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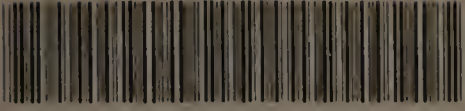
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**RELATIVE SUSCEPTIBILITY OF ENDOPHYTIC AND NON-ENDOPHYTIC
TURFGRASSES TO PARASITIC NEMATODES**

A Thesis Presented

by

NORMAN R. LAFAILLE

**Submitted to the Graduate School of the
University of Massachusetts Amherst in partial
fulfillment of the requirements for the degree of**

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Department of Plant and Soil Sciences

**RELATIVE SUSCEPTIBILITY OF ENDOPHYTIC AND NON-ENDOPHYTIC
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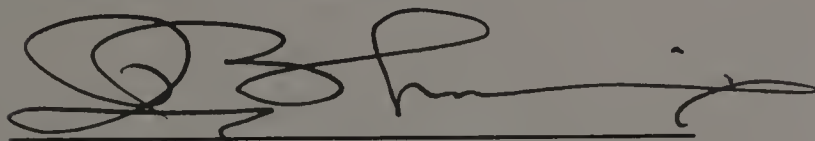
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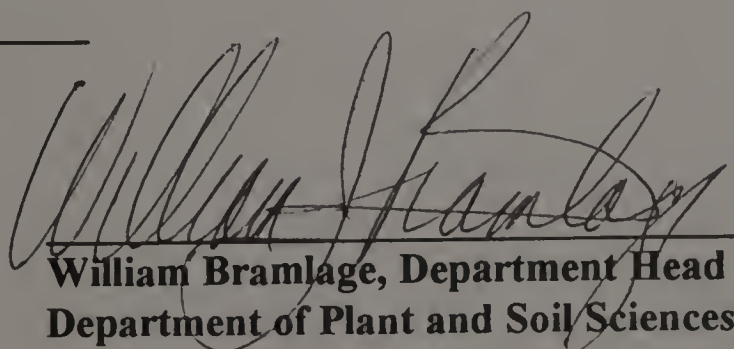
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CHAPTER I

ENDOPHYTIC AND NON-ENDOPHYTIC TURFGRASS RESPONSE TO PLANT PARASITIC NEMATODES

Introduction

Plant parasitic nematodes have been recognized as pests of horticultural and agricultural crops for many years. Turfgrass managers of warm season turfs have recognized the potential danger of plant parasitic nematodes and consider them a major pest problem. Managers of cool season turfgrasses have only recently begun to acknowledge the fact that plant parasitic nematodes can be a significant problem. Recognizing damage from plant parasitic nematodes can be difficult since above ground symptoms are not always apparent. Plant parasitic nematodes are microscopic and can easily be confused with free living non-parasitizing nematodes which are abundant in most soils and play an important role in soil ecosystems feeding on bacteria, fungi and algae (Nelson, 1995). The first report of parasitic nematodes as a northern turfgrass problem dates back to 1954 (Troll and Tarjan, 1954). Since that time turfgrass managers have become increasingly aware of the problems associated with nematodes on both warm and cool season turfs.

Red fescue (Festuca rubra L.) Tall fescue (Festuca arundinacea Schreb.) and perennial ryegrass (Lolium perenne L) are commonly used turfgrasses on golf courses as well as on home lawns, athletic fields and park areas. The presence of endophytic fungi (Acremonium spp.) living symbiotically within some varieties of red and Tall fescues, and perennial ryegrass have been shown to act as a biological control agent against many

surface feeding insects (Breen, 1994; Mathias, 1990) as well as some fungal pathogens (Siegel and Latch 1991). Endophytic turfgrasses are also known to have higher levels of growth, overall vigor and competition compared to non-endophytic turfgrass (Clay, 1987). Endophytic fungi reside intercellularly within host plant tissue and interact with the host plant to produce a number of alkaloid-type compounds which accumulate only in above ground tissues (Bacon et al. 1986). Endophytic fungi of the Genus Acremonium are the most common and are obligate, seed-borne fungi that cause no visible pathogenic symptoms on the host plant (Morgan-Jones and Gams 1982; Rykard et al. 1985; Siegel et al. 1987). As previously noted, a major problem with many turfgrass stands is the presence of large populations of plant-parasitic nematodes which have been shown to weaken plants to a point that insects and disease causing fungi have a direct advantage for feeding and infection. Research has been extremely limited on the interaction of endophytic grasses and plant parasitic nematodes, and it is the principle hypothesis of this thesis that a turfgrass stand infected with endophytic fungi may be more resistant to plant parasitic nematodes.

The objectives of this study are 1) to determine if plant parasitic nematodes reduce shoot and root biomass of different cultivars of Lolium perenne L., Festuca rubra L., and Festuca arundinacea Schreb; 2) to determine if cultivars of Lolium perenne L., Festuca arundinacea Schreb., and Festuca rubra L. differ in their susceptibility to stunt nematodes (Tylenchorhynchus spp.) and lance nematodes (Hoplolaimus spp.); 3) to determine whether resistance to stunt and lance nematodes is increased in turfgrass stands of endophytic cultivars of Lolium perenne L., Festuca arundinacea Schreb., and Festuca

rubra L.; and 4) to assess nematode reproduction and the usefulness of the experimental system used.

Literature review

Endophyte Characteristics

A mutualistic relationship between fungal endophytes and their hosts exists in hundreds of species of grasses used for food, forage and turfgrass (Bacon and DeBattista 1991; Clay 1988, 1989, 1990; Funk and Clarke 1989; Siegel et al. 1984a, 1985, 1987a, 1989; White et al. 1987; White and Morgan Jones 1987). Benefits to the host plant include increased stress tolerance, insect and disease resistance and overall growth and vigor (Siegel and Latch 1991; Kelley and Clay 1987; Latch et al. 1985b). The fungus benefits from this by obtaining photosynthate, mineral nutrients and a mechanism of dissemination through the host plant (Smith et al. 1985).

Endophytic fungi of grasses are members of the family Clavicipitaceae in the tribe Balansiae (Clay 1989). The Balansiae are ascomycetes, systemic in plants, perennial and are known to infect the grass families of Poaceae, Cyperaceae, and Junaceae (Clay 1989). The Balansiae tribe is comprised of the genera Balansiopsis, Atkinsonella, Myriogenospora, Balansia, and Epichloe. (Clay 1986a; Siegel et al. 1987). Epichloe typhina is most commonly responsible for the infection of important genera of turfgrasses including Agrostis, Dactylis, Festuca, Hordeum, and Lolium (Bacon et al. 1977). Acremonium spp. endophytes, originally classified as Sphacelia typhina (Latch et al. 1984; Morgan Jones and Gams 1982; White and Cole 1985a,b), are the anamorphic state of Epichloe typhina. Dissemination of Acremonium spp. occurs via host seed (Siegel et al.

1987; Clay 1986a). Hyphae of the endophyte grow intercellularly along the longitudinal axis of the host epidermal cells, and at the time of flowering mycelia grow into the developing ovule of the host (Bacon et al. 1986; Siegel et al. 1987). The endophyte remains viable in the aleurone layer of the seed. During germination of seed, the fungus infects the endosperm followed by colonization of the developing leaf sheaths (Philipson and Christy 1986; Siegel et al. 1985, 1987).

Over 90% of the pastureland in the U.S. is thought to be infected with Acremonium. Initial interest in endophytes stemmed from disease syndromes of livestock feeding on infected grasses known as the "ryegrass staggers" and "fescue foot". Symptoms included decreased weight gain, lower milk production, gangrene, vasoconstriction, and spontaneous abortions (Bacon et al. 1977, 1986; Clay et al. 1985; Hoveland et al. 1983). Several alkaloids produced by the fungus or by the plant in response to the fungus are known to be responsible for these symptoms. Production of ergot alkaloids are associated with Acremonium coenophialum (Bacon et al. 1981, 1986; Bacon 1985, 1988; Porter et al. 1985). Ergovaline and ergotamine are the ergot alkaloids largely responsible for mammal toxicosis. Ergot alkaloids have also been shown to cause reduced feeding and antibiosis effects on the fall armyworm (Spodoptera frugiperda). Loline alkaloid derivatives were the specific ergot alkaloids found to be responsible for reduced feeding of fall armyworm, European cornborer (Ostrinia nubilalis), and a variety of other insects (Riedell et al. 1990). Reduced survival and weight gain of hairy chinch bug (Blissus leucopterus hirtus) and different species of billbug (Sphenophorus spp.) feeding on endophyte infected grasses has been well documented (Clay et al. 1985; Rowan and Gaynor 1986; Johnson-Cicalese

and White 1990). In laboratory choice experiments, hairy chinch bug and other insects chose a non-endophytic variety over a variety hosting endophyte (Mathias et al. 1990), and studies by Bacon et al. (1986) showed that the majority of the alkaloids are present in leaf sheaths where endophytes reside. Only 10-15% of the alkaloids produced were detected in the roots (Siegel et al. 1989).

Counts of spiral nematodes (Helicotylenchus spp.) were lower in soil fractions taken from a tall fescue stand infected with Acremonium coenophialum (Pedersen et al. 1988), indicating a possible endophyte effect on nematode population. Pedersen and Rodriguez-Cabana (1986) also demonstrated that numbers of spiral nematodes were reduced by 66% in soil containing endophyte-infected Tall fescue roots compared to endophyte-free Tall fescue roots. Populations of Pratylenchus scribneri (Steiner) and Tylenchorhynchus acutus (Allen) were substantially lower in endophyte infected versus endophyte-free Tall fescue (West et al. 1988), additional studies by Kimmons et al. (1988) showed reduced populations of Pratylenchus scribneri and Meloidogyne marylandi in turfgrass field soils containing Acremonium-infected Tall fescue.

Alkaloid levels can vary among different host-endophyte combinations. Plant age and environmental conditions can also influence alkaloid production thus affecting the relative resistance of endophyte infected plants to insect and parasite damage (Eichenseer et al. 1991; Siegel et al. 1989).

Nematode / Disease Interaction

The effects of high populations of plant parasitic nematodes and fungal infection have been documented. Studies by Vargas and Laughlin (1972) found reduced top growth

of Merion Kentucky bluegrass infected with both Fusarium roseum and Tylenchorhynchus dubius Filipjev. Infection with these plant parasitic nematodes exposed the bluegrass to more severe damage by this well known disease of Merion Kentucky bluegrass. In separate studies exposure of the fungus alone did not result in reduced top growth, therefore, it was believed that the nematode presence was predisposing the turfgrass to infection. In addition, Fusarium moniliforme will cause wilting on corn only when Hoplolaimus indicus is present in the soil system (Dropkin, 1989). The damage made by nematode feeding is thought to be a route of entry for fungal disease infection.

Nematodes in Turfgrass

Interest in damage caused by nematodes in turf began in the early 1950's. Troll and Tarjan (1954) found large populations of nematodes in putting green turf/soil samples sent to them for disease diagnosis. Of 41 putting greens sampled nine different genera of plant parasitic nematodes were found including Tylenchorhynchus, Hoplolaimus, and Heterodera. These findings were followed by the efforts of Good, Christie, and Nutter (1956) who examined turf samples taken from bermudagrass, zoysiagrass, St. Augustinegrass, and centipedegrass. In their study Hoplolaimus and Belonolaimus were found to be the most damaging. They also found large populations of Rotylenchus, Trichodorus, and Criconemoides. A study of 21 lawns in Mississippi yielded relative frequency data showing Tylenchorhynchus spp. in 8 lawns; Trichodorus spp. in 6; Rotylenchus spp. and Criconemoides spp. in 5; Hoplolaimus spp. in 4; Xiphinema spp. in 3; Paratylenchus spp. 2; and Pratylenchus in 1 lawn. Twenty-six bentgrass putting greens

were sampled in Illinois by Taylor, Britton and Hechler (1963) who found 100% of the greens containing nematodes of the genus Tylenchorhynchus and 62% containing the genus Hoplolaimus. Other genera found were Criconemoides, Helicotylenchus, Paratylenchus, Pratylenchus, and Trichodorus. At least two different species of Tylenchorhynchus as well as Helicotylenchus dihystra Cobb, were found infecting Kentucky bluegrass lawns in Lincoln, Nebraska (Sumner, 1967). Sumner (1967) also demonstrated a decline in the nematode population during the winter months, with highest infection from April through September. A study conducted by Smolik and Malek (1972) from 81 samples of Kentucky bluegrass taken from 65 location in South Dakota showed that 46% of the samples yielded significant populations of stunt nematodes. Thirty two species were found including Hoplolaimus, Helicotylenchus, and Pratylenchus. Another study conducted by Lucas, Blake, and Barker (1974) recorded information on nematodes associated with bentgrass and bermudagrass golf greens in North Carolina. Their results indicated the presence of a number of nematode genera on bermudagrass including Criconemoides, Helicotylenchus, Trichodorus, Meloidogyne, Tylenchorhynchus, Hoplolaimus, and Belonolaimus (in ascending order of infection). Trichodorus, Hoplolaimus, Tylenchorhynchus, and Helicotylenchus were found in soils taken from bentgrass greens. Busey, Giblin-Davis and Center (1993) worked with different varieties of St. Augustine grass and found that with diploid varieties, root weights were 33% lower due to nematode presence compared to polyploid varieties. Nematode populations were only slightly increased in polyploid varieties compared with diploid varieties. This general resistance may be attributed to the thicker primary roots of polyploid plants. Another

study by Davis, Wilkinson, and Noel (1994) was undertaken to assess root growth of bentgrass and Annual bluegrass as influenced by coinfection with Tylenchorhynchus nudus and Magnaporthe poae, at three different temperature levels; 24, 28, and 30 C. Root length of both grasses were reduced significantly in the presence of Tylenchorhynchus nudus at all temperature levels. Magnaporthe poae only suppressed root growth at 30 C, suggesting that nematode damage was an independent factor of decline. A review by Riedel (1981) outlined the association of different genera of nematodes with cool season host plants. Agrostis hosts 11 genera, Festuca hosts 6, Lolium hosts 7, Poa annua hosts 2 and Poa pratensis hosts 11 different genera. Of all the genera which infect cool season grasses, Riedel (1981) attributes the greatest percentage damage to be caused by Helicotylenchus, Tylenchorhynchus, Criconemoides, and Pratylenchus.

Nematode Characteristics

General Characteristics:

Soil inhabiting nematodes are aquatic wormlike organisms which thrive in water-filled soil pore spaces within the soil. The phylum Nematoda is second only to the phylum Insecta in size and diversity. Nematodes can be found living in both plants and animals but only a small percentage of all nematodes are plant parasites and an even smaller percentage of those being parasites of turfgrass species (Nelson, 1995). Nematodes occur in all soil types but are best adapted to sandy soils. Damage to plants occurs by nematodes feeding on plant cells and/or burrowing through tissue in search of a feeding site (Dropkin, 1989; Siddiqi, 1993). Nematodes have been considered a pest of warm season turfgrass species for over fifty years and have been recorded as far back as the early eighteenth

century as significant agricultural pests infecting crops such as soybean, corn, rice, cotton, potato, tobacco, alfalfa, cereals and grasses used for food and forage. They are also common pests of many fruits and woody ornamentals (Nickle, 1984). Turfgrass managers of cool season turf have recently begun to understand that nematodes can be a significant biotic stress.

Genus Tylenchorhynchus

Nematodes belonging to the Genus Tylenchorhynchus are members of the order Tylenchida; suborder Tylenchina; family Tylenchorhynchidae and subfamily Tylenchorhynchinae (Siddiqi, 1993; Dropkin, 1989). Adaptation is partially dependent on geographical and ecological considerations. Most species have a wide host range and are between 0.5 - 1.5 mm in length. Stylet lengths range from 15-27um long with prominent basal knobs. Common ectoparasites of roots, these nematodes feed on epidermal cells in the region of cell elongation (Dropkin, 1989). Individual feeding usually occurs for less than 10 minutes in any one location. When a cell wall is punctured by the stylet, enzymes are released into the cell for partial digestion to occur after which cell contents are consumed by the nematode. If populations become high, roots may be severely injured resulting in plant stunting and reduction in yield. Smolik and Malik (1973) demonstrated that Tylenchorhynchus nudus reduced clipping weights of Kentucky bluegrass by 28% and root and crown weights by 36%. Four months following inoculation, there was an 8 fold increase in nematodes. Sandy loam soils appear to be most conducive for maximum infection. Stress levels associated with nematodes was increased when plants were in a state of nutrient deficiency as well. Tylenchorhynchus dubius suppressed secondary stolon

formation in 'Toronto' creeping bentgrass. The nematode also resulted in shortened internodes and premature inflorescence formation (Laughlin and Vargas 1972). Foliar and root weights were reduced in 'Merion' Kentucky bluegrass in the presence of this specie (Laughlin and Vargas, 1972). Studies by Noel and Lownsbery (1977) indicated a higher reproduction rate of Tylenchorhynchus clarus as temperature increased. Reproduction was greatest at 24 and 27^o C compared to 21^o C.

Genus Hoplolaimus

The genus Hoplolaimus is a member of the order Tylenchida; suborder Tylenchina; superfamily Tylenchoidea; family Hoplolaimidae; and subfamily Hoplolaiminae (Dropkin, 1989; Siddiqi, 1993). Hoplolaimus are cylindrical in shape and range in size from 1-2mm. They have a thick cephalic framework and large stylet 33-52 μ m in length. They are usually ectoparasites, but some individuals completely enter the root causing even greater damage due to destruction of tissues. These wounds also enhance infection by other pathogenic organisms (Dropkin, 1989). Symptoms of injury include severe wilting and stunted plants. Grasses susceptible to infection include species of Festuca, Agrostis spp., Cynodon spp., Phalaris spp. and Dactylis glomerata (Cook and Yeates, 1993).

Materials and methods

Experiment 1

Plant Materials and Growth Conditions:

Cultivars of (Festuca rubra L.) including the endophytic and non-endophytic varieties of 'SR 5200', 'SRX 3322' and 'SRX 3324' and an endophytic and non-endophytic variety of 'K-31' (Festuca arundinacea Schreb.) were used in this study. Plants were

grown from seed in 200 cell propagation trays with fifteen seeds placed in each cell and twenty five cells per cultivar. Seeds were germinated in Promix™ potting soil. Propagation trays were placed into a mist house and maintained at temperatures between 19 and 22° C for 21 days. After 21 days of growth all cells were thinned to 10 viable plants. Soil plugs from cells were transplanted into Conetainers™ which are cone shaped plastic containers with drainage holes at the bottom.

Nematodes:

Stunt (Tylenchorhynchus spp.) and lance (Hoplolaimus spp.) nematodes were initially obtained from a local golf course putting green. Initial counts were determined by the centrifugal flotation method (Dropkin, 1989). Three separate soil samples were used to determine the average initial populations; 492 stunt and 117 lance nematodes/100 cm³ of soil.

Experimental System:

Disposable foam plugs Dispo-plugs™ were placed into the bottom of each Conetainer and 100 cm³ of soil with or without nematodes was added into each Conetainer. Nematode free soil was obtained by a heat treatment of 49° C for a period of fifteen minutes of half of the nematode infested soil (soil used was a 50% native soil containing nematodes and 50% USGA greens mix). Turfgrass plugs from propagation trays were then transplanted into Conetainers at a depth of one inch. Conetainers were then placed in the greenhouse and maintained at temperatures between 19 and 22° C. Racks of Conetainers were watered by submerging the racks into a tub of 1/4 strength hoaglands solution to a depth of 2/3 the height of the conetainers. Watering was

conducted twice weekly for 10 minute intervals. The foam plugs placed in the bottom of each Conetainer prevented disruption of the soil.

Data Collection and Experimental Design:

Four cultivars had four treatments each: 1) Endophytic with nematodes; 2) endophytic without nematodes; 3) non-endophytic with nematodes and; 4) non-endophytic without nematodes. Each treatment was replicated 12 times. Data was subjected to Duncans multiple range tests.

Fresh weights of leaf clippings were recorded weekly for a period of 12 weeks. To ensure equal clipping heights, blades were removed by clipping flush to the top of each Conetainer.

Six of the twelve replications for each treatment were used to determine final dry weights of roots and shoots. Soil was carefully removed from the roots by placing in a pan of water and gently massaging the soil from the roots. The shoots were separated from the roots at the crown. Roots and shoots were placed in paper bags and dried for 48 hrs. at 36⁰ C.

Nematode populations were determined by sugar flotation (Dropkin, 1989). Six individual extraction were made for each treatment. These six counts were then summed and averaged for each treatment.

Experiment II

Plant Material and Growth Conditions:

'AFE' and 'AFA' Tall fescue (Festuca arundinacea Schreb.) replaced K-31 in this study. Due to transplant inconsistency, seeds were sown directly into Conetainers and

germinated in a mist house maintained at 19-22⁰ C. Twenty one days after sowing, individual plugs were thinned to 7 viable plants.

Nematodes:

Soil was obtained from a putting green in Northfield, MA. And contained 504 lance nematodes and 476 stunt nematodes per 100 cm³ of soil.

Experimental System:

Nematode free soil was obtained by subjecting the infested soil to two heat treatments of 49⁰C. The additional soil heat treatment was added after a 24 hr. cooling period. This resulted in over 95% nematode decline for use in our nematode free treatments.

Racks of Conetainers were watered at a rate of 1/2-3/4" per week by placing them under a mist chamber on the second and fifth days of the week, 15 ml of 1/4 strength Hoagland's solution was added by hand to each Conetainer weekly before watering.

Data Collection and Experimental Design

Data collection for this study remained the same as experiment I except for the following changes: Five cultivars had four treatments each: 1) Endophytic with nematodes; 2) endophytic without nematodes; 3) non-endophytic with nematodes and; 4) non-endophytic without nematodes. Each treatment was replicated 10 times. Data was subjected to Duncans multiple range tests.

Fresh weights of leaf clippings were recorded at 3, 6 and 9 weeks after establishment (30 days from seeding). To ensure equal clipping heights, blades were removed by clipping flush to the top of each Conetainer.

Five of the ten replications for each treatment were used to determine final dry weights of shoots and roots. The remaining five replications were used for final nematode extractions.

Results

Experiment I

The differences in growth between all species and cultivars without endophyte or nematode effects was variable with regard to statistical significance. Shoot fresh weights were significantly higher for 'K-31' Tall fescue and 'SR-5200' creeping red fescue three weeks after establishment (30 days from seeding) compared to 'SRX-3322' and 'SRX-3324' hard fescues. After six weeks, 'K-31' and 'SR-5200' produced significantly higher fresh weights compared to the other cultivars and only 'SR-5200' was significantly higher after nine weeks. The highest shoot fresh weights after twelve weeks of growth were observed for 'SR-5200' followed by 'K-31', 'SRX-3322', and 'SRX-3324' (Table 1).

Week X Treatment interaction among all cultivars indicated no effect of endophyte at three weeks of growth. After six weeks of growth, treatment 1 (with endophyte and with nematodes) was significantly higher than treatment 2 (with endophyte and without nematodes) treatment 3 (without endophyte and with nematodes), and treatment 4 (without endophyte and without nematodes), with treatment 4 yielding the least fresh weight, suggesting the possibility of increased growth within treatment 1 due to endophyte presence. Treatment 1 (+E, +N) significantly outperformed treatment 4 (-E, -N) again at week nine, but treatment 2 (+E, -N) and 3 (-E, +N) showed no significant differences compared to treatment 1 (+E,+N). This trend diminished by week

twelve with treatment 3 (-E, +N) significantly higher than treatments 4 (-E, -N) and 1 (+E, +N) (Table 2). A more accurate representation of overall growth was achieved by weighing final dry weights of shoots and roots for all cultivars 'K-31' and 'SRX-3322' produced significantly higher dry weights of shoots compared to 'SR-5200' and 'SRX-3324'. When comparing the effect of nematode infestation or endophyte presence among the cultivars no significant differences were found which indicates no effect by nematode or endophyte presence on final dry weights of shoot system (Table 3)

'SR-5200' achieved highest final root dry weights compared to the other cultivars (Table 3). When the interactive effects of endophytic turfgrass varieties (+E) and non-endophytic varieties (-E) in the presence (+N) or absence (-N) of lance and stunt nematodes was measured, treatment 4 (-E, -N) produced significantly higher root dry weights suggesting suppression of root growth by nematode infestation (Table 4).

Differences in lance and stunt nematode population growth, between cultivars with (+E) and without (-E) endophyte presence, was measured after soil inoculation of 12 weeks (only treatments 1 and 3 contained nematodes). An initial population of 117 Hoplolaimus (lance) nematodes per Container was determined at the onset of the experiment. 'SRX-3322' with endophyte resulted in a Mean of 168, just slightly above the initial population and 'SRX-3322' without endophyte produced a Mean of 408. For 'SR-5200', 'SRX-3324' and 'K-31', the opposite occurred with the endophytic varieties allowing a greater nematode reproduction rate than the non-endophytic varieties (Table 5). This data indicates that 'SRX-3322' without endophyte would be a model system for experiments where reproduction of lance nematodes was important.

An initial population of 492 Tylenchorhyncus (stunt) nematodes was determined at the beginning of the experiment, Hard fescues 'SRX-3322' and 'SRX-3324' produced averages well below the initial population of 492. 'SR-5200' with endophyte produced a Mean of 548 and without endophyte a Mean of 636. 'K-31' resulted in the largest final counts with 905 for the endophytic variety and 655 for the non-endophytic variety (Table 6).

Table 1. Differences in growth between all species and cultivars without endophyte or nematode effects. Data represents growth measured in fresh weight of turfgrass clippings taken at 3, 6, 9 and 12 weeks after establishment (30 days from seeding).

<u>Cultivar</u>	<u>Week 3</u>	<u>Week 6</u>	<u>Week 9</u>	<u>Week 12</u>
	-----mg-----			
SRX 3322	71 c	85 c	36 b	27 c
SR 5200	99 b	108 a	48 a	37 a
SRX 3324	78 c	95 b	38 b	26 c
K-31	162 a	107 a	38 b	32 b

* Means followed by the same letters within columns are not significantly different at the .05 level.

Table 2. The interactive effects of endophytic turfgrass varieties (+E) and non-endophytic varieties (-E) in the presence (+N) or absence (-N) of lance and stunt nematodes. Data represents growth measured in fresh weight of turfgrass clippings taken 3, 6, 9 and 12 weeks after establishment (30 days from seeding).

<u>Treatment</u>	<u>Week 3</u>	<u>Week 6</u>	<u>Week 9</u>	<u>Week 12</u>
	-----mg-----			
Trt 1: +E+N	95 b*,**	106 a	42 a	28 c
Trt 2: +E-N	115 a	98 bc	40 ab	31 ab
Trt 3: -E+N	91 b	100 ab	41 ab	33 a
Trt 4: -E-N	109 a	91 c	38 b	30 cb

* Means followed by the same letters within columns are not significantly different at the .05 level.

** Data represents the mean value of all species and varieties tested.

Table 3. Differences in growth between all species and cultivars without endophyte or nematode treatment effects. Data represents 12 weeks of growth after establishment (30 days from seeding) and measured as total root and shoot dry weights.

<u>Cultivar</u>	<u>Shoot mean (\bar{x}) dry wt.</u>	<u>Root mean (\bar{x}) dry wt.</u>
	-----mg-----	
K-31	79.6 a*	78.2 b
SRX 3322	72.3 a	93.7 b
SR 5200	59.2 b	119.9 a
SRX 3324	57 b	73.9 b

*Means followed by the same letters within columns are not significantly different at the .05 level.

Table 4. The interactive effects of endophytic turfgrass varieties (+E) and non-endophytic varieties (-E) in the presence (+N) or absence (-N) of lance and stunt nematodes. Data represents the total Mean (\bar{x}) root dry weights taken 12 weeks after establishment (30 days from seeding).

<u>Treatment</u>	<u>Root Mean (\bar{x}) dry wt. (mg)</u>
Trt 4: -E-N	99.5 a*
Trt 2:+E-N	92.6 ab
Trt 1:+E+N	87.2 b
Trt 3:-E+N	86.4 b

*Means followed by the same letters within columns are not significantly different at the .05 level.

Table 5. Differences in lance nematode population growth between cultivars with (+E) and without (-E) the endophytic fungus. Data represents the total population of lance nematodes measured 12 weeks after soil inoculation.

<u>Cultivar</u>	<u>Trt 1: +E +N</u>	<u>Trt 3: -E +N</u>
SRX 3322	168 a*,**	408 a
SR 5200	369 a	286 ab
SRX 3324	256 a	185 b
K-31	253 a	162 b

* Means followed by the same letters within a column are not significantly different at the .05 level.

** Data represents total population/100 cm³ of soil.

Table 6. Differences in stunt nematode population growth between cultivars with (+E) and without (-E) the endophytic fungus. Data represents the total population of stunt nematodes measured 12 weeks after soil inoculation.

<u>Cultivar</u>	<u>Trt 1: +E +N</u>	<u>Trt 3: -E +N</u>
SRX 3322	212 a*,**	361 a
SR 5200	548 a	636 a
SRX 3324	179 a	256 a
K-31	905 a	655 a

* Means followed by the same letters within a column are not significantly different at the .05 level.

** Data represents total population/100 cm³ of soil.

Experiment II:

When comparing growth between all species and cultivars without endophyte or nematode effects, 'Gator' perennial ryegrass produced significantly higher clipping fresh weights than all other species three weeks after establishment (30 days from seeding). 'AFA/E' Tall fescue produced significantly higher weights than 'SR-5200', 'SRX-3322' and 'SRX-3324'. The strong creeping red fescue 'SR 5200' produced significantly higher fresh weights compared to the two hard fescues 'SRX 3322' and 'SRX 3324'. At weeks six and nine 'SR 5200' was significantly higher than the other cultivars and 'SRX 3322', 'Gator' and 'SRX 3324' were statistically higher than 'AFA/E' (Table 7).

The interactive effects of endophytic turfgrass varieties (+E) and non-endophytic varieties (-E) in the presence (+N) or absence (-N) of nematodes only caused significant differences at week six after establishment (30 days from seeding). Treatment 2 (+E, -N) produced significantly lower clipping fresh weight compared to treatments 1 (+E, +N), 3 (-E, +N) and 4 (-E, -N) (Table 8).

When comparing differences in growth, measured as total shoot and root dry weights, between all species and cultivars without nematode or endophyte effects, 'AFA/E' Tall fescue produced significantly higher dry weights of shoots compared to the other cultivars. Hard fescues 'SRX 3322' and 'SRX 3324' and 'Gator' perennial ryegrass all produced dry weights slightly below AFA/E Tall fescue, but significantly higher compared to strong creeping red fescue 'SR 5200' (Table 9).

When comparing differences in overall dry weights of root system among species and cultivars, 'Gator' perennial ryegrass and AFA/E Tall fescue had significantly higher

root production compared to 'SRX 3322', 'SRX 3324' and 'SR 5200' (Table 9). The presence of nematodes or endophytic fungi had no effect on this experiment.

Soil used in experiment II had initial populations of 504 lance and 476 stunt nematodes per 100 cm³ of soil. At the end of twelve weeks all cultivars and varieties had lower populations of lance nematodes. AFA Tall fescue maintained the highest population with a Mean of 173 for the +E variety and 66 for the -E variety. Final populations for SRX 3322, 3324 and SR-5200 (+E) were 108, 33 and 136 respectively, and for the -E varieties were 51, 97 and 61. Gator perennial ryegrass maintained populations of 98 (+E) and 92 (-E) (Table 10). Stunt nematode populations increased on +E varieties of SR-5200 and SRX-3324 with population Means of 670 and 666. populations on -E varieties were 441 and 403. Populations for Gator perennial ryegrass were lowest with 87 for the +E variety and 90 for the -E variety. SRX-3322 had populations of 346 for +E and 297 for the -E variety. AFA/E populations were 431(+E) and 475 (-E) (Table 11).

Table 7. Differences in growth between all species and cultivars without endophyte or nematode effects. Data represents growth measured in fresh weight of turfgrass clippings taken at 3, 6 and 9 weeks after establishment (30 days from seeding).

<u>Cultivar</u>	<u>Week 3</u>	<u>Week 6</u>	<u>Week 9</u>
	-----mg-----		
SRX 3322	59 d	90 b	114 bc
SR 5200	68 c	111 a	147 a
SRX 3324	54 d	85 b	125 b
AFA/E	121 a	74 b	101 b
GATOR	141 a	89 b	115 bc

* Means followed by the same letters within columns are not significantly different at the .05 level.

Table 8. The interactive effects of endophytic turfgrass varieties (+E) and non-endophytic varieties (-E) in the presence (+N) or absence (-N) of lance and stunt nematodes. Data represents growth measured in fresh weight of turfgrass clippings taken 3, 6 and 9 weeks after establishment (30 days from seeding).

<u>Treatment</u>	<u>Week 3</u>	<u>Week 6</u>	<u>Week 9</u>
	-----mg-----		
Trt 1: +E+N	93 a ^{*,**}	92 a	118 a
Trt 2: +E-N	85 a	82 b	122 a
Trt 3: -E+N	90 a	96 a	125 a
Trt 4: -E-N	86 a	89 ab	116 a

* Means followed by the same letters within columns are not significantly different at the .05 level.

** Data represents the mean value of all species and varieties tested.

Table 9. Differences in growth between all species and cultivars without endophyte or nematode treatment effects. Data represents 9 weeks of growth after establishment (30 days from seeding) and measured as total root and shoot dry weights.

<u>Cultivar</u>	<u>Shoot mean (\bar{x}) dry wt.</u>	<u>Root mean (\bar{x}) dry wt.</u>
	-----mg-----	
AFA/E	20.6 a*	30.7 b
GATOR	14.1 b	35.6 a
SRX 3324	12.3 b	19.8 c
SRX 3322	12 b	20.7 c
SR 5200	8.8 c	19.7 c

*Means followed by the same letters within columns are not significantly different at the .05 level.

Table 10. Differences in lance nematode population growth between cultivars with (+E) and without (-E) the endophytic fungus. Data represents the total population of lance nematodes measured 12 weeks after soil inoculation.

<u>Cultivar</u>	<u>Trt 1: +E +N</u>	<u>Trt 3: -E +N</u>
SRX 3322	108 a*, **	51 a
SR 5200	136 a	61 a
SRX 3324	33 b	97 a
AFA/E	173 a	66 a
GATOR	98 a	92 a

* Means followed by the same letters within a column are not significantly different at the .05 level.

** Data represents total population/100 cm³ of soil.

Table 11. Differences in stunt nematode population growth between cultivars with (+E) and without (-E) the endophytic fungus. Data represents the total population of stunt nematodes measured 12 weeks after soil inoculation.

<u>Cultivar</u>	<u>Trt 1: +E +N</u>	<u>Trt 3: -E +N</u>
SRX 3322	346 a*,**	297 a
SR 5200	670 a	441 a
SRX 3324	666 a	403 a
AFA/E	431 a	475 a
GATOR	87 b	90 b

* Means followed by the same letters within a column are not significantly different at the .05 level.

** Data represents total population/100 cm³ of soil.

Discussion

Aggressive growth habits of certain turfgrasses make them suitable to outcompete pest activity including nematode infestations. Turfgrasses which host endophyte typically have higher levels of growth, overall vigor and competition compared to non-endophytic turfgrasses. When comparing treatment effects on fresh weight clipping yields for experiment I, endophytic varieties did not differ from the non-endophytic varieties. Nematode effects were also variable with regard to fresh weight of clippings. At week three, treatments 2 (+E, -N) and 4 (-E, -N) produced higher clipping weights. This trend diminished by week six, possibly due to a low nematode population or overall growth by the cultivars utilized in the experiment masked the damage caused by nematode presence. When comparing root dry weights treatments 2 (+E, -N) and 4 (-E, -N) produced significantly higher dry weights of root systems indicating a reduction in root growth where nematodes were present. The endophyte presence did not seem to effect the root dry weight results.

Variable data was recorded for differences in growth between all species and cultivars without endophyte or nematode treatment effects in experiment II. Interestingly, fresh weights of clippings increased over time for SRX-3322, 3324 and SR-5200 and decreased for AFA/E and Gator. When comparing the treatment effects, treatment 2 (+E, -N) had lower fresh weights at week 6. This trend diminished by week 9.

An important point to consider was whether or not the Conetainer system was conducive to nematode reproduction. All cultivars and species in experiment I showed an increase in populations of lance nematodes. In experiment II populations declined.

Populations of nematodes are affected by the carrying capacity of the host and the environment suggesting that the initial population in experiment I (117) was conducive for increased reproduction, whereas initial populations in experiment II (504) may have caused a reduction in reproduction.

SR-5200 (+E and -E) and K-31(+E and -E) showed increased population levels of stunt nematodes in experiment I. The non endophytic variety of SR-5200 allowed greater reproduction than the endophytic variety suggesting the possibility of a slight suppression caused by endophyte presence. SR-5200 (+E) and SRX-3324 (+E) had higher population levels in experiment II. This data suggest that SR-5200 would be a suitable host for reproduction of stunt nematodes within the Conetainer system.

CHAPTER II

EFFECTS OF PLANT PARASITIC NEMATODES ON ROOT GROWTH OF CREEPING BENTGRASS

Introduction

Most golf course greens in the northern U.S. are seeded to predominantly creeping bentgrass (Agrostis stolonifera L.). This species of turfgrass is able to withstand mowing heights as low as 1/8", which has been the trend in putting green maintenance. The turfgrass manager is faced with increased problems from disease, insect and environmental stresses due to this increased level of maintenance. Plant parasitic nematodes, primarily considered a warm season turfgrass problem, are becoming a more significant reason for failure in cool season turfgrasses, especially in view of the higher intensity of stressful management practices currently being imposed.

Nematodes move in thin water films around soil particles and can move more freely in coarse sandy soils compared to fine textured silt or clay soils. As such, it is common to see high populations of nematodes in sand based greens which are typical of most turf greens built in the last twenty years. Also, frequent top-dressing of greens has resulted in layers of sandy soil. Frequent irrigation and coarse particle sizes of sand creates an ideal habitat for nematode reproduction. Plant parasitic nematodes are obligate parasites, feeding only on living host cells and without the host, will die within a few months or at least not be able to reproduce. Plant parasitic nematodes injure the root system and above ground symptoms reflect that. Visual symptoms include an overall yellowing of the turf with no apparent pattern; premature wilting in hot weather, and; sporadic thinning of the

turfgrass stand (Nelson; 1995). These symptoms could easily coincide with common disease patterns which could make diagnosis difficult. Cyst nematodes (Heterodera spp.) have been found to cause root infections on many species of turfgrass including perennial ryegrass (Lolium perenne L.), annual ryegrass (Lolium multiflorum Lam.), Tall fescue (Festuca arundinacea Schreb.), colonial bentgrass (Agrostis capillaris L.), and creeping bentgrass (Agrostis stolonifera L.) (Nelson; 1995). However, screening for comparative susceptibility between cultivars or within these species has not been reported.

The objectives of this study were 1) to determine if cyst nematodes reduce shoot and root biomasses of different cultivars of Agrostis stolonifera L; 2) to determine if cultivars of Agrostis stolonifera L. differ in their susceptibility to cyst nematodes (Heterodera spp.); and 3) to assess nematode activity in Conetainers.

Literature review

Genus Heterodera:

The Genus Heterodera is of the order Tylenchida; suborder Tylenchina; superfamily Heteroderoidea; family Heteroderidae; subfamily Heteroderinae (Dropkin, 1989). Adult females, are lemon shaped and are between 0.5-1mm in length. A thick white cuticle in young adults darkens to brown or black as the cyst matures to protect the eggs inside (Hooper and Evans, 1993). Juveniles undergo their first molt in the egg. Second stage juveniles which leave the egg and exit the cyst are between 400-500 μ m long. These are elongate nematodes with a strong cephalic skeleton (Dropkin, 1989). Cyst nematodes are capable of surviving long periods in the soil in the absence of a host. When a stimulus

reaches the second stage juveniles, which have already molted once within the egg, they are released from the hardened cyst, en-route to a feeding site. The feeding site, called a syncytium, is comprised of enlarged and interconnected cells close to the stele. Three moltings will occur at this site and after the final molt the nematodes will emerge to the root surface as a result of cell destruction around the swollen body. The head and neck regions will remain in the root (Dropkin, 1989; Hooper and Evans, 1993). Cyst nematode infection results in poorly developed root systems which can cause plant moisture stress and possible nutrient imbalances.

Materials and methods

Plant Material:

Cultivars of creeping bentgrass (*Agrostis palustris* Huds *A. stolonifera* L.) including L-93, Providence and Penn Eagle were used in this study. Plants were grown from seed in propagation trays. Each cell contained 0.5g of seed. Twenty cells of each variety were established in a mist house maintaining temperatures between 19 and 22° C. Media used for establishment was a standard 80% sand and 20% peatmoss topdressing mixture. At 21 days soil/turf plugs were transplanted into Conetainers for the remainder of the study. Plugs were transplanted at a 1/4" depth to allow for accurate fresh weights of clippings. Conetainers were placed in a greenhouse and maintained at temperatures ranging from 19-22° C.

Nematodes:

Soil containing cyst nematodes was obtained from Bedford Golf Course. Initial counts were determined using a modified version of the centrifugal/sugar flotation method

described by Dropkin (1989). Three samples were used to determine the initial population of 20 females per 100cc of soil.

Experimental Design and Data Collection:

All tissue weight assessments were the same as described in the previous materials and methods section. The experiment was a complete random design utilizing three cultivars of bentgrass each with two treatments, with (+N) and without (-N) nematodes. Each treatment had six replications. At week 12 nematode extractions of three of the six replications were conducted to obtain final counts of nematodes. At week 16 the remaining three replications were extracted.

Results and discussion

Fresh weights of clippings at 3, 6, 9 and 12 weeks after establishment (30 days from seeding) yielded inconclusive results for both cultivar comparison and treatment (with/without nematodes) effects on growth. At week nine, although the treatment effect was non-significant, Providence and Penn Eagle outperformed L-93 on overall fresh weights of clippings (Table 12).

Final populations of cyst nematodes were lower in all cultivars after twelve and sixteen weeks. A heat exposure given to half of the replications for each cultivar did not sufficiently kill cyst nematodes (Table 13). This reduction of hardened cyst was possibly due to the hatching of the juveniles in response to the stimulus of root growth near the feeding site, however under microscopic observation low numbers of juveniles were

present. In view of these results, it is concluded that the experimental system did not support cyst nematode reproduction.

Table 12. The effects of nematode free (-N) and nematode infested (+N) soils on growth, measured in fresh weights of turfgrass clippings, taken 3, 6, 9 and 12 weeks after establishment (30 days from seeding).

	<u>Week 3</u>		<u>Week 6</u>		<u>Week 9</u>		<u>Week 12</u>	
	<u>-N</u>	<u>+N</u>	<u>-N</u>	<u>+N</u>	<u>-N</u>	<u>+N</u>	<u>-N</u>	<u>+N</u>
	-----mg-----							
<u>L-93</u>	3.2 a*	3.5 a	4.9 a	4.7 a	4.6 b	3.9 b	18 a	16.6 a
<u>Providence</u>	3.7 a	4.4 a	5.9 a	6.3 a	5.7 a	5.0 a	19.9 a	17.1 a
<u>Penn Eagle</u>	3.7 a	3.8 a	6.1 a	5.9 a	5.9 a	5.2 a	18.5 a	19.5 a

* Means followed by the same letters with a column are not significantly different at the .05 level.

Table 13. Differences in female cyst nematode populations with and without a heat treatment. Data represents the final Mean (\bar{x}) of female cyst per 100 cm³ of soil. (experiment began with 20 female nematodes per 100 cm³)

<u>Cultivar</u>	<u>Treatment</u>	<u>Mean (x)</u>
<u>L-93</u>	Heat	2.67
<u>L-93</u>	No Heat	3.67
<u>Providence</u>	Heat	3.67
<u>Providence</u>	No Heat	2.67
<u>Penn Eagle</u>	Heat	3.00
<u>Penn Eagle</u>	No Heat	3.00

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