summed to determine the total root length of each root sample. In 2003 at the final sampling date, *H. galeatus* in roots were staged and counted to determine number of nematodes/gram of root in fresh-weighed samples using a modified acid-fuchsin staining-destaining procedure (Byrd et al., 1983).

Total root lengths and cumulative shoot dry weights were compared for both evaluation periods. The one-way analysis of variance (ANOVA) procedure performed using SAS software (SAS Institute, Cary, NC) was used to analyze these two responses for differences between inoculated and uninoculated treatments within each grass species.

Data for *B. longicaudatus* and *H. galeatus* were analyzed separately, even when the two experiments shared controls (2003 experiment).

The one-way ANOVA procedure also was used to determine differences in susceptibility between grass species by comparing final nematode population densities, total root length percent reduction, and cumulative shoot dry weight percent reduction. Total root length percent reduction was calculated by  $[100 \times (\text{total root length of inoculated} - \text{total root length of uninoculated}) / \text{total root length of uninoculated}]$  and cumulative shoot dry weight percent reduction was calculated by  $[100 \times (\text{cumulative shoot dry weight of inoculated} - \text{cumulative shoot dry weight of uninoculated}) / \text{cumulative shoot dry weight of uninoculated}]$ . Again, data for *B. longicaudatus* and *H. galeatus* were analyzed separately, even when the two experiments shared controls (2003 experiment). A difference in the susceptibility of grasses to the nematodes was determined to be significant when  $P \leq 0.05$ .

### Results

The population of *B. longicaudatus* had a high degree of association with root reduction in both turfgrasses while the population of *H. galeatus* caused root reduction at only one sampling date for each grass (Tables 2-2, 2-3). In the 2002 trial, the *B. longicaudatus* population caused a reduction in total root growth in both seashore paspalum and bermudagrass ( $P \leq 0.05$ ) at 120 days after inoculation, but only in seashore paspalum at the 60-day sampling (Table 2-2). In 2003, *B. longicaudatus* extensively stunted total root growth ( $P \leq 0.05$ ) in seashore paspalum and bermudagrass at 60 and 120 days after inoculation (Table 2-2). In both years, lengths of small diameter roots ( $< 0.02$  mm-diam.) on plants inoculated with *B. longicaudatus* were less than ( $P \leq 0.01$ )

Table 2-2. Cumulative shoot dry weight and total root lengths of 'SeaIsle 1' seashore paspalum and 'Tifdwarf' bermudagrass 60 and 120 days after initial inoculations of  $107 \pm 8$  (2002) and  $211 \pm 10$  (2003) *Belonolaimus longicaudatus*.

Cultivar	DAI	Treatment	Dry shoot weight (g)	Root length (mm)
2002 trial				
'Tifdwarf'	60	U	$6.2 \pm 3.4$	$951 \pm 212$
		I	$6.6 \pm 2.5$	$762 \pm 234$
	120	U	$13.2 \pm 3.8$	$1,597 \pm 312^*$
		I	$11.7 \pm 3.0$	$947 \pm 589$
'SeaIsle 1'	60	U	$13.9 \pm 3.2$	$1,489 \pm 287^*$
		I	$11.6 \pm 3.0$	$1,150 \pm 281$
	120	U	$24.4 \pm 6.7$	$1,647 \pm 152^{***}$
		I	$21.5 \pm 4.2$	$1,044 \pm 337$
2003 trial				
'Tifdwarf'	60	U	$4.6 \pm 1.2$	$1,525 \pm 192^*$
		I	$4.3 \pm 1.9$	$1,107 \pm 386$
	120	U	$13.3 \pm 4.0$	$2,385 \pm 468^{***}$
		I	$13.4 \pm 3.2$	$1,380 \pm 354$
'SeaIsle 1'	60	U	$7.9 \pm 2.3$	$1,602 \pm 301^{**}$
		I	$5.3 \pm 1.3$	$984 \pm 456$
	120	U	$21.6 \pm 4.5$	$2,217 \pm 315^{***}$
		I	$20.6 \pm 4.3$	$1,185 \pm 467$

Data are means of 8 (2002) or 10 (2003) replicates  $\pm$  standard deviations.

DAI = Days after inoculation.

I = inoculated and U = uninoculated.

\*, \*\*, \*\*\* Indicate uninoculated different from inoculated significant at  $P \leq 0.05$ ,  $P \leq 0.01$ , and  $P \leq 0.001$  respectively, according to the one-way analysis of variance.

uninoculated plants for both grasses and sampling dates. Lengths of small diameter roots on seashore paspalum inoculated with *B. longicaudatus* ranged from  $488.96 \pm 217.26$  to  $574.26 \pm 227.91$  mm compared to uninoculated plants having lengths ranging from  $669.82 \pm 103.53$  to  $1037.26 \pm 126.31$  mm. Bermudagrass followed a similar trend, with lengths of small diameter roots ranging from  $469.58 \pm 108.99$  to  $740.81 \pm 146.93$  mm and  $623.60 \pm 133.24$  to  $1218.87 \pm 175.42$  mm for inoculated and uninoculated plants, respectively. Cumulative shoot dry weight was not different ( $P > 0.05$ ) from controls when either turfgrass was inoculated with *B. longicaudatus*. *Belonolaimus longicaudatus*

reproduced with ease on both grasses, indicating they were suitable hosts. In both trials, *B. longicaudatus* reproduction data were variable among replicates, with bermudagrass supporting higher populations than seashore paspalum at all sampling dates ( $P \leq 0.05$ ), except the 120-day evaluation period in 2002 (Table 2-4).

Throughout the majority of sampling times in both experiments, the population of *H. galeatus* was not associated with root reduction in either grass (Table 2-3). However, in the second year of the study, *H. galeatus* did reduce root growth ( $P \leq 0.05$ ) in seashore paspalum and bermudagrass after 60 and 120 days of exposure, respectively (Table 2-3). Cumulative shoot dry weight was not different from controls when either turfgrass was inoculated with *H. galeatus*. Both grasses were suitable hosts for *H. galeatus* with moderate reproduction occurring both years. Bermudagrass was a more suitable host at two sampling dates, supporting higher soil populations than seashore paspalum ( $P \leq 0.05$ ) after 60 (2003) and 120 (2002) days of feeding and reproducing (Table 2-4). Root extraction of *H. galeatus* by Baermann tray extraction returned low recovery in 2002. However, in 2003, roots were stained through a modified acid-fuschin staining process (Byrd et al., 1983), revealing that *H. galeatus* readily entered the roots of both grasses. Nematodes were exclusively found within the root cortex and tended to aggregate in random, nonspecific areas of the roots. Final root population means were  $63 \pm 69$  and  $88 \pm 55$  per gram of root fresh weight for bermudagrass and seashore paspalum respectively. Populations of *H. galeatus* entering the roots did not indicate a difference ( $P > 0.05$ ) in host suitability between the two grasses.



Table 2-3. Cumulative shoot dry weight and total root lengths of 'SeaIsle 1' seashore paspalum and 'Tifdwarf' bermudagrass 60 and 120 days after initial inoculations of 100 (2002) and 199 ± 13 (2003) *Hoplolaimus galeatus*.

Cultivar	DAI	Treatment	Dry shoot weight (g)	Root length (mm)
2002 trial				
'Tifdwarf'	60	U	6.0 ± 1.9	927 ± 130
		I	7.2 ± 2.2	920 ± 203
	120	U	14.1 ± 3.0	936 ± 344
		I	11.9 ± 1.9	993 ± 192
'SeaIsle 1'	60	U	11.1 ± 2.0	1,244 ± 272
		I	11.5 ± 2.0	1,216 ± 257
	120	U	22.8 ± 2.9	1,170 ± 223
		I	23.0 ± 3.8	1,164 ± 275
2003 trial				
'Tifdwarf'	60	U	4.6 ± 1.2	1,525 ± 192
		I	4.6 ± 1.9	1,697 ± 490
	120	U	13.3 ± 4.0	2,385 ± 468*
		I	11.9 ± 1.8	2,050 ± 425
'SeaIsle 1'	60	U	7.9 ± 2.3	1,602 ± 301*
		I	5.9 ± 1.6	1,276 ± 350
	120	U	21.6 ± 4.5	2,217 ± 315
		I	21.0 ± 4.0	2,098 ± 410

Data are means of 8 (2002) or 10 (2003) replicates ± standard deviations.

DAI = Days after inoculation.

I = inoculated and U = uninoculated.

\* Indicate uninoculated different from inoculated significant at  $P \leq 0.05$  according to the one-way analysis of variance.

Root length percent reductions and cumulative shoot dry weight reductions calculated from root and shoot growth data were used to compare the effects of *B. longicaudatus* or *H. galeatus* between 'SeaIsle 1' seashore paspalum and 'Tifdwarf' bermudagrass. Differences in cumulative shoot dry weight percent reductions between grasses were not found ( $P > 0.05$ ) in either year for *B. longicaudatus* or *H. galeatus*. *Hoplolaimus galeatus* did not cause reductions in root growth in the 2002 trial. Therefore root length percent reductions showed no differences ( $P > 0.05$ ) between the two grasses. In the 2003 trial, *H. galeatus* did stunt seashore paspalum root growth even

though soil populations were below reported threshold levels for bermudagrass (Crow et al., 2003). Root length percent reductions demonstrated a difference ( $P \leq 0.05$ ) between the two grass species 60 days after inoculation, with seashore paspalum showing a 19.4% root length reduction, and bermudagrass showing a 14% increase in root length compared to uninoculated plants.

*Belonolaimus longicaudatus* reduced root growth in both grasses, but root length percent reductions did not indicate a difference ( $P > 0.05$ ) in susceptibility between 'SeaIsle 1' seashore paspalum and 'Tifdwarf' bermudagrass. In 2002, root length percent reduction averaged 13.7% for bermudagrass, and 20.2% for seashore paspalum at the 60-day sampling. At 120 days after inoculation, root length percent reduction averaged between 35% and 40% for both grasses. In the second year of the study, root length percent reduction after 60 days was 26.7% and 38.4% for bermudagrass and seashore paspalum respectively. At the final sampling date, root reductions continued on the same trend with root length percent reductions above 39% for both grasses.

Table 2-4. Final population densities (nematodes/100 cm<sup>3</sup> of soil) on 'SeaIsle1' seashore paspalum and 'Tifdwarf' bermudagrass 60 and 120 days after initial inoculations of 100 (2002) and 199 ± 13 (2003) *Hoplolaimus galeatus* and 107 ± 8 (2002) and 211 ± 10 (2003) *Belonolaimus longicaudatus*.

Cultivar	2002		2003	
	60 DAI	120 DAI	60 DAI	120 DAI
<i>B. longicaudatus</i>				
'Tifdwarf'	445 ± 330*	495 ± 530	273 ± 220*	1,236 ± 714*
'SeaIsle 1'	107 ± 86	148 ± 81	90 ± 97	559 ± 366
<i>H. galeatus</i>				
'Tifdwarf'	30 ± 16	312 ± 188*	58 ± 28**	171 ± 134
'SeaIsle 1'	22 ± 13	80 ± 47	15 ± 8	116 ± 33

Data are means of 8 (2002) or 10 (2003) replicates ± standard deviations.

DAI = Days after inoculation.

\*, \*\* Indicate 'Tifdwarf' different from 'SeaIsle 1' significant at  $P \leq 0.05$  and  $P \leq 0.01$  respectively, according to the one-way analysis of variance.

### Discussion

Results indicate slight differences in host suitability, but no major differences in susceptibility between the two grasses. 'Tifdwarf' bermudagrass tended to be a more suitable host for *B. longicaudatus* and *H. galeatus* throughout the experiment. Lower final population densities with root length percent reductions equal to 'Tifdwarf' bermudagrass suggest that 'SeaIsle 1' seashore paspalum could be a more susceptible or less tolerant host of *B. longicaudatus*. Since *H. galeatus* was not found to consistently cause root stunting in either grass, seashore paspalum cannot be assumed more susceptible than bermudagrass.

Population densities of *B. longicaudatus* after 120 days were very high in 2003 as opposed to 2002. Higher inoculation levels and the earlier starting date in 2003 could have resulted in enhanced reproduction. Furthermore, populations in the glasshouse may peak during the late spring and early summer months, and thereafter decline as late summer and early fall temperatures become the norm. Robbins and Barker (1974) reported that reproduction of two populations of *B. longicaudatus* were greatest at 25 to 30 °C, and minimal reproduction occurred at 20 °C. Neither grass had a reduction in cumulative top growth even though root growth was stunted by *B. longicaudatus*. Johnson (1970) and Giblin-Davis et al. (1992b) reported similar results for top growth tissue weights for 'Tifdwarf' bermudagrass. The proposed hypothesis is that root stunting caused by *B. longicaudatus* could stimulate the production of more photosynthetic material to overcome the damaged root system (Giblin-Davis et al., 1992b). In these experiments, mowing frequency and height did not simulate typical golf course mowing practices. Golf courses tend to have intense aboveground defoliation, causing an

immense amount of stress on the root system. This only amplifies the root problems that *B. longicaudatus* is already causing.

Both grass species were suitable hosts for the nematodes. The lack of root stunting caused by *H. galeatus* is very different from the reaction of the two grasses to the more virulent nematode, *B. longicaudatus*. *Hoplolaimus galeatus* caused a slight root reduction in both grasses in the second year of the study, even though reproduction appeared to be relatively low. In February and March 2003, during the 60-day experimental time period, temperatures and day-lengths were not optimal for warm-season turfgrass growth. These environmental factors may have slowed root growth and allowed the higher inoculum levels of *H. galeatus* to reduce root lengths compared to uninoculated controls. In 2003, statistical analysis showed that there was a blocking effect ( $P \leq 0.05$ ) in the *H. galeatus* 120-day bermudagrass root length data. A significant blocking effect means the randomized complete block design was useful and accounted for confounding factors in the glasshouse. Time course experiments with 'Floritam' and 'FX-313' St. Augustinegrass indicated that *H. galeatus* had no effect on plant growth even though soil counts of *H. galeatus* exceeded 40 nematodes/100 cm<sup>3</sup> of soil, the proposed action threshold (Crow et al., 2003), for 84 days within the course of the experiment (Giblin-Davis et al., 1995).

Tarjan and Busey (1985) reported that 'Tifdwarf' and 'Tifgreen' bermudagrass inoculated with a mixed sample of phytoparasitic nematodes (including *B. longicaudatus* and *H. galeatus*) caused 36% to 39% root dry weight reductions. These root reductions appeared to correlate with increases in *H. galeatus* population levels. In our experiments, *B. longicaudatus* alone caused approximately the same amount of root reduction, while

*H. galeatus* failed to consistently affect root growth. However, *H. galeatus* was able to freely enter the roots of both grasses. In field situations, many other pathogens and climatic stresses can enhance the pathogenic effects of nematodes on turfgrasses. Glasshouse and laboratory work with soybean suggested that greater plant damage resulted from inoculation with both *H. galeatus* and fungi in the genera *Rhizoctonia*, *Fusarium*, and *Macrophomina* than with either pathogen alone (McGawley et al., 1984). Further investigation is needed to determine if *H. galeatus* may be having a synergistic effect with other pathogens and/or nematodes in the soil environment in causing damage to turfgrass roots.

In conclusion, our research suggests that the susceptibility of 'SeaIsle 1' seashore paspalum to *B. longicaudatus* and *H. galeatus* is not less than 'Tifdwarf' bermudagrass. There are limitations in extrapolating damaging thresholds from glasshouse pot experiments to field situations, consequently action thresholds cannot be predicted from our data. Glasshouse pot experiments eliminate the ability of climatic stresses and pathogens other than the plant-parasitic nematodes to affect plant growth. Exposing the plant to one pathogen and/or stress at a time allows us to determine damage potential exclusive of all other negative effects on plant growth. Further experimentation is needed to determine the effects of *B. longicaudatus* and *H. galeatus* on seashore paspalum growth in field conditions.

CHAPTER 3  
INFLUENCE OF SALT CONCENTRATION IN IRRIGATION WATER ON  
PATHOGENICITY AND DEVELOPMENT OF *Belonolaimus longicaudatus* AND  
*Hoplolaimus galeatus* ON SEASHORE PASPALUM

Introduction

Salt tolerant turfgrasses are becoming essential in many areas because of salt accumulation in soil, restrictions on groundwater use, and salt-water intrusion into groundwater (Carrow and Duncan, 1998; Parker, 1975). Seashore paspalum (*Paspalum vaginatum* Swartz) is a warm-season turfgrass adapted for saline conditions (Malcolm and Laing, 1969; Morton, 1973). Breeding for cultivars with fine leaf texture, and tolerance to drought and high salinity irrigation has allowed seashore paspalum to become frequently used in highly managed turfgrass sites (Dudeck and Peacock, 1985; Duncan, 1999a). One major limitation of cultivating turfgrasses in the sandy soils of the southeastern United States is the destruction of roots by phytoparasitic nematodes (Perry and Rhoades, 1982). The sting nematode (*Belonolaimus longicaudatus* Rau) and the lance nematode (*Hoplolaimus galeatus* (Cobb) Thorne) are destructive pathogens on a variety of agronomic crops and turfgrasses (Ahmad and Chen, 1980; Perry and Rhoades, 1982; Perry et al., 1970; Smart and Nguyen, 1991).

While *B. longicaudatus* is usually limited to the coastal plains of the southeastern United States (Christie, 1959; Holdeman, 1955; Robbins and Barker, 1974), *H. galeatus* has a much wider distribution (Williams, 1973). *Belonolaimus longicaudatus* damages lateral roots as soon as they are formed, causing stunted root growth, decreased water and nutrient uptake, and decreased rates of evapotranspiration (Busey et al., 1991; Johnson,

1970; Perry and Rhoades, 1982). *Hoplolaimus galeatus* enters the root cortex and may damage the roots by feeding and physical tunneling through the cell walls (Krusberg and Sasser, 1956; Williams, 1973). Both nematodes have been reported as important pathogens of turfgrasses in the southeastern United States (Christie et al., 1954; Kelsheimer and Overman, 1953; Perry and Rhoades, 1982). *Belonolaimus longicaudatus* and *H. galeatus* have been reported as pathogens of many bermudagrass (*Cynodon dactylon* (L.) Pers. and *Cynodon* spp. hybrids) and St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze) cultivars (Busey et al., 1991; Giblin-Davis et al., 1992a; 1992b; Perry et al., 1970). In more recent studies, root and shoot growth of diploid and polyploid St. Augustinegrasses were not affected by *H. galeatus*, even though the plants supported high population levels (Giblin-Davis et al., 1995; Henn and Dunn, 1989). Two populations of *B. longicaudatus* readily reproduced on 'Tifdwarf' bermudagrass, and caused extensive root damage (Giblin-Davis et al., 1992a, Johnson, 1970). A forage grass study determined that there is differential host suitability and susceptibility to *B. longicaudatus* in *Digitaria* spp., *Paspalum* spp., and *Chloris* spp. introductions (Boyd and Perry, 1969).

Post-plant nematicides labeled for turfgrass are becoming limited and alternatives must be found to replace recently discontinued nematicides. Salinity had a detrimental effect on population densities of nematodes on some annual crops (Edongali et al., 1982; Heald and Heilman, 1971). Soil salinity has demonstrated negative effects on the hatching and infectivity of *Meloidogyne incognita* Kofoid and White, *M. javanica* (Treub) Chitwood, and *M. arenaria* (Neal) Chitwood juveniles (Bird, 1977; Dropkin et al., 1958; Lal and Yadav, 1975; Maqbool et al., 1987). Khan and Khan (1990) found that

after only 7 days of salinity exposure, *M. incognita* and *M. javanica* had reductions in hatching and increased mortality. Population levels of *Aphelenchus avenae* Bastain, *Pratylenchus thornei* Sher and Allen, *Helicotylenchus* spp., and *Rotylenchulus reniformis* Linford and Oliveira were found to be lower at increased salt concentrations compared to nonsaline treatments (Lal and Yadav, 1976). Some species of root-knot nematodes (*M. spartinae* Rau and Fassuliotis) and sting nematodes (*B. maritimus* Rau) may be well adapted to high-salinity conditions (Rau, 1963; Rau and Fassuliotis, 1965). After personal communication with two turfgrass managers in south Florida, Morton (1973) hypothesized that low nematode counts may be associated with seashore paspalum irrigated with high saline water. Further investigation is necessary to confirm this hypothesis.

Seashore paspalum is known to be tolerant of high salinity irrigation, but the reaction of nematodes parasitizing seashore paspalum to the salinity is unknown. Therefore, an experiment was performed to examine the effects of salinity irrigation on *B. longicaudatus* and *H. galeatus*. Objectives of the experiment were to: i) determine the effects of high salinity irrigation on the reproduction of a population of *B. longicaudatus* and a population of *H. galeatus*, ii) establish simple models relating irrigation salinity levels to population levels of *B. longicaudatus* and to population levels of *H. galeatus*, iii) compare the effects of high salinity irrigation alone to the combined effects of high salinity irrigation and *B. longicaudatus* on shoot and root growth.

#### Materials and Methods

Four separate experiments were performed, one with *B. longicaudatus* and one with *H. galeatus* in each of two years (2002, 2003). Experiments were conducted in 2002



from April to September and repeated in the spring and summer of 2003 at the University of Florida Turfgrass Envirotron Glasshouse in Gainesville, FL.

In preparation for these experiments, 'SeaIsle 1' seashore paspalum, a commercially available cultivar of *Paspalum vaginatum*, was obtained from R. R. Duncan at the University of Georgia. Nematode-free plugs of grass were obtained by rooting aerial cuttings of stolons from each grass in tapered RLC-7 (UV Stabilized) Super "Stubby" Cells (cell depth = 14 cm; diam. = 3.8 cm; volume = 115 ml) (Ray Leach Single Cell Cone-tainer, Oregon) filled with 140 g of uninfested soil. Soil used for growth media consisted of 100% United States Golf Association (USGA) sand. The soil texture was analyzed using the sieving method for testing a USGA root zone mix (USGA Green Section Staff, 1993) (Table 2-1). An absorbent cotton ball was placed at the bottom of each cell to prevent soil from escaping through the drain holes. The soil was then thoroughly wetted to allow for settling.

A depression was made in each cell and two aerial stolons were planted on opposing sides of the depression. Stolons (5 to 8 cm long) were terminal cuttings with two or three nodes. During the fall and winter months, the cells were placed on a glasshouse bench 1.25 m below an enclosed high bay 1,000-watt metal halide growth lamp (Hi-Tek Series, Lithonia Lighting, Conyers, GA) set on a 12-hour cycle to simulate the longer daylengths required for optimal growth. The grass was fertilized using a fertilizer solution (20%-20%-20% (N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O) plus trace elements) (Peters Professional All-Purpose Plant Food, Spectrum Brands, St. Louis, MO) at a rate equivalent to 49.0 kg N/ha/month, 21.6 kg P/ha/month, and 40.7 kg K/ha/month. A substantial root system was allowed to develop for four weeks.

Plugs of seashore paspalum obtained from the cells were transferred into 14.5 × 16-cm-diam. clay pots (1,500 cm<sup>3</sup>) filled with 100% USGA specification sand (Table 2.1). Insect screening (7 × 5 lines/cm<sup>2</sup>) was cut into 5 × 5-cm squares and placed at the bottom of each pot to prevent soil from escaping through the drain hole. Roots were washed free of soil and trimmed to approximately 5 cm below the crown to promote fresh root growth. Two depressions were made in each pot on opposite sides and two plugs of turfgrass were planted per pot. These experimental units were placed in an environmentally controlled glasshouse and irrigated with tap water as needed for 14 days to allow for adjustment to the new environment.

A population of *B. longicaudatus* originally from unmanaged field soil in the Sanford, FL area was obtained from R. M. Giblin-Davis at the Fort Lauderdale, FL Research and Education Center and allowed to reproduce on 'FX 313' St. Augustinegrass. A population of *H. galeatus* was obtained from a 'Floradwarf' bermudagrass putting green at the G. C. Horn Turfgrass Field Laboratory in Gainesville, FL. Inocula were extracted from soil using a modified Baermann funnel method (McSorley and Federick, 1991). In 2002, the *H. galeatus* population was contaminated with other plant-parasitic nematodes, therefore handpicking was necessary to obtain an uncontaminated population. One hundred *H. galeatus* of mixed life stages were inoculated into each pot of seashore paspalum. Since the *B. longicaudatus* population was free of other plant-parasitic nematodes, handpicking was not necessary. A solution of *B. longicaudatus* at various life stages and tap water was calibrated by counting nematodes from 1-ml aliquots on a grided counting slide (Hawksley and Sons Limited, Lancing, Sussex, United Kingdom) replicated ten times. Approximate numbers of nematodes were measured with a pipette

from water suspensions of inocula. A total of  $111 \pm 16$  *B. longicaudatus* were added to each of the inoculated pots. In 2003, *H. galeatus* and *B. longicaudatus* were obtained from the previous year's experiment and again, inocula was obtained using a modified Baermann funnel method (McSorley and Federick, 1991). Solutions were made for each nematode and standardized to deliver approximately 250 nematodes per pot. A total of  $243 \pm 13$  *B. longicaudatus* per pot and  $238 \pm 8$  *H. galeatus* per pot were inoculated for two separate experiments. A higher level of nematodes was used in the second year to achieve higher reproduction. In both years, nematodes were suspended in 50 ml of tap water and equally distributed into four cavities formed in the soil near the base of the plant. After inoculation, the cavities were then pinched closed with surrounding soil. Uninoculated controls received 50 ml of tap water. Tap water was applied as needed for two weeks to allow nematodes to adjust to their new environment before experimental treatments were initiated. The 30 pots inoculated with each nematode were then separated into five randomized blocks and treated identically except for salt concentration in irrigation water. Separate experiments were performed for each nematode with each experiment having six treatments with five replications.

Salinity irrigation treatments were formulated using Instant Ocean Synthetic Sea Salt (Aquarium Systems, Inc., Mentor, OH). Ionic composition of Instant Ocean is primarily  $\text{Na}^+$  and  $\text{Cl}^-$  and designed to mimic closely that of seawater (Atkinson and Bingman, 1998; He and Cramer, 1992). Six 50-liter carboys were used to mix salinity treatments. A 3-liter plastic laboratory pitcher filled with 2 liters of deionized water, and a 6-cm stir bar was used with a magnetic stirrer to get the artificial sea salt into solution. After adding the concentrated solution to each carboy, they were diluted to 35 liters with

deionized water. An electrical conductivity meter (YSI Incorporated, Yellow Springs, OH) equipped with a  $14.6 \times 1.3$ -cm-diam. dip-type plastic cell was used to test the accuracy of each treatment. The electrical conductivity meter was calibrated using a 10 dS/m standard solution and temperature chart. More salt or deionized water was added to adjust the electrical conductivity to the proper treatment level.

In 2002, irrigation treatments were deionized water concentrated to five salinity levels, (5, 10, 25, 40, and 55 dS/m) and deionized water (0 dS/m) to serve as a control. Irrigation treatments were adjusted in 2003 reflecting the results of the 2002 trials and were formulated by concentrating deionized water to five salinity levels, (5, 10, 15, 20, and 25 dS/m) and deionized water to serve as a control. Each day, 150 ml of each irrigation treatment was applied to the appropriate pots, excluding days before and after leaching events. The experimental units were leached on a weekly (2002) or biweekly (2003) basis to prevent build up of salts and to deliver fertilizer without changing treatment levels. 'SeaIsle 1' seashore paspalum was fertilized on a weekly basis with 20 ml of a solution consisting of 5,100 mg  $\text{NH}_4\text{NO}_3$  (34% N), 6,354 mg KCl, 252 mg  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ , 435 mg  $\text{CaSO}_4$ , 246 mg  $\text{MgSO}_4$ , 1.55 mg  $\text{H}_3\text{BO}_3$ , 0.34 mg  $\text{MnSO}_4$ , 0.58 mg  $\text{ZnSO}_4$ , 0.13  $\text{CuSO}_4$ , and 3.5 mg  $\text{FeSO}_4$  per 1 liter of deionized water. In 2003, 40 ml of the fertilizer solution was applied on a biweekly basis. Since salinity levels were lower in the 2003 experiment, leaching was done every two weeks as opposed to every week. Total N applied for the duration of the experiment was 624 mg/pot. Liquid fertilizer was added to 580 ml or 560 ml of deionized water and varying amounts of artificial sea salt was added to concentrate the solution to each irrigation treatment. All 600 ml of solution was poured into each pot to flush out residual salts, and replace with fertilizer solution.

During the course of the 2002 experiments, which lasted from 30 May 2002 to 10 October 2002, average monthly high and low air temperatures in the glasshouse ranged from 29 to 34 °C, and 21 to 26 °C, respectively. In 2003, the *B. longicaudatus* experiment began 8 April 2003 and ended 6 August 2003 and the *H. galeatus* experiment began 23 May 2003 and ended 20 September 2003. Average monthly high and low air temperatures ranged from 28 to 34 °C and 22 to 27 °C, respectively. Using a 1.18-liter pump sprayer, an insecticide/miticide (Mavrik Aquaflow, Wellmark International, Schaumburg, IL) was applied at the labeled rate twice during the course of 2003 experiments for control of bermudagrass mites (*Eriophyes cynodoniensis* Sayed).

After 120 days, experimental units were brought into the laboratory for analysis. The contents of each pot were emptied into their individually labeled plastic bags and thoroughly hand mixed. A 100-cm<sup>3</sup> soil sample was taken from each bag and processed using a modified centrifugal-sugar flotation technique (Jenkins, 1964). Nematodes were counted from the entire sub-sample using an inverted light microscope at × 32 magnification. For the 2003 *B. longicaudatus* trial, second-stage juveniles were counted separately from the other life stages. The two categories were summed to determine the total population of each soil sample. In addition to soil extraction, *H. galeatus* in roots from the 2003 experiment were staged and counted to determine number of nematodes/gram of root in fresh-weighed samples using a modified acid-fuchsin staining-destaining procedure (Byrd et al., 1983). Effects of salinity were evaluated by regressing log transformations of final nematode population densities on salinity irrigation levels.

In 2003, the sting nematode portion of the experiment was adjusted because *B. longicaudatus* was determined to stunt root growth in seashore paspalum (Chapter 2).

Effects of high salinity irrigation alone were compared to the combined effect of high salinity irrigation and *B. longicaudatus* on shoot and root growth. Thirty additional experimental units were irrigated with salinity treatments and left uninoculated to serve as controls. Using fabric scissors (Fiskars Brand Inc., Madison, WI), the grass was trimmed biweekly to approximately 2 cm above the soil surface for all experiments. In 2003, tissue clippings were only collected from the sting nematode experiment. Tissue was placed in 15 cm × 23 cm catalog envelopes (Quality Park products, St. Paul, MN) using a spouted 2.84-liter sample pan (40.6 cm × 30.5 cm × 5 cm) (Seedburo, Chicago, IL), and dried at 75 °C for 48 hours to obtain cumulative shoot dry weight.

Final destructive analysis also was adjusted from the previous years' experiments, with the inclusion of uninoculated treatments in the *B. longicaudatus* experiment. Shoots were trimmed as close to the soil as possible and saved for cumulative shoot dry weight analysis. Using a stainless steel T-sampling tool, a root core (approximately 4-cm-diam. × 14 cm deep) was taken from the center of each pot to compare root lengths at each salinity level with inoculated treatments. Root cores were washed free of soil on a sieve with 1.7-mm pore openings nested within a sieve with 75- $\mu$ m pore openings. Roots were removed from any aboveground growth and placed into 50-ml disposable plastic centrifuge tubes containing 30 ml of tap water. The 75- $\mu$ m-pore sieve was then submerged in 5 cm of tap water to allow the finer roots to float out and separate from the soil. These fine roots were collected using laboratory forceps and placed into the 50-ml plastic centrifuge tubes. Five drops (0.25 ml) of a 1% methylene blue mixture was added to the 30 ml of tap water to stain the roots. After a minimum of 24 hours in the solution, the roots were removed, placed on a 75- $\mu$ m-pore sieve, and washed free of excess dye.

Stained roots were placed into a glass-bottom tray and scanned using a HP ScanJet 2cx desktop scanner (Hewlett Packard, Boise, ID) to create a black and white bitmap image of the roots (Kaspar and Ewing, 1997; Pan and Bolton, 1991). The GSRoot (Louisiana State University, Baton Rouge, LA) software program was used to analyze the bitmap images. This program measures root lengths and surface areas from scanned images. Root length data were recorded for seven diameter ranges (< 0.05 mm, 0.05 to 0.10 mm, 0.10 to 0.20 mm, 0.20 to 0.30 mm, 0.30 to 0.40 mm, 0.40 to 0.50 mm, and > 0.50 mm). The resulting values were summed to determine the total root length of each root sample.

In 2003, total root length measurements and cumulative shoot dry weights from the sting nematode experiment were compared at each salinity level using a one-way analysis of variance (ANOVA) procedure. Inoculated plants were compared to uninoculated at each salinity level to determine if root and/or shoot growth reduction occurred.

Orthogonal contrasts were used to compare groups of final population means or individual means for selected sets of treatments. In our experiments, the highest three salinity treatments were compared to the lower three treatments, and selected individual treatments (10 dS/m and 15 dS/m) were compared to the 0 dS/m. Transformation by  $\log_{10} x$  was performed to normalize nematode-count data and achieve a better trendline fit (Proctor and Marks, 1975). Using SAS software, (SAS Institute, Cary, NC) the quadratic least squares procedure was used to fit a linear or quadratic model to the data relating log-transformations of final nematode population levels to salinity irrigation treatment levels. Linear and quadratic regressions were drawn using Excel software (Microsoft Corporation, Redmond, WA). One-way ANOVA procedure, quadratic least squares procedure, and orthogonal contrasts were performed using SAS software.

## Results

Reproduction of *H. galeatus* and *B. longicaudatus* was affected by increasing salinity levels (Figs. 3.1, 3.2). Final populations of *H. galeatus* decreased linearly ( $P \leq 0.01$ ) with increasing salinity irrigation treatments (Fig. 3-1). The high  $R^2$ -values, 0.92 (2002) and 0.83 (2003), indicate that the data followed the linear regression lines very closely. Lower salinity treatments, 0 and 5 dS/m, resulted in higher nematode reproduction than the higher salinity irrigation treatments (Fig. 3-1). Final population densities were very low at the treatment level of 25 dS/m in both years. Treatment levels of 40 and 55 dS/m were included in the first year of the study and resulted in population levels at or near zero. Final population means of *H. galeatus* were  $1 \pm 1.4$  and  $2 \pm 1.4/100 \text{ cm}^3$  of soil for the 40 and 55 dS/m treatments respectively. The ability of *H. galeatus* to enter the root cortex as migratory endoparasites also decreased as salinity levels increased. Final population densities within the root cortex were lower ( $P \leq 0.05$ ) in the 15, 20, and 25 dS/m treatments ( $19 \pm 13.6 \text{ H. galeatus/gram}$  of root fresh weight) when compared to the 0, 5, and 10 dS/m treatments ( $167 \pm 146.9 \text{ H. galeatus/gram}$  of root fresh weight).

The relationship between final population densities of *B. longicaudatus* and salinity treatment levels demonstrated a quadratic regression curve in both years of the experiment (Fig 3-2). The quadratic regression was clearly defined when final population data were transformed using  $\log_{10} x$  (Fig. 3-2). Non-transformed data also showed a quadratic regression (Data not shown), but log transformation improved  $R^2$ -values and goodness of fit. In 2002, final populations of *B. longicaudatus* demonstrated a quadratic relationship from 0 to 25 dS/m, with the 25 dS/m treatment supporting a lower



population level than the 5 and 10 dS/m treatments ( $P \leq 0.01$ ). In both years, the 10 dS/m treatment supported a higher population of *B. longicaudatus* than the 0 dS/m treatment ( $P \leq 0.05$ ) causing the regression lines to be quadratic (Figure 3.2). Treatment levels of 40 and 55 dS/m were included in the first year of the study and again resulted in population levels at or near zero. *Belonolaimus longicaudatus* final population means were  $3 \pm 3.7$  and  $< 1/100 \text{ cm}^3$  of soil for the 40 and 55 dS/m treatments respectively. In each trial, the orthogonal contrast test revealed that *B. longicaudatus* final population levels in the 25 dS/m treatment were lower ( $P \leq 0.01$ ) than those in the less saline treatments.

Final *B. longicaudatus* population densities were higher at 10 and 15 dS/m than 0 dS/m ( $P \leq 0.05$ ) in the second year of the study (Fig. 3.2). At 15 and 20 dS/m, second-stage juveniles (J2) were the predominant portions of the overall population, making up an average of 68 and 60% of the total population, respectively (Fig. 3.3). At lower salinity levels (0, 5, and 10 dS/m), adults, third-stage juveniles (J3), and fourth-stage juveniles (J4), were the larger portions of the final population densities (Fig. 3.3). Shoot growth was not affected ( $P > 0.05$ ) by inoculation with *B. longicaudatus*. Root length comparisons revealed that *B. longicaudatus* caused root reduction ( $P \leq 0.05$ ) at low salinity levels, 0 to 10 dS/m. However at higher salinity irrigation treatments, 15 to 25 dS/m, root lengths were not different ( $P > 0.05$ ) between inoculated and uninoculated plants (Fig. 3-4). These results indicate that the ability of *B. longicaudatus* to stunt root growth was negatively affected at salinity levels of 15 dS/m and above.

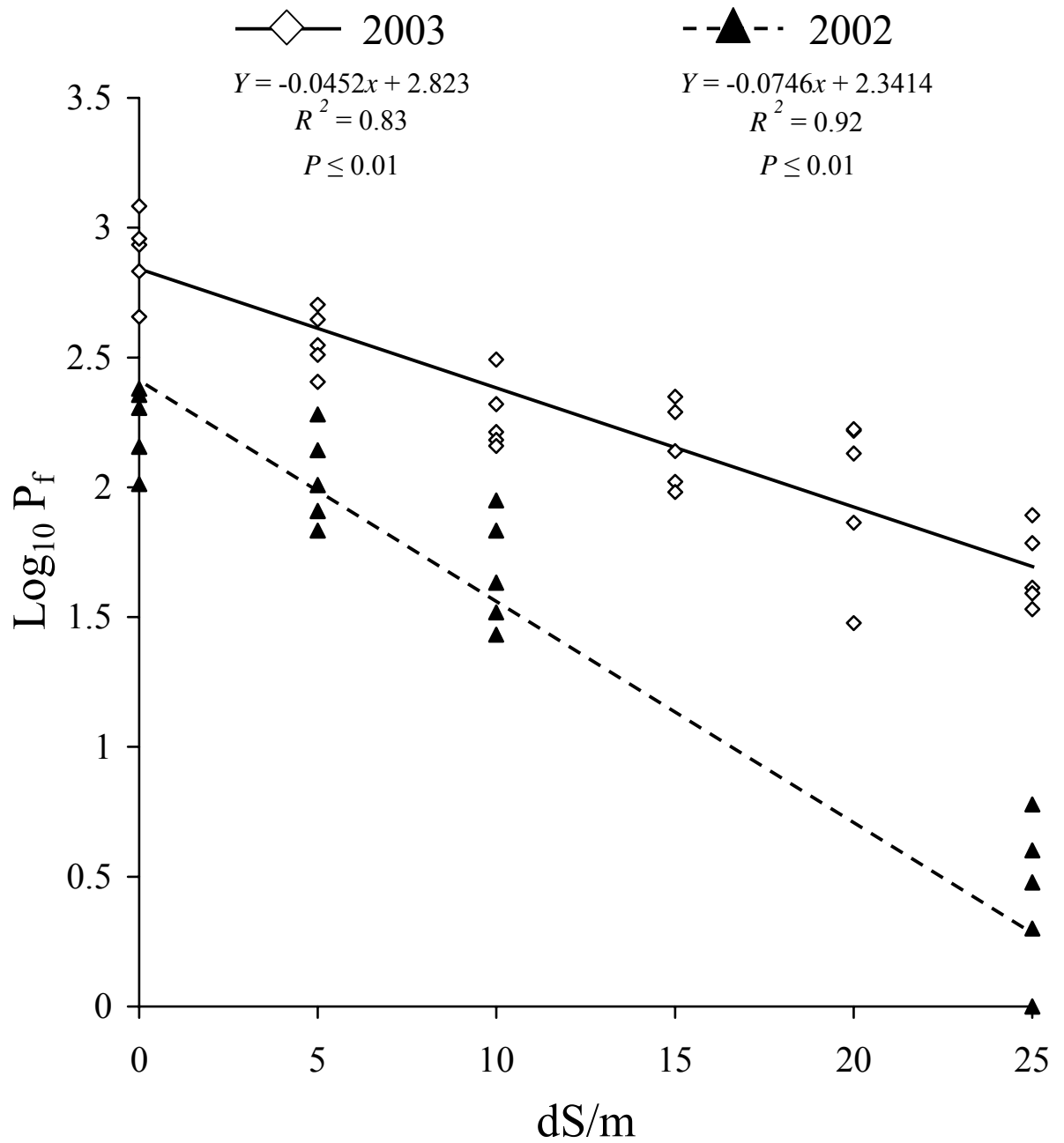


Fig. 3-1. Relationship between log transformation of final population densities ( $P_f$ ) of *H. galeatus* (nematodes/100 cm<sup>3</sup> of soil) ( $Y$ ) and salinity treatment ( $x$ ) in 2002 and 2003 glasshouse experiments.



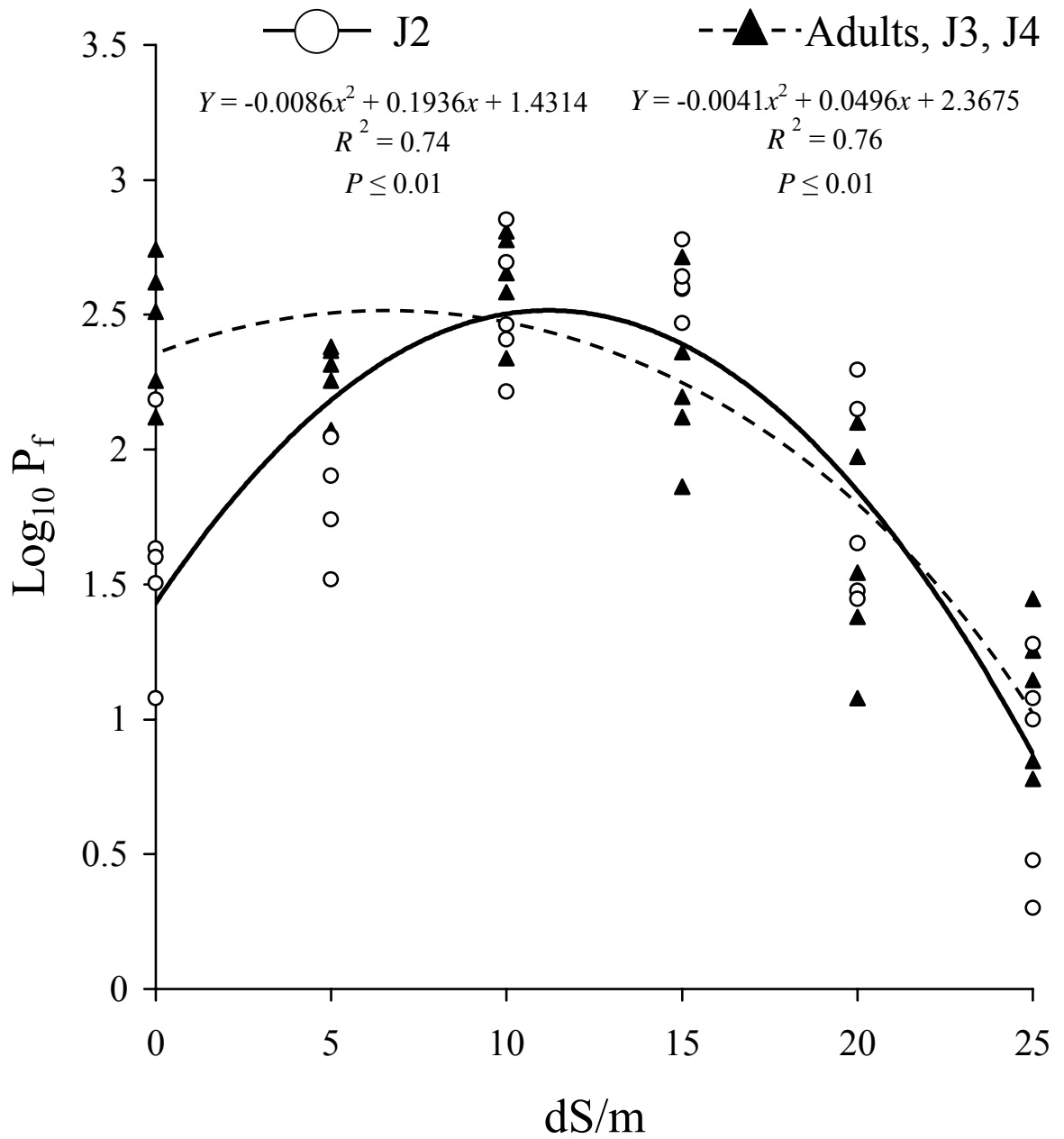


Fig. 3-3. Relationship between log transformation of J2 or adults, J3, and J4 final population densities ( $P_f$ ) of *B. longicaudatus* (nematodes/100 cm<sup>3</sup> of soil) ( $Y$ ) and salinity treatment ( $x$ ) in the 2003 glasshouse experiment.

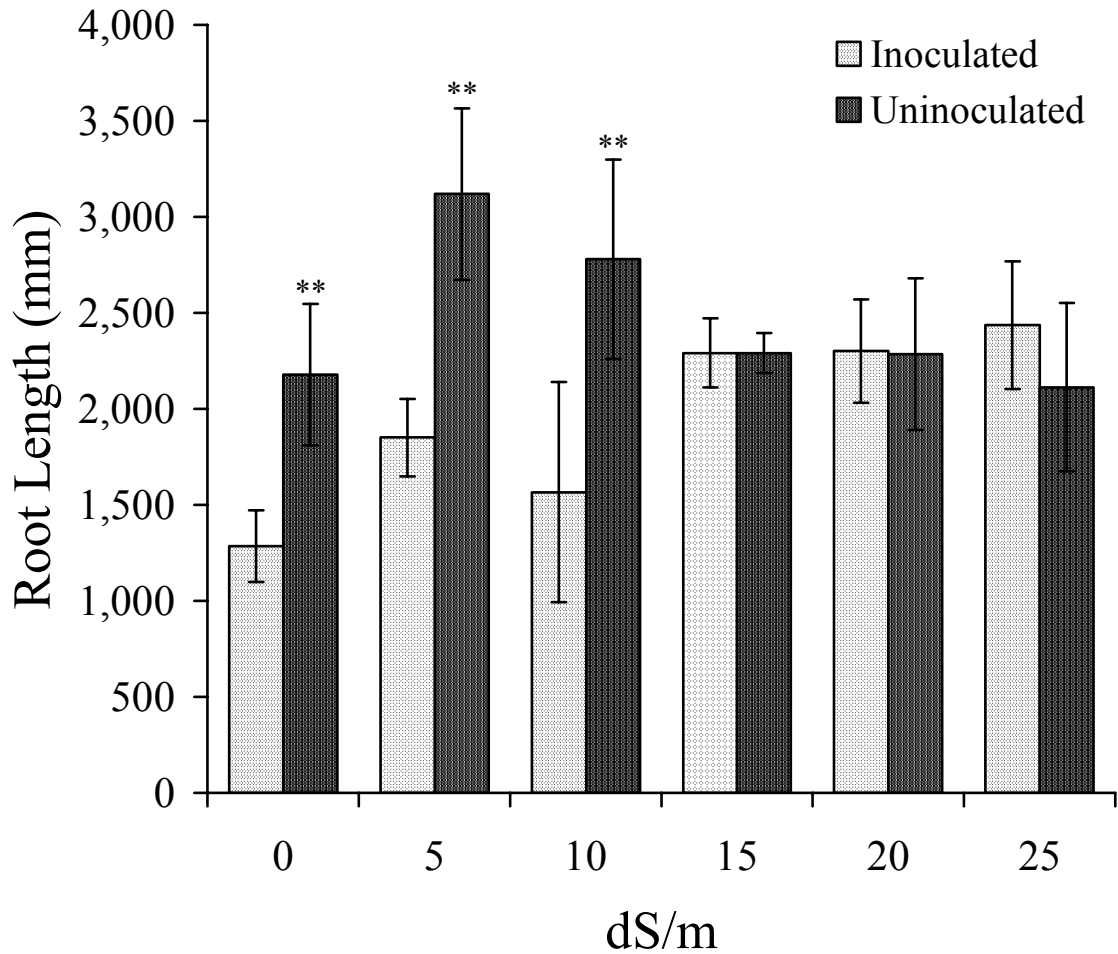


Fig. 3-4. Effects of *Belonolaimus longicaudatus* on root length of 'Seasle 1' seashore paspalum (*Paspalum vaginatum*) at increasing salinity levels. Inoculated plants received  $243 \pm 13$  *B. longicaudatus*, while uninoculated plants received no nematodes. The error bars show standard deviation of individual population means. \*\* Indicates difference from inoculated at  $P \leq 0.01$ , according to the one-way ANOVA procedure.

#### Discussion

Irrigation with poor quality water is becoming more common as increasing water restrictions are forcing turfgrass managers to obtain water from alternative sources. In our experiments, high salinity irrigation affected these particular populations of the two nematodes differently. Lower salinity levels (5, 10, and 15 dS/m) caused an increase in *B. longicaudatus* reproduction, while the population of *H. galeatus* steadily decreased as

salinity levels increased. Irrigation with low salinity levels (5 and 10 dS/m) resulted in denser, more vigorous root and shoot growth than the 0 dS/m treatment. Irrigating with deionized water may have caused nutrient deficiencies and elevated evapotranspiration rates. With increased root growth, more feeding sites were available for *B.*

*longicaudatus*.

In 2003, second-stage juveniles (J2) of *B. longicaudatus* comprised a majority of the population in the 15 and 20 dS/m treatments. The abundance of J2 at moderate salinity levels resulted in elevated total population numbers compared to lower salinity levels, causing the regression line to be quadratic (Fig. 3.2, 3.3). The nematodes counted as J2 had a clear body cavity indicating they were probably unable to feed. Usually, J2 can easily be separated from other life stages by their dark color and stout body shape (Han, 2001). Root length data in 2003 support this hypothesis, with root reduction not occurring at the 15, 20, or 25 dS/m treatments as opposed to the lower salinity treatment levels (Fig. 3.4). Reproduction and maturation of the nematodes at higher salinity treatments probably occurred early in the experiment before the salinity was able to build up in the soil. Han (2001) stated that it is unknown whether or not feeding is necessary for J2 to develop into J3. However, J2 began molting into J3 3 to 5 days after hatching (Han, 2001).

Shoot growth was not affected by inoculations with *B. longicaudatus*, but at salinity levels above 10 dS/m, shoot growth became less vigorous. In a previous study, shoot growth began to decline in response to soil electrical conductivity levels  $\geq 8$  dS/m, with shoot growth being reduced by 25% and 50% at 14 and 34 dS/m, respectively (Lee, 2000). Cumulative shoot dry weight data collected from previous susceptibility

experiments also showed that inoculation with *B. longicaudatus* did not affect aboveground plant growth (Chapter 2). Salinity treatment levels equaling 25 dS/m and above reduced *B. longicaudatus* populations to extremely low levels in 2002, but these high salinity levels had detrimental effect on the growth of the grass. In 2003, inoculum levels were much higher and the same quadratic regression occurred, with 25 dS/m reducing reproductions compared to treatment levels ranging from 0 to 15 dS/m ( $P \leq 0.05$ ).

*Hoplolaimus galeatus* are classified as migratory endoparasites on turfgrasses. Their ability to enter the roots gives them the capability to escape the effects of most nematicides (Giblin-Davis et al., 1995). In our experiment, both soil and root populations were reduced as salinity irrigation levels increased from 0 to 25 dS/m. The nematodes were probably not able to escape the effects of the salinity by entering the roots because the root cortex tissue does not exclude the elevated ion concentrations associated with saline water. Published action thresholds that justify post-plant nematicide treatment for *H. galeatus* on bermudagrass are 40/100 cm<sup>3</sup> of soil (Crow et al., 2003). Soil populations more than exceeded these numbers for both experiments at 15 dS/m and less. In previous experiments, *H. galeatus* had no effect on seashore paspalum growth even though soil counts exceeded 40/100 cm<sup>3</sup> of soil throughout the experiments (Chapter 2). Time course experiments (Giblin-Davis et al., 1995) with 'Floritam' and 'FX-313' St. Augustinegrass also indicated that *H. galeatus* had no effect on plant growth.

In 2002, treatment levels of 40 and 55 dS/m caused near complete mortality for both nematodes, but the shoot growth of the grass was stunted and yellowed. Even though *B. longicaudatus* and *H. galeatus* were effectively controlled, the turfgrass was

not visually acceptable. Seashore paspalum can be irrigated with seawater (54 dS/m) in the field when soil conditions allow for sufficient leaching to occur and turfgrass managers fertilize, amend, and cultivate the soil properly (Carrow and Duncan, 1998). In our experiments, prolonged exposure to these high salinity levels was detrimental to turfgrass quality. In the glasshouse, we were unable to provide sufficient leaching, proper amendments, and cultivation of soil necessary for seashore paspalum survival at salinity levels near that of seawater.

Results from glasshouse experiments are difficult to extrapolate to field conditions, but we can conclude that salinity irrigation had an effect on *B. longicaudatus* and *H. galeatus* nematode reproduction. The treatment salinity levels were at a continuous level throughout the experiment, and never allowed the nematodes to recover from salinity stress. A discontinuous high salinity irrigation situation would probably be more similar to irrigation with poor quality water under field conditions where rainfall can leach salt from the soil profile (Mashela et al., 1992a). Irrigation with pure seawater or with seawater as a high percentage of the blended irrigation water may have potential as an effective option for control of *B. longicaudatus* and *H. galeatus*. This information may be vital to turfgrass managers currently maintaining seashore paspalum known to have a nematode problem. Further investigation is necessary to determine if frequency and timing of high salinity irrigation, in addition to amount of salinity, can have an effect on nematode reproduction and feeding.



CHAPTER 4  
SURVEY OF PLANT-PARASITIC NEMATODES ASSOCIATED WITH SEASHORE  
PASPALUM

Introduction

New turfgrass cultivars or ecotypes are commonly introduced into the turfgrass industry, but in rare cases, a new species of turfgrass begins to become more widely utilized. Seashore paspalum (*Paspalum vaginatum* Swartz) is a warm season grass adapted for saline conditions (Malcolm and Laing, 1969; Morton, 1973). Breeding for fine leaf textured cultivars, and its natural tolerance to drought and high salinity irrigation has allowed seashore paspalum to become frequently used in highly managed turfgrass sites (Dudeck and Peacock, 1985; Duncan, 1999a). One major limitation of cultivating turfgrasses in the sandy soils of the southeastern United States is the destruction of roots by plant-parasitic nematodes (Perry and Rhoades, 1982).

Surveys are necessary to determine the distribution, frequency, and abundance of phytoparasitic nematodes associated with particular plants. These surveys provide indispensable information on the probability and severity of crop losses due to plant-parasitic nematode damage. Results of surveys can help to determine more confident action thresholds allowing the grower to use soil and root nematode assays to formulate a management strategy. This will help avoid unnecessary post-plant nematicide treatments or failure to apply a needed one. High population levels of some plant-parasitic nematode genera can cause significant damage to turfgrass areas (Di Edwardo, 1963; Johnson, 1970; Lucas et al., 1974; Winchester and Burt, 1964; Rhoades, 1962; 1965;

Kelsheimer and Overman, 1953, Christie et al., 1954). Therefore, a survey was conducted for golf courses and home lawns with established seashore paspalum in Florida. This survey was performed to determine the plant-parasitic nematodes commonly associated with seashore paspalum.

#### Materials and Methods

A majority of the survey soil samples were taken during the spring and summer months of 2002 and 2003 from coastal areas in central and south Florida. Soil samples were collected from golf courses in or near Fort Myers, Miami Beach, Delray Beach, Vero Beach, and Naples, FL. Home lawn samples were collected from many areas in central and south Florida, primarily in the Tampa, Miami, Fort Myers, Naples, and Daytona Beach areas. Seashore paspalum is well adapted to these areas because of its tolerance to high salinity irrigation and saltwater intrusion (Malcolm and Laing, 1969; Morton, 1973). Areas sampled were in close proximity to each coast and all samples were from sandy soils. A total of 20 home lawns and 8 golf courses were sampled. These sample sizes may appear low for a survey, but a high percentage of golf courses and home lawns growing seashore paspalum in Florida were sampled. According to a United States Golf Association Florida Regional Agronomist, 12 to 15 golf courses in Florida are currently growing seashore paspalum on > 90% of their maintained areas (Lowe, pers. comm.). A non-biased approach was practiced when selecting the home lawns and golf courses to be sampled. At each golf course, four greens and four fairways were individually sampled for plant-parasitic nematodes. Two golf courses were not growing seashore paspalum over the entire course, thus fewer samples were collected. Home lawns were sampled separately for plant-parasitic nematodes. Two to seven samples were taken at each lawn. More samples were collected from larger lawns and

lawns with noticeable aboveground damage. A 2.5-cm-diam. cone-shaped soil sampler was used to collect 20 soil cores (2.5-cm-diam × 10 cm deep) at approximately equal intervals in a zig-zag pattern across the sampling area. If yellowing or declining areas were noticed, then separate samples were taken from these areas.

Each sample was emptied into individually labeled plastic bags and thoroughly hand mixed. A 100-cm<sup>3</sup> soil sample was removed from each bag and nematodes were extracted using a modified centrifugal-sugar flotation technique (Jenkins, 1964). Plant-parasitic nematodes were identified to genus and counted using an inverted light microscope at × 32 magnification. After counting, the nematodes were preserved in a 5% formalin solution. Using a compound microscope at × 40 and × 100 magnifications, some preserved nematodes were slide mounted and identified to the species level with reasonable confidence. Morphology keys and original nematode descriptions were used for species identification (Esser, 1971; 1973; Esser and Vovlas, 1989; Golden and Taylor, 1956; Handoo and Golden, 1992; Loof and Luc, 1990; Rau, 1958; Sher, 1961; 1963; 1966; Sledge and Golden, 1964).

### Results

Plant-parasitic nematodes associated with seashore paspalum were the typical range of genera found parasitizing other warm-season turfgrasses. Ten genera of plant-parasitic nematodes were extracted from 60 seashore paspalum soil samples from eight golf courses (Table 4-1). In addition to the same ten genera found on seashore paspalum golf courses, two more plant-parasitic nematode genera, a total of 12, were extracted from 51 soil samples from 20 home lawns (Table 4-2). Nematodes in the genera *Hemicriconemoides* and *Helicotylenchus* are not frequently discovered in such high

populations on turfgrasses in Florida as were found on seashore paspalum (Crow, pers. comm.). As with a majority of turfgrass samples in Florida, *Hoplolaimus* spp. was commonly found in seashore paspalum samples. The genera most frequently found were *Hoplolaimus*, *Mesocriconema*, *Hemicriconemoides*, and *Helicotylenchus*, which were found on 100, 100, 88, and 88% of the golf courses surveyed and on 75, 95, 70, and 85% of the home lawns sampled, respectively (Table 4-1, 4-2). *Xiphinema*, *Pratylenchus*, and *Tylenchorhynchus*, were found on less than 30% of the golf courses and less than 45% of the home lawns sampled (Table 4-1, 4-2). *Peltamigratus* and *Hemicycliophora* were not found in any of the golf course soil samples and were only found associated with a low percentage of the home lawns (Table 4-2).

Various morphological measurements, identifying cuticular markings, and body shape were used for species identification. Species identified from soil samples taken from the rhizosphere of seashore paspalum were *Belonolaimus longicaudatus* Rau, *Hoplolaimus galeatus* (Cobb) Thorne, *Helicotylenchus pseudorobustus* (Steiner) Golden, *Meloidogyne graminis* (Sledge and Golden) Whitehead, *Hemicriconemoides annulatus* Pinochet and Raski, *Trichodorus proximus* Allen, and *Peltamigratus christiei* (Golden and Taylor) Sher.

Previous surveys indicate that a variety of nematodes are associated with turfgrasses in the southeastern United States (Lucas et al., 1974; Parris, 1957; Sikora et al., 2001). Even though these surveys were primarily for nematodes associated with bermudagrass and bentgrass (*Agrostis* spp.), many of the nematodes we found were from the same genera. *Mesocriconema*, *Hoplolaimus*, and *Helicotylenchus* were all common in our survey, which is similar to observations throughout the southeast. The relatively

high frequency of *B. longicaudatus* found in soil samples from golf courses (50%) and home lawns (40%) was expected because of the sandy soils associated with coastal areas in Florida. *Belonolaimus longicaudatus* is most commonly found in Florida because it is well adapted to soils with > 80% sand content and < 10% clay content (Robbins and Barker, 1974). *Belonolaimus longicaudatus* is recognized as the most pathogenic ectoparasitic plant nematode to turfgrasses in Florida. Acceptable visual quality is often not possible without treatment of the *B. longicaudatus* infestation.

Table 4-1. Frequency of occurrence and population density of plant-parasitic nematode genera in soil samples collected from seashore paspalum golf courses in Florida.

Nematode genus	Golf courses with nematode (%) <sup>a</sup>	Nematodes/100 cm <sup>3</sup> of soil	
		Mean <sup>b</sup>	Maximum <sup>c</sup>
<i>Hoplolaimus</i>	100	162	643
<i>Mesocriconema</i>	100	69	563
<i>Hemicriconemoides</i>	88	97	1,014
<i>Helicotylenchus</i>	88	469	2,808
<i>Trichodorus</i>	63	30	150
<i>Tylenchorhynchus</i>	63	7	16
<i>Meloidogyne</i>	63	39	123
<i>Belonolaimus</i>	50	16	114
<i>Pratylenchus</i>	25	76	167
<i>Xiphinema</i>	25	79	253

<sup>a</sup>Percentage of golf courses with at least one infested green or fairway.

Percentage based on eight golf courses with one to four fairways and zero to four greens sampled on each golf course for a total of 60 soil samples.

<sup>b</sup>Mean population levels of nematodes recovered from samples in which the nematode was found.

<sup>c</sup>Maximum population levels found from individual soil samples.

Table 4-2. Frequency of occurrence and population density of plant-parasitic nematode genera in soil samples collected from seashore paspalum home lawns in Florida.

Nematode genus	Home lawns with nematode (%) <sup>a</sup>	Nematodes/100 cm <sup>3</sup> of soil	
		Mean <sup>b</sup>	Maximum <sup>c</sup>
<i>Mesocriconema</i>	95	123	552
<i>Helicotylenchus</i>	85	175	743
<i>Hoplolaimus</i>	75	73	253
<i>Hemicriconemoides</i>	70	92	428
<i>Trichodorus</i>	60	49	234
<i>Tylenchorhynchus</i>	40	42	388
<i>Belonolaimus</i>	40	11	72
<i>Meloidogyne</i>	28	14	45
<i>Pratylenchus</i>	25	5	15
<i>Hemicycliophora</i>	25	22	91
<i>Peltamigratus</i>	5	29	57
<i>Xiphinema</i>	5	7	7

<sup>a</sup>Percentage of home lawns with at least one infested soil sample. Percentage based on 20 home lawns with one to five samples taken at each lawn for a total of 51 soil samples.

<sup>b</sup>Mean population levels of nematodes recovered from samples in which the nematode was found.

<sup>c</sup>Maximum population levels found from individual soil samples

### Discussion

Action threshold levels have not yet been established for seashore paspalum, but according to levels established for bermudagrass (Crow et al., 2003), 26 of the 51 home lawn samples and 34 of the 60 golf course samples had nematode populations above action threshold levels. *Belonolaimus*, *Hoplolaimus*, *Helicotylenchus*, *Trichodorus*, *Hemicriconemoides*, and *Mesocriconema* were the genera found above action threshold levels for bermudagrass (Crow et al., 2003). *Hoplolaimus galeatus*, *B. longicaudatus*, and *T. proximus* were associated with damaged areas on golf courses and home lawns in the current survey. Drought stress during the summer was amplified when these nematodes were present. Seashore paspalum was slow to recover from dieback

associated with drought. Damaged areas were more common on home lawns than on golf courses. Golf courses are usually well maintained through proper fertilization and adequate irrigation, possibly masking root damage caused by high nematode populations. Declining seashore paspalum was found when populations of *T. proximus* exceeded 60 nematodes/100 cm<sup>3</sup> of soil, *H. galeatus* exceeded 50 nematodes/100 cm<sup>3</sup> of soil, and when *B. longicaudatus* numbers were above 10 nematodes/100 cm<sup>3</sup> of soil. High populations of *Helicotylenchus* spp. (> 500 nematodes/100 cm<sup>3</sup> of soil) were often found associated with seashore paspalum. Damaged areas were not noticed in areas where high population levels of *Helicotylenchus* spp. were recovered from soil samples. In Florida, *Helicotylenchus* spp. are rarely found in turfgrass soil samples at these high population levels (Crow, pers. comm.). Their ability to damage turfgrass in Florida is not widely known.

A wide range of plant-parasitic nematodes was found associated with seashore paspalum golf courses and home lawns in Florida. Some nematode genera tended to have population levels higher than those usually found associated with bermudagrass (*Cynodon* spp.) in Florida. The high population levels of *Helicotylenchus* spp. and *Hemicriconemoides* spp. found in this survey suggest seashore paspalum is an excellent host for these nematodes. Approximately half of the seashore paspalum samples had nematode populations at damaging levels according to bermudagrass threshold levels. These threshold levels are approximate and can fluctuate due to host suitability, soil temperature, soil texture, soil moisture, and a variety of other soil conditions. Threshold levels used for bermudagrass seem reasonable for diagnosis of samples with *B. longicaudatus*, *H. galeatus*, and *T. proximus*, but adjustments may be necessary for

*Helicotylenchus* and *Hemicriconemoides*. As seashore paspalum becomes more widely grown, a better understanding of its relationship with plant-parasitic nematodes can be ascertained.



## CHAPTER 5 SUMMARY

With more than 1,300 public and private golf courses and over three million acres of residential lawns (Haydu and Hodges, 2002; Hodges et al., 1994), Florida is not only a prime location for highly managed turfgrass areas, but also an ideal habitat for *Belonolaimus longicaudatus* and *Hoplolaimus galeatus*. The data reported herein indicates that this population of *B. longicaudatus* is detrimental to the growth of 'SeaIsle 1' seashore paspalum in the southeastern United States. The reaction of seashore paspalum to the population of *H. galeatus* was very different with only a limited effect on plant growth detected in our research. Our data show that 'SeaIsle 1' seashore paspalum is not any less susceptible to *B. longicaudatus* or *H. galeatus* than is 'Tifdwarf' bermudagrass.

The population of *B. longicaudatus* had a high degree of association with root growth stunting ( $P \leq 0.05$ ) in 'SeaIsle 1' seashore paspalum and 'Tifdwarf' bermudagrass while *H. galeatus* failed to reduce root or shoot growth in either grass ( $P > 0.05$ ) (Chapter 2). Results indicate that there are slight differences in host suitability, but no differences in root length percent reduction between the two grasses. 'Tifdwarf' bermudagrass tended to support higher populations of *B. longicaudatus* and *H. galeatus* during the experiments ( $P \leq 0.05$ ) (Chapter 2). Shoot growth was not affected ( $P > 0.05$ ) by either nematode on either grass. 'SeaIsle 1' seashore paspalum and 'Tifdwarf' bermudagrass root growth was reduced by 35 to 45% when exposed to *B. longicaudatus* for 120 days. The population of *H. galeatus* was associated with a slight abbreviation of root development in seashore

paspalum and bermudagrass during the second year of the study (Chapter 2). Even though final population densities of *H. galeatus* more than exceeded the reported action threshold of 40 nematodes/100 cm<sup>3</sup> of soil for bermudagrass (Crow et al., 2003), neither seashore paspalum nor bermudagrass had a consistent reduction in root growth, although *H. galeatus* were found within the root cortex of both grasses (Chapter 2). These results indicate that there is no reason to believe that the susceptibility of 'SeaIsle 1' seashore paspalum to *H. galeatus* and *B. longicaudatus* is lower than 'Tifdwarf' bermudagrass.

The two nematodes were affected differently by high salinity irrigation (Chapter 3). Moderate salinity levels (10 and 15 dS/m) caused an increase in *B. longicaudatus* reproduction when compared to 0 dS/m ( $P \leq 0.05$ ). Visual inspection revealed that irrigation with these low salinity levels resulted in a denser, more vigorous root and shoot growth than 0 dS/m. With increased root growth, more feeding sites were available for *B. longicaudatus*. At high salinity levels of 25 dS/m and higher, *B. longicaudatus* populations were reduced to non-damaging levels. In 2003, root reductions were not detected ( $P > 0.05$ ) at salinity levels of 15 dS/m and higher when plants inoculated with *B. longicaudatus* were compared to uninoculated plants with equivalent irrigation treatments (Chapter 3). A high percentage of final populations at these salinity levels consisted of second-stage juveniles (J2). Further research must be performed to determine if salinity interferes with host locating receptors of *B. longicaudatus*. Moderate salinity levels may also be stimulating egg hatch resulting in high populations of J2.

In our experiments, soil and root populations of *H. galeatus* were reduced as salinity irrigation levels increased from 0 to 25 dS/m (Chapter 3). Final population

densities of *H. galeatus* demonstrated a negative sloping linear regression ( $P \leq 0.01$ ) when compared to increasing salinity irrigation treatments. Lower salinity treatments, 0 and 5 dS/m, resulted in higher reproduction capabilities than the higher salinity irrigation treatments ( $P \leq 0.05$ ) (Chapter 3). Final populations were very low at the 25 dS/m treatment level and above. The  $R^2$ -values were 0.92 (2002) and 0.83 (2003), showing that the data followed a linear regression very closely. High salinity irrigation may be effective in controlling *H. galeatus* in the soil, but these nematodes are also able to enter the root cortex as migratory endoparasites. Our results indicate that the ability of *H. galeatus* to completely enter and tunnel within the root cortex does not provide a barrier from the effects of the high salinity irrigation (Chapter 3).

The survey of nematodes associated with established seashore paspalum revealed a majority of genera typically associated with maintained turfgrass areas (Chapter 4). *Hoplolaimus* spp., *Helicotylenchus* spp., *Mesocriconema* spp., and *Hemicriconemoides* spp. were common in home lawns and golf courses. Some genera (*Hemicriconemoides* and *Helicotylenchus*) tended to have population levels higher than those usually found associated with bermudagrass in Florida. The relatively high frequency of *B. longicaudatus* at golf courses (50%) and home lawns (40%) should have been expected because of the sandy soils associated with coastal areas in Florida. Damaged areas could be found associated with parasitism by *B. longicaudatus*, *H. galeatus*, and *Trichodorus proximus* during the summer months when the grass was under the most environmental stress (Chapter 4).

Nematode management in turfgrass areas is becoming increasingly difficult because of the elimination of a majority of post-plant nematicides. Alternatives in the

form of different chemical formulations, biological control, or resistant plant cultivars must be found to be able to maintain acceptable turfgrass quality in many areas. With the development of salt-tolerant grasses such as seashore paspalum, high salinity irrigation could be implemented as part of a management regiment for nematodes if irrigation periods were timed properly. Water use for landscape irrigation is becoming a critical issue in Florida. Maintaining turfgrass of acceptable quality is increasingly difficult when forced to reduce water consumption or switch to alternative water sources. In Florida, recycled water was the primary source for almost half of all golf courses in 2000 and has increased from 8% in 1974 to 21% in 1994 to 49% in 2000 (Haydu and Hodges, 2002). Seashore paspalum is well adapted to irrigation from these alternative water sources and could become more prevalent as water restrictions increase. Irrigation with water containing high levels of dissolved salts reduces shoot growth of seashore paspalum (Lee, 2000), but since high yield is not essential for turfgrass managers this could be an effective nematode management technique. Further research must be performed to determine the timing, frequency, and duration of high salinity irrigation events.

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Adam C. Hixson was born 25 April 1978 in Waco, TX, and grew up in China Spring, TX. He graduated from China Spring High School in 1996, and began studies at Texas A&M University, College Station, TX in the fall of 1996. He was a member of the Corps of Cadets for four years while pursuing his Bachelor of Science degree in entomology. After graduation from Texas A&M University in 2000, he began studies for his Master of Science degree at University of Florida, Gainesville, Florida. The title of his thesis is "*Belonolaimus longicaudatus* and *Hoplolaimus galeatus* on Seashore Paspalum (*Paspalum vaginatum*)."