ABSTRACT

GALLE, GLENN HARRISON. Biology and Management of Plant-Parasitic Nematodes of Turfgrass. (Under the direction of Dr. James P. Kerns and Dr. Charles H. Opperman).

The most damaging plant-parasitic nematode of golf courses throughout North Carolina is sting nematode (*Belonolaimus longicaudatus*). This nematode is impactful throughout the entire Southeastern United States and is a particular problem because it can cause significant amounts of root damage at very low numbers. Much of the previous research on sting nematode has been conducted in Florida, and information on sting nematode in North Carolina is limited. Very little is known about the biology and successful management of sting nematode in turfgrass settings.

Sting nematode populations were sampled from golf courses throughout North Carolina to determine when populations were at their highest. Populations throughout the state were found to peak in August or September after a steady rise in population counts starting from their lowest point in February. Vertical distribution data was also collected, with nematodes being extracted from the 30 cm-deep soil core broken into 10 cm segments. Sting nematodes were observed to move deeper than 20 cm during the summer months of June, July and August. Populations were found to move to a shallower depth during the fall and stay within the top 10 cm during the winter and spring months. Root-knot nematode populations were also sampled and remained only in the top 10 cm of the soil column during the entire year.

Nematicide field trials were conducted from 2014 to 2018 to determine an effective nematicide management program utilizing the newest available products. Both abamectin and fluopyram (active ingredients) were the focus of these experiments, and applications were made from April through July. Abamectin was not found to reduce sting nematode populations nor to improve turfgrass quality during all 5 years. However, fluopyram application were found to quickly decrease nematode populations throughout a given season. In 2016, populations decreased

from 500 nematodes in the non-treated controls to 30 nematodes in fluopyram-treated plots. In 2017, the focus was on determining the most effective timing for fluopyram applications. Earlier applications in May were found to be most effective at reducing September populations, with the low rate of 0.62 l/ha working just as effectively as the 1.25 l/ha rate.

In vitro studies were conducted to examine the impact of temperature on nematode movement. In the first set of experiments, agarose plates (with or without plants) were inoculated with sting nematodes, and placed at one of five temperatures: 10, 15, 20, 25, and 30°C. Movement distance was recorded each day for seven days. A second study was conducted to investigate effects on movement when the inoculated plates were moved to either a warmer or cooler environment. Sting nematode movement distance was significantly greater at 30 than at 10°C, indicating that nematode movement is higher at warmer temperatures. When subjected to temperature change, there was a significant increase in movement when nematodes were moved from 10 to 15°C. .

Experiments was conducted to investigate turfgrass species responses to sting nematode feeding damage. Five cultivars of creeping bentgrass (*Agrostis stolonifera* L.) and ultradwarf bermudagrass (*Cynodon dactylon* x *C. transvaalensis* Burtt-Davy) each were inoculated with different amounts of sting nematodes, and the nematodes were allowed to feed and cause root damage. The plants were then stressed with a simulated drought, and the differences in turf quality were observed. The newest cultivars of creeping bentgrass were able to maintain the highest quality during the stress period, and creeping bentgrass as a species was able to tolerate up to 20 nematodes feeding without a decrease in quality. Bermudagrass as a species was less tolerant to nematode feeding with a decrease in quality occurring at 10 nematodes, and no differences were observed between the cultivars.

Biology and Management of Plant-Parasitic Nematodes of Turfgrass

by
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DEDICATION

To my wife, Ruth, and the rest of my family for all of the support you have given me through this process.

BIOGRAPHY

Glenn was raised by Judd and Amy Galle in Cedar Springs, MI. After graduating from Cedar Springs high school in 2009, he attended Lake Superior State University in Sault Ste. Marie, MI. While obtaining a bachelor's degree in biology with a minor in chemistry, Glenn discovered an interest in nematodes in an animal parasitology class his junior year. Combined with an interested in research as well as plants, Glenn discovered the field of plant pathology and decided that after completing his degree in 2013 he would pursue a Ph.D. in plant pathology at North Carolina State University in Raleigh, NC.

Upon starting at North Carolina State, Glenn was put into a rotation program to determine what project might be best suited for him. Immediately he was interested in a project focusing on *Belonolaimus longicaudatus* on turfgrass and joined the Kerns lab in the spring of 2014. In the winter of 2015, Glenn married his wife Ruth. Along with his research, Glenn has participated in the Plant Pathology Graduate Student Association as a recruitment chair and has been the graduate student member on the Plant Pathology Society of North Carolina's board. Glenn also obtained funding for his research from the United States Golf Association.

Upon completion of his degree, Glenn hopes to continue his research on nematodes within the agrichemical industry. Outside of work, Glenn enjoys playing disc golf with his wife and dog, volunteering at his church, and spending time with friends and family.

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER THREE

APPENDIX A

LIST OF FIGURES

CHAPTER TWO

CHAPTER THREE

CHAPTER FOUR

CHAPTER FIVE

APPENDIX A

CHAPTER ONE

Review of the literature

THE IMPORTANCE OF TURFGRASS TO NORTH CAROLINA

The turf industry is a significant part of the agricultural sector in North Carolina. The most recent survey in 1999 showed an economic impact of $4.7 billion annually, with growth each year suggesting it is significantly higher in 2019 (North Carolina Turfgrass Survey 1999). When last surveyed, land coverage comprised 2 million acres, or 6% of the state (North Carolina Turfgrass Survey 1999). The largest sector of the turfgrass industry is home lawns and commercial landscaping, but golf courses make up a significant component to this industry as well. North Carolina is home to 534 golf courses distributed throughout the state, and each comes with its own unique challenges for turf management.

North Carolina is a very difficult state in which to grow healthy turf. It lies in a transition zone, or an area of the country where it is challenging to grow warm or cool-season turfgrasses (Patton 2012). This state is home to hot, humid summers and cool, dry winters. North Carolina is around the upper limit for growing warm-season grasses like bermudagrass. These grasses prefer hot humid summers, but do not tolerate cold winters well. The plants will go dormant during cold winter months, and if temperatures are low enough will suffer from severe freeze damage and may not recover in the spring (Beard 1973). North Carolina is also a difficult location to grow creeping bentgrass. This grass thrives during fall, winter and spring months because it is adapted to cold temperatures, but it struggles during the summer months. Prolonged temperatures at or above 30°C reduce its growth, and it is highly susceptible to heat and drought stress that is common during this time (Beard 1973). While creeping bentgrass can recover well during the cooler fall temperatures, the poor quality during the summer months is a problem as this is a time when golf course traffic tends to peak for much of the state.

On top of poor adaptability to the climate, these grasses also struggle with the wide variety of diseases found throughout the state. North Carolina is home to a wide variety of fungal diseases on turfgrass, from dollar spot of cool-season grasses to spring dead spot on the warm-season grasses (Turgeon 1999). This state is also host to a wide variety of nematode parasites which can cause significant damage to turfgrass plants throughout the entire year (Lucas et al. 1974, Zeng et al. 2012). The three most damaging plant-parasitic nematode species on turfgrass can all be found in North Carolina: root-knot nematode (*Meloidogyne* spp.), lance nematode (*Hoplolaimus galeatus* Cobb) and sting nematode (*Belonolaimus longicaudatus* Rau) (Zeng et al. 2012). All can cause significant management issues for superintendents, although none more than sting nematode.

TAXONOMY AND MORPHOLOGY

Belonolaimus longicaudatus is a nematode belonging to the order Tylenchida. The genus Belonolaimus was first described on longleaf and slash pine in the Ocala National Forest in Florida by Steiner (1949). The original species described was *B. gracilis*, and it wasn't until ten years later when a second species was described. Rau (1958) described *B. longicaudatus* as having a longer stylet and shorter tail than *B. gracilis*. A total of six species have been described belonging to the genus Belonolaimus, although *B. longicaudatus* is the most prevalent and destructive species of them all (Decraemer & Geraert 2006)

The morphology of *B. longicaudatus* is fairly distinct, and its two defining characteristics are its large size and long, slender stylet. Females of this sexually dimorphic species range in length from 2-3 mm, while males tend to be slightly shorter than 2 mm. The stylet of both sexes is long and thin, with very distinct knobs at the base. The oesophageal glands overlap the anterior end of the intestines. The head region is distinctly rounded, with six distinct lips when viewed en face. Their bodies are strongly annulated, and the lateral field is marked with a single line. In females, the ovaries are outstretched from a central vulva, with a distinct spermathecae. In males, the testis are overstretched and the bursa envelopes the entire tail end with curved spicules present. All of the descriptions above come from Rau (1958).

LIFE CYCLE

The life cycle of *B. longicaudatus* is well described from *in vitro* assays performed by Huang and Becker (1999) on excised *Zea mays* roots. The life cycle takes approximately 24 days to complete under ideal conditions. Starting after the first molt in the egg (from a first-stage juvenile (J1) to a second-stage juvenile (J2)), the J2 hatches and then feeds for 12 to 24 hours before the second molt occurs over the next 2 days. The third stage juvenile (J3) feeds immediately after emerging from the old cuticle, and at 7 days molts for another 2 days before emerging as a fourth-stage juvenile (J4). The fourth and final molt begins 13 days after the emergence of the J2 from the first molt. For males, the testis, spiculae, and bursa formed entirely in the 2 day molt from a J4 to adult. For females, the 4th stage molt was found to take 3 days, at which time all reproductive organs develop.

After molting to adults, the males were found to immediately surround the females. Sexual reproduction is obligatory for *B. longicaudatus* (Perry and Rhoades 1982). The males rub against the outside of the female, and steadily move forward until the bursa touched the female body. They continue to move forward until the bursa reached the vulval region, and copulation takes 6 to 10 minutes to complete. Egg development within the uteri begins shortly thereafter, and eggs are first laid starting at 19 days after J2 emergence. The first molt from stage-one juvenile to J2 occurs 4 days after the egg was laid, and takes one day before the J2 would emerge. Feeding was found to occur at all stages of the life cycle, including the adult males and females after mating.

DISTRIBUTION AND HOST RANGE

Sting nematode is primarily found in warmer climates, as it is native to the southeastern United States and is throughout to have a Center of Origin in Florida. It was first described in Florida (Rau, 1958), but is has it has since been discovered in California in the United States and more recently has been reported in Mexico (Mundo-Ocampo et al. 1994; Mundo-Ocampo et al. 2017). It has also been described in several island nations in the Caribbean including Bermuda and Costa Rica (Perry and Rhoades 1982). A significant limitation for this nematode is the requirement for high sand content soils (>80%) and low organic material (<10%) (Robbins & Barker 1974). In North Carolina, soils like this are found throughout the central and eastern portions of the state. This also may explain why sting nematodes are a significant threat to putting green turf. Putting greens built to United States Golf Association specification must have a minimum of 85% sand, and therefore is the ideal environment for sting nematode (USGA 2004).

B. longicaudatus has a very wide host range exceeding 200 crop species, and has been shown to be problematic on a variety of crop species including Chinese Elm (*Ulmus parvifolia*), johnsongrass (*Sorghum halepense*), muscadine grape (*Vitis rotundifolia*), pecan (*Carya illinoensis*), strawberry (*Fragaria virginiana*), white clover (*Trifolium repens*), corn (*Zea mays*), crabgrass (*Digitaria sanguinalis*), potato (*Solanum tuberosum*), pearl millet (*Pennisetum glaucum*), soybean (*Glycine max*), barley (*Holdeum vulgare*), crimson clover (*Trifolium incarnatum*), rye (*Secale cereale*), wheat (*Triticum aestivum*), bush bean (*Phaseolus vulgaris*), curled dock (*Rumex crispus*), eggplant (*Solanum melongena*), highbush blueberry (*Vaccinium corymbosum*), lettuce (*Lactuca sativa*), turnip (*Brassica rapa*), and loblolly pine (*Pinus taeda*) (Robbins & Barker 1973). Further, it is a known issue on all species of turfgrass, and in particular is damaging on the common putting green turf species of creeping bentgrass (*Agrostis solonifera*) and bermudagrass *(Cynodon dactylon*) (Crow 2005). Weeds are also viable hosts, and as a result can be very difficult to manage and reduce populations (Robbins & Barker 1973).

CHEMICAL MANAGEMENT

Sting nematode can cause severe damage to turf stands, and if left unmanaged can result in a multitude of issues for golf courses. A diseased putting green requires more nitrogen and water inputs to compensate for the damaged root system, which results in higher costs and more ecological impact (Luc et al. 2007). Symptoms of sting nematode feeding damage on turfgrass are not distinct from nematode feeding damage caused by other species, and begins with yellowing of the turf (Winchester and Burt 1964). Symptoms are often overcome by the high maintenance of putting green systems in general, but becomes more apparent as the turf is stressed by heat or drought (Lucas et al. 1974). Further progression includes thinning of the turf stand, browning of the turfgrass and as previously mentioned death can result if the damage is severe enough (Christie et al. 1952). The damage threshold on putting green turf for sting nematode is quite low. Populations of as few as 1-19 nematodes per 100 cc of soil are thresholds, and it is common advice that if sting nematode is detected chemical interventions are necessary (Clemson 2000).

B. longicaudatus is most active during the spring, summer and fall months when soil temperatures are between 20-30°C. Population increases often coincide with increases in root mass as the grass plants are actively growing (Smiley et al. 2005). Populations can vary greatly over short distances, and vertical migration is significant during the summer months when extremely high soil temperatures are common at the soil surface. In studies on soybean and maize, populations can move as deep as 45 or 75 cm, respectively (McSorley & Dickson 1990; Todd 1989). This is unlikely to occur on putting greens due to construction depth limitations, but sting nematode can migrate as deep as 30 cm on putting greens which is deeper than the normal root depth of turfgrass used for golf course putting greens (Bekal & Becker 2000).

Chemical management of sting nematode is very effective. Fenamiphos (Nemacur – Bayer CropScience) was commonly used on turfgrass since the 1970's.. This product was highly effective and turf managers making spring applications could be confident in the population reduction of a variety of nematode species including sting and lance (*Hoplolaimus galeatus*) nematodes on their putting greens (Peacock et al. 2005) . However, in 2007 Nemacur was voluntarily withdrawn from the market and no longer available for use as of 2017 (Keigwin 2014)

The two most common products used for management of sting nematode today are abamectin under the trade name Divanem (Syngenta Crop Protection) and fluopyram under the trade name Indemnify (Bayer CropScience). These products are effective at reducing populations of sting nematode in the soil (Gu & Crow 2018; Watson & Desaeger 2019). Both products can be applied with a overhead sprayer causing minimal disruption to the putting green surface, and immediate re-entry onto the putting green is allowed due to the safety of these products.

Abamectin is a naturally derived product that was originally used as a miticide and insecticide. It acts to block neurotransmitter channels, resulting in an ion imbalance and paralysis (Jansson and Dybas 1998). It acts as a contact nematicide, and nematode recovery does not occur after exposure (Faske and Starr 2006). The half-life in the soil is between 20 to 47 days depending on organic matter content. The biggest drawback to this product is the high affinity to organic matter, and in particular the thatch layer present on putting greens. Movement beyond 2.5 cm is difficult, even with heavy irrigation, and therefore multiple applications may be needed to effectively manage nematode populations (Gannon et al. 2016).

Fluopyram was originally discovered as a fungicide, and was commonly used for management of brown patch and dollar spot. It is a succinate-dehydrogenase inhibitor, acting to stop cellular respiration (Kuhn 1984). As a nematicide, its mode of action is similar (Heiken 2017). It has a half-life between 180 and 360 days (New York State 2017). It acts as a contact nematicide, although unlike abamectin recovery after exposure is possible (Faske and Hurd 2015). Fluopyram is effective against most nematode species, but has little to no activity on lance nematode. For sting nematode, however, fluopyram is one of the most commonly applied products in the Southeastern United States.

A variety of other chemical nematicides as well as some biological control options have been investigated over the years for management of sting nematode. Furfural under the trade name Multiguard Protect has had mixed results at reducing sting nematode populations, and is a potentially effective tool for use by superintendents (Crow & Luc 2014). It also is highly phytotoxic. The biological product Nortica (*Bacillus firmus* I-1582) was found to be effective at reducing nematode populations and improving root health (Crow 2014). This product provides a protective layer to the turf root, and is shown to also producing secondary metabolites resulting in nematode paralysis and mortality (Mendoza et al. 2008). Its use is limited, but could be effective at managing populations at early or late stages when feeding activity is not as aggressive (Crow 2014).

Currently no known turfgrass cultivars are completely resistant to plant-parasitic nematode feeding damage, including sting nematode. Some cultivars of bermudagrass including ‘Tifdwarf’, ‘Emerald Dwarf’, ‘TifSport’, ‘Riviera’, ‘Princess 77’ and ‘Celebration’ have shown moderate tolerance to nematode feeding (Pang et al. 2011a; Pang et al. 2011b). However, no studies have examined feeding tolerance of commonly used putting green species of creeping bentgrass and the ultradwarf bermudagrass hybrids. This information is necessary for the turf managers in North Carolina and could be an important factor for those renovating their putting greens in the future.

SUMMARY

Overall, sting nematode is a very problematic nematode for a variety of crops including turfgrass on golf courses. This nematode is highly damaging, and control options are limited to just a few products. For superintendents, more information is necessary to more effectively mitigate turf damage and use the products they have more effectively. For this project, the ultimate objective was to conduct research on applicable ways to make *B. longicaudatus* management more efficacious. This included surveying golf courses to look at population structure and dynamics, investigation into application timing of products as they became available in the wake of the loss of Nemacur and screening cool- and warm-season cultivars of putting green turf for tolerance to nematode feeding and stress. The combination of this information would help to define and describe the populations of sting nematode in the state of North Carolina.

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CHAPTER TWO

Population dynamics of *Belonolaimus longicaudatus* and *Meloidogyne* spp. in North Carolina

ABSTRACT

Sting nematode (*Belonolaimus longicaudatus* Rau 1958) and root-knot nematode (*Meloidogyne* spp.) are important pathogen of creeping bentgrass (*Agrostis stolonifera* L.) and hybrid bermudagrass (*Cynodon dactylon* x *Cynodon transvaalensis* Burtt-Davy) putting greens in the climate transition zone in the USA. These nematodes cause severe damage on putting greens hindering playability. Sting nematode is an ectoparasitic nematode that feeds on plant roots inhibiting growth and development at low population numbers. Root-knot nematode is an endoparasitic nematode, feeding and reproducing within the turf root system and decreasing overall root function. However, little is known about the population dynamics and nematode movement in soil throughout the year for either. A sampling study was initiated in 2014 to identify nematode populations numbers throughout the year, and to understand the vertical distribution of the nematodes within putting green soil. Four golf courses were sampled throughout central North Carolina, and nematode samples were taken monthly at three different depths. The overall activity of *B. longicaudatus* populations were consistent at all three golf courses, with numbers ranging from 40-250 nematodes per 500 cc. soil. Root-knot nematodes were sampled at a single course, and numbers of stage 2 juveniles ranged from 20-120 nematodes per 500 cc. soil. For sting nematode, populations were primarily located within the top 10 cm of soil during the winter, but during the summer populations shifted to 20+ cm deep in soil. In contrast, root-knot nematode populations remained within the top 10 cm throughout the entire year. Both sting and root-knot increased in April as temperatures started to increase. This finding indicates very different

population dynamics depending upon feeding tactics, with high variability between ectoparasitic and endoparasitic nematode species.

INTRODUCTION

Turfgrass management is a challenge for golf course superintendents throughout the United States, and North Carolina golf courses are no exception. North Carolina is a transition zone state with hot, humid summers and cool, dry winters. No turfgrass species is well adapted to this varied climate, rendering them susceptible to a wide variety of diseases, including nematodes, during stressful time periods. A wide variety of nematode species can cause turf damage, but two of the most prevalent in North and South Carolina are sting nematode (*Belonolaimus longicaudatus* Rau 1958) and root-knot nematode (*Meloidogyne* spp.) (RKN). They were found to occur on 46.5% and 27.1% of golf courses, respectively (Zeng et al. 2012). The feeding of these nematodes damages the root system of turfgrass resulting in poor uptake of water and nutrients. The impaired root system predisposes the plant to other stresses, including heat or drought stress periods common during the North Carolina summers (Lucas 1982).

Sting nematode is a migratory ectoparasite on turfgrass and is extremely damaging to turfgrass at low numbers. The economic damage threshold for sting nematode on bermudagrass is 1-16 nematodes/100 cc soil, indicating that simple detection of this nematode in a soil sample is a problem for growers (Clemson Extension 2000). These nematodes feed primarily on root tips, and if left unmanaged they can quickly decimate a root system (Christie et al. 1952). Sting nematode populations are found predominately in soil consisting of >80% sand (Robbins and Barker 1974). This makes putting greens constructed to United States Golf Association (USGA) standards an ideal environment, as they must be constructed with approximately 85% sand or greater (USGA 2004). Sting nematode is most commonly found in the central and eastern regions of the state, where the overall sand content of the soil is very high.

A variety of RKN species parasitize turfgrass roots, although *M. graminis* (Sledge & Golden, 1968) and *M. marylandi* (Jepson & Golden 1987) are the most common species in North Carolina (Ye et al. 2015). These are sedentary endoparasites, and their development of a feeding site is disruptive to a turf root system. Root-knot nematodes are found in a much wider array of soil types than sting nematode, including those with higher clay content. It has also been found to be more widespread throughout the state of North Carolina, from the mountains to the coast, increasing the potential for RKN to be a significant pest for a wide range of golf courses (Zeng et al. 2012).

Management of these nematodes species is critical for the health of turfgrass and the golf course industry in the state of North Carolina. Our research and others indicate that chemical management of these species can be successful with products such as abamectin and fluopyram, but application timing is critical when making these applications (Gu and Crow 2018; Galle unpublished). Research in Florida on sting nematode found that populations increased most from March to May, which may be an ideal time to apply nematicides (McGroary et al. 2009). However, research in Florida may not be directly applicable to North Carolina due to differences in climate. The average soil temperature for the Florida study was 14°C, but in North Carolina soil temperatures can drop as low as 5°C during the winter (NC Cronos 2019). Soil temperature has been shown to influence nematode activity, with low temperatures delaying increases in populations compared to other warmer climates (Lucas et al. 1978).

One objective for this study was to investigate population dynamics of sting nematode and RKN throughout the year on golf course putting greens, with a particular focus on the vertical distribution within the soil column. Monthly sampling was performed at multiple golf courses throughout the central region of North Carolina. A second objective was to compare the population dynamics of sting nematode between an annual and perennial cropping system. A maize field just outside of Clayton, NC was sampled for sting nematodes as the annual crop and compared to the population counts for the previously mentioned golf courses.

MATERIALS AND METHODS

This study was performed at four golf courses throughout central and eastern North Carolina: Raleigh Golf Association (Raleigh, NC), Benvenue Country Club (Rocky Mount, NC), Wilson Country Club (Wilson, NC) and Sedgefield Country Club (Greensboro, NC). Raleigh Golf Association putting greens were planted with the 'L-93' cultivar of creeping bentgrass (*Agrostis stolonifera* L.) and Wilson Country Club with the 'Penn A-1/A-4' cultivars of creeping bentgrass. Benvenue Country Club and Sedgefield Country Club both were planted with 'Champion' bermudagrass (*Cynodon dactylon* x. *C. transvaalensis* Burtt-Davy) putting greens. Typical golf course cultivation practices occurred as customary, and all fertility, fungicide and nematicide applications were made as scheduled. The study was also conducted at a maize field at the NCSU Central Crops Research Station (Clayton, NC). Sting nematode populations were collected from all sites except Sedgefield Country Club, which was the only site for sampling root-knot nematode populations. The maize field used was planted in May of both years for a variety trial to investigate sting nematode feeding tolerance. No fertility, herbicide, or irrigation was applied to the field throughout the entire year. At the end of the season, the plants were turned into the soil using a cultivator and left untouched until cultivation in April of the following spring.

Soil cores were collected on a monthly basis from 2015-2017 from each site. Three putting greens at each site were selected based upon previous sampling data for high sting or root-knot nematode prevalence. In the case of sampling the maize field, the field was arbitrarily split into thirds and samples were taken along the edge of each section. Cores were taken from the perimeter of each putting green using a 1-cm diameter soil probe. Cores were taken as deep as possible, up to 30 cm. They were separated into three 10-cm sections, and all cores from each depth were collected into a single bag for each research site.

Sting and root-knot nematode juveniles were extracted from the soil using a modified sieving and centrifugal-flotation method (Jenkins 1964). Soil samples were first homogenized by hand, and three 100 cc samples were taken from each bag. The soil was initially sieved using a #100 mesh sieve placed over a #400 mesh sieve, and the suspension collected from the #400 mesh sieve was then transferred to centrifuge tubes. Nematodes were centrifuged at 4,000 rpm for 5 minutes, and the supernatant was poured off. The pellet was mixed with 60% sucrose solution and centrifuged at 4,000 rpm for 30 seconds. The supernatant was poured through a #500 mesh sieve, and the nematodes were rinsed for 10 to 15 seconds with water. Nematodes were then identified and counted via light microscopy. For root-knot nematodes, roots were examined for galling.

All data was analyzed using the GLIMMIX procedure using SAS 9.4 (SAS Institute, Cary, NC). Data was separated by golf course, and years were analyzed both together and separately. Mean separation was tested using Tukey's HSD ($\alpha = 0.5$).

RESULTS

Sting Nematode Populations

Among individual golf courses and the maize field, population dynamics followed a similar trend for 2015 through early 2017. Throughout much of the winter and early spring months, nematodes were found predominately in the top 10 cm of the soil profile at all three golf courses as well as the maize field. Starting in May, populations rose steadily and peaked in late September. At all locations except for Benvenue Country Club, populations would steadily decrease to seasonal lows in February. Populations at Benvenue Country Club were slightly different in that they peaked in late summer and had a sharp decrease in populations throughout the fall. The highest overall sting nematode population total was at Raleigh Golf Association in November of 2015 when counts reached 251 nematodes per 500 cc soil.

Year and month had a significant interaction at Raleigh Golf Association ($p=0.0003$), Benvenue Country Club ($p<0.0001$), and Central Crops Research Station ($p<0.0001$) but not at Wilson Country Club ($p=0.2671$). At Raleigh Golf Association, the highest populations were in the months of June, September, and November in 2015, and December of 2016 (Figure 2.1). Similarly, the highest counts were found in July of 2015 and September of 2016 at Wilson Country Club (Figure 2.2). The highest counts were found at Benvenue were in September of 2015, but a positive spike in the population was observed in March of 2015 (Figure 2.3). The Central Crops maize field, however, was different in that the highest counts were from 2016, occurring from May through October (Figure 2.4). All of the locations showed similar results to the combined data, but there was some variation between locations by year.

When focusing in on vertical distribution, most of the majority of sting nematode populations were found in the top 10 cm of the soil from January to April across sampled sites.

During May and June, populations began to shift to the middle 10 cm of the soil profile, with some of the population still occurring in the top 10 cm. Sting nematodes could be found predominately in the bottom 20 cm during much of the late summer at Raleigh Golf Association and the Central Crops maize field (Figures 2.5 and 2.6). Nematode populations were more evenly distributed across all three-soil sections during late summer at Benvenue and Wilson country clubs (Figures 2.7 and 2.8). During the fall months, populations would typically shift back towards the top 10 cm, with few sting nematodes being found in the bottom 10 cm section by January at all 4 locations.

Analysis between turf types indicated differences between creeping bentgrass and bermudagrass nematode populations. Overall at these locations, bentgrass putting greens had four times as many nematodes as bermudagrass; averaging 36 nematodes compared to 10 nematodes/500 cc soil ($p < 0.0001$). Nematode populations at bentgrass locations also increased earlier in the season, starting in April as opposed to June in bermudagrass. Nematode counts for both grasses peaked in late September, although bentgrass populations remained elevated throughout the fall while bermudagrass populations decreased very quickly starting in October and November. The nematode number in the maize field were consistently at a count of approximately 25 (Figure 2.9) and peaked two months earlier in July than the turfgrasses that peaked in September. However, after this peak populations remained elevated throughout the fall months similar to creeping bentgrass nematode populations.

Root Knot Nematode Populations

Root knot nematode populations were unique in that all nematodes were found within the top 10 cm of the soil column at all sampling times. Populations were at their lowest in February and peaked at approximately 130 nematodes/500 cc soil during September. Populations steadily rose from March through September, and after the peak steadily decreased to the lowest population totals in the winter months. A rapid population increase was observed in early April followed by a slight drop in counts in May before continuing to rise throughout the summer (Figure 2.10). There was no significant interaction between year and month, in contrast to that observed in the experiments with sting nematode.

DISCUSSION

Overall, populations of sting nematode were highest during the summer months followed by a gradual decline during the fall to the lowest populations counts in January and February. Soil temperatures likely drive this population trend as described in similar studies from California and Florida (Bekal and Becker 2000; McGroary et al. 2009). However, no correlation between soil temperature and total nematode count was observed due to the high variability found in field based nematode studies. A weak correlation between soil temperature and the depth of nematode populations was observed, indicating that higher soil temperatures facilitate nematode movement deeper into soil. An experiment by Robbins and Barker (1974) examining temperature effects on sting nematode populations found that populations start to increase at 25°C. Our research as well as a study based in California found that sting nematode populations increased at 15°C (Bekal and Becker 2000; Galle et al. unpublished). These differences can be attributed to differences in experimental design as the Robbins and Barker study was performed in a greenhouse while the current and California studies were field samplings.

The observed vertical nematode population shift is important for management of sting nematode as currently used nematicides are readily bound in organic material in the thatch layer after application. Traditionally, superintendents in North Carolina make their nematicide applications during late May and June, with some even delaying application until July. This is when it is thought that nematode populations are increasing rapidly. These data demonstrate that nematode populations are migrating deeper into the soil in June and July, which can hinder management as K_{oc} (soil adsorption coefficient) of nematicides are quite high. Abamectin, one of the most commonly used nematicides in golf course turf and moves poorly beyond the top 2.5 cm of soil (Gannon et al. 2016). If applied in June, July and August, a majority of the sting population is below 2.5 cm and it is unlikely that the nematicide is reaching the nematodes. Therefore, one implication from this research is to begin applications earlier in the spring. Due to the slight differences between cultivars, it is recommended that nematicides should be applied in April on creeping bentgrass courses in the central and eastern portions of North Carolina. On bermudagrass, due to the later increase in populations it is recommended to make nematicide applications in May, although the increase in March would warrant an additional application be made in March if possible.

The high population counts of sting nematode throughout the fall from October to December were unexpected. Temperatures are decreasing at this time, and it was anticipated that populations would decline at this time as well. This does, however, coincide with the timeframe when creeping bentgrass rooting recovers from summer decline (Lyons et al. 2011). It is possible that this increase in roots provides sting nematodes with a food source and keeps their populations elevated until soil temperatures drop considerably in January and February. A fall increase also occurred on the bermudagrass course, where a small increase in the nematode population occurred in November and December. Unlike most bermudagrass cultivars, 'Champion' bermudagrass also produces roots in the fall right before it becomes dormant. Similar to the creeping bentgrass courses, it is likely that this food source could enable a slight increase in the sting nematode population. Taken as a whole, these results strongly suggests that both temperature and root development influence sting nematode population counts in North Carolina golf course greens.

An increase in nematode populations in the fall months is a troubling prospect, because it suggests that root parasitism is occurring, and damage may occur. Chemical management is rarely deployed in the fall by superintendents, likely because symptoms rarely show up due to low environmental stresses during North Carolina's more temperate fall and winter months. This may not be the best management scenario and fall applications should become more common for courses heavily infested with sting nematodes. The vertical distribution observed for sting nematode shows that a majority of the population is near the surface during this time, and it may be ideal to apply nematicides to target reproducing individuals, potentially reducing the carry-over population heading into the spring and summer of the following year. Some nematicides like fluopyram have a long half-life and fall applications may be beneficial and should be investigated further for management of sting nematode populations (NY State 2017).

Comparing a perennial cropping system like turfgrass with an annual system like maize also provides an interesting insight into the population dynamics of sting nematode. Evidence from fall population increases previously discusses shows a strong potential for nematode populations to be significantly linked to plant root production. This occurs in the spring for turfgrass and would occur in May when the maize field was planted. Similar to the bermudagrass populations, sting nematode numbers started to decrease in the fall when the maize plants were cultivated into the soil. Populations still remained elevated (14-16 nematodes /500 cc soil) above what was expected during the winter. Unlike in a perennial turf system where plants have a consistent food source throughout the year, this was unexpected in a fallow field system (Crow 2005). Little is known about life expectancy of many plant pathogenic nematodes including sting nematode, but it is possible that this nematode can survive at cool temperatures for long periods of time without food and may survive as the initial population in the following spring months (Barker et al. 1969).

Because the RKN population appears only to be found in the top layer of the soil column, nematicide application should be effective. However, the endoparasitic life cycle of RKN creates another set of issues because the nematodes are protected by being inside the roots the majority of the time. Most contact nematicides are unable to reach the developing and mature RKN in the roots, and therefore management should be focused on the second stage juveniles found outside of the roots. Populations begin to increase most rapidly the months of April and May and stay elevated throughout much of the summer. This observation was consistent with another study and suggests that nematode parasitism may occur throughout the entire summer (Morris et al. 2013). Early applications are likely to reduce the population initially, and multiple application throughout the summer are necessary to continue to keep the population numbers suppressed.

In summary, our results reveal that sting nematode populations maintain a much wider range of vertical distribution throughout the summer months than previously reported. At each location sampled, sting nematode populations increased between March and May. Therefore, for effective management a nematicide application should coincide with this increase. We also observed that sting nematode populations remained elevated in October and a majority were in the top 10 cm supporting another nematicide application at this time. Although sting nematode populations were high in June, July and August, the majority of the population was found below 10 cm deep in soil. This presents a challenge for chemical management of sting nematode as nematicides are not readily mobile in soil. Thus, turfgrass managers should focus on cultural practices during this time period, including fertility, mowing height and potentially cytokinin applications as these can increase root length and density (Tucker et al. 2006; Liu and Huang 2002).

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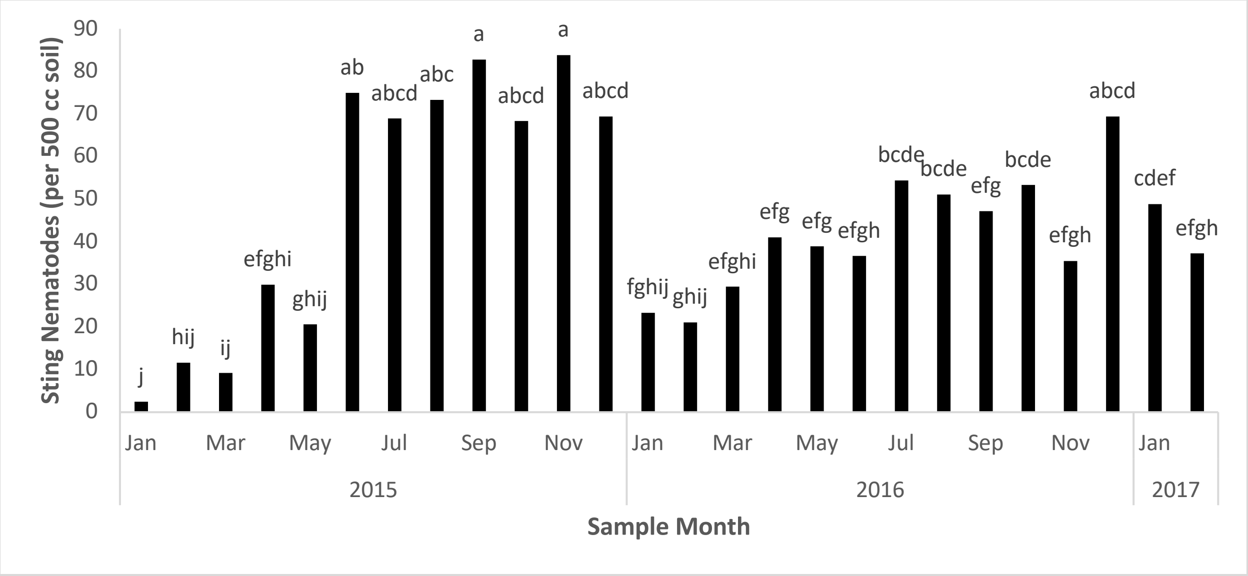

Figure 2.1. The total population counts of sting nematode at Raleigh Golf Association from 2015-2017. Each bar is the aggregate count from all three depths sampled. Different letters indicate differences in means between the different months according to Tukey's HSD (α=0.05).

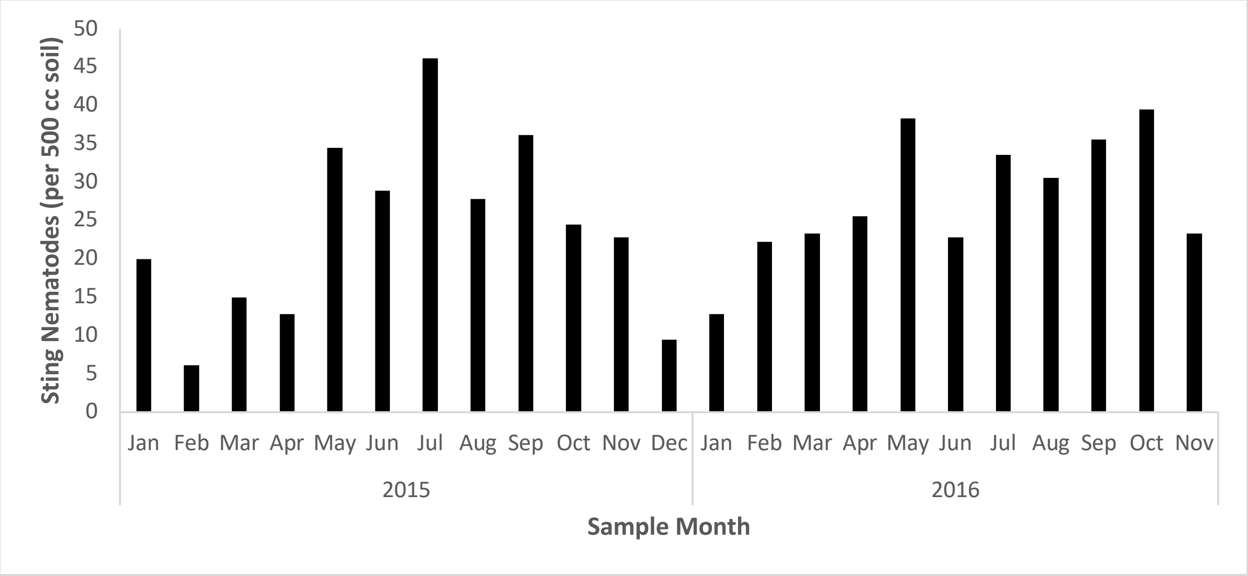

Figure 2.2. The total population counts of sting nematode at Wilson Country Club from 2015-2016. Each bar is the aggregate count from all three depths sampled. No significant differences between the months was determined.

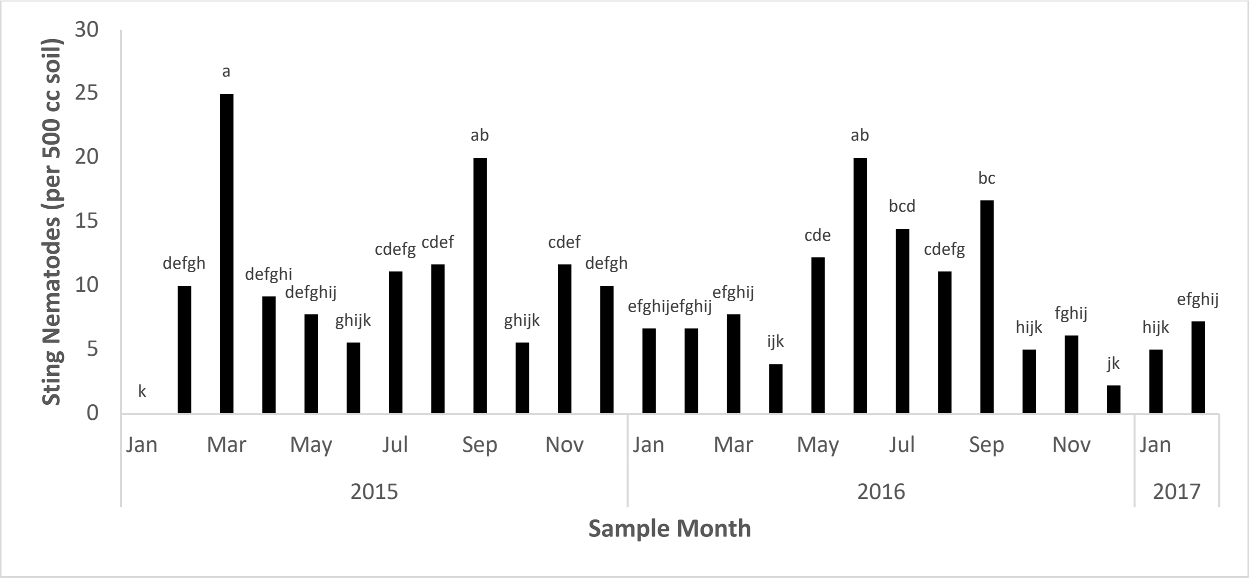

Figure 2.3. The total population counts of sting nematode at Benvenue Country Club from 2015-2017. Each bar is the aggregate count from all three depths sampled. Different letters indicate differences in means between the different months according to Tukey's HSD (α=0.05).

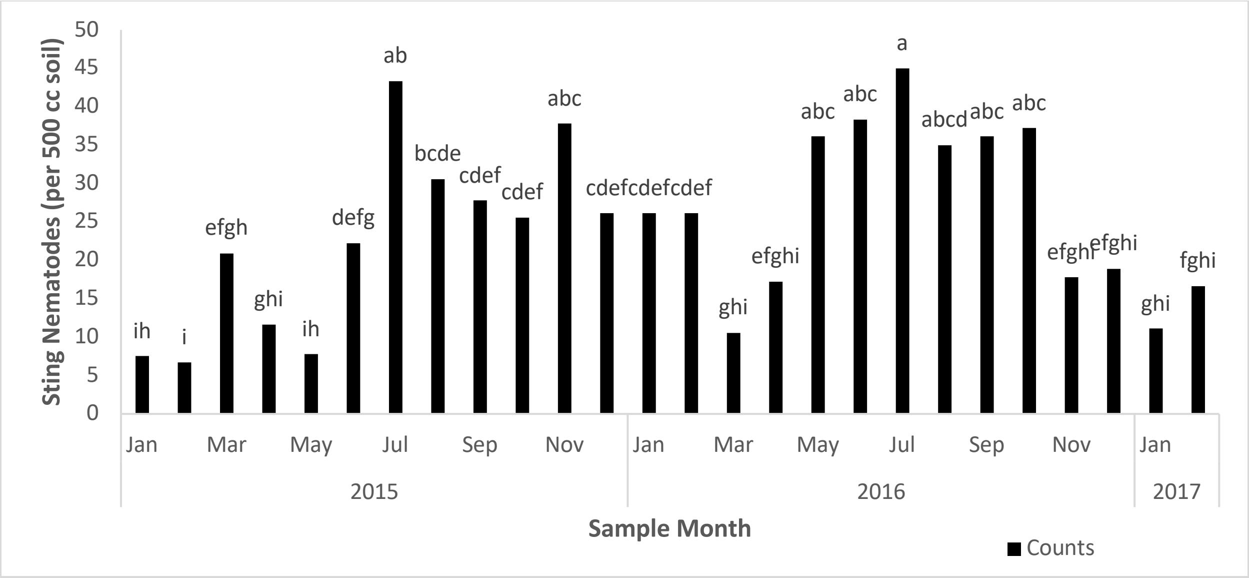

Figure 2.4. The total population counts of sting nematode at Central Crops Research Station on maize from 2015-2017. Each bar is the aggregate count from all three depths sampled. Different letters indicate differences in means between the different months according to Tukey's HSD (α=0.05).

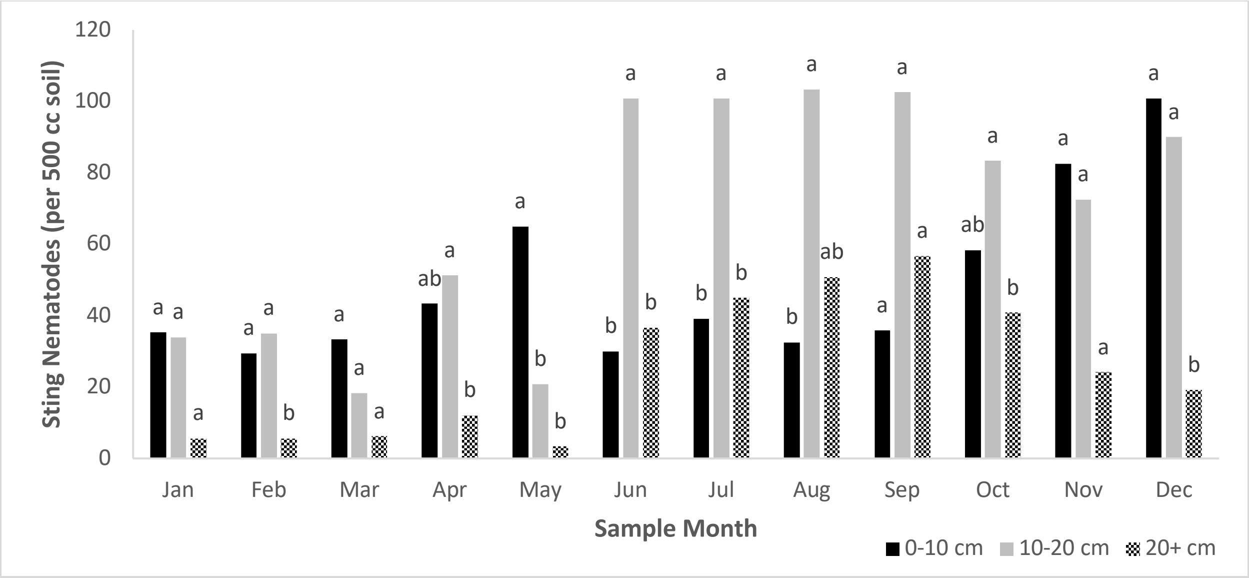

Figure 2.5. The aggregate monthly populations of sting nematode at Raleigh Golf Association on creeping bentgrass from 2015-2017. The black bars represent the population counts in the 0-10 cm subsection. The gray bars represent the population counts in the 10-20 cm subsection. And the dotted bars represent the population counts in the 20+ cm subsection. Different letters indicate differences in means between the different depths within each month according to Tukey's HSD(α=0.05).

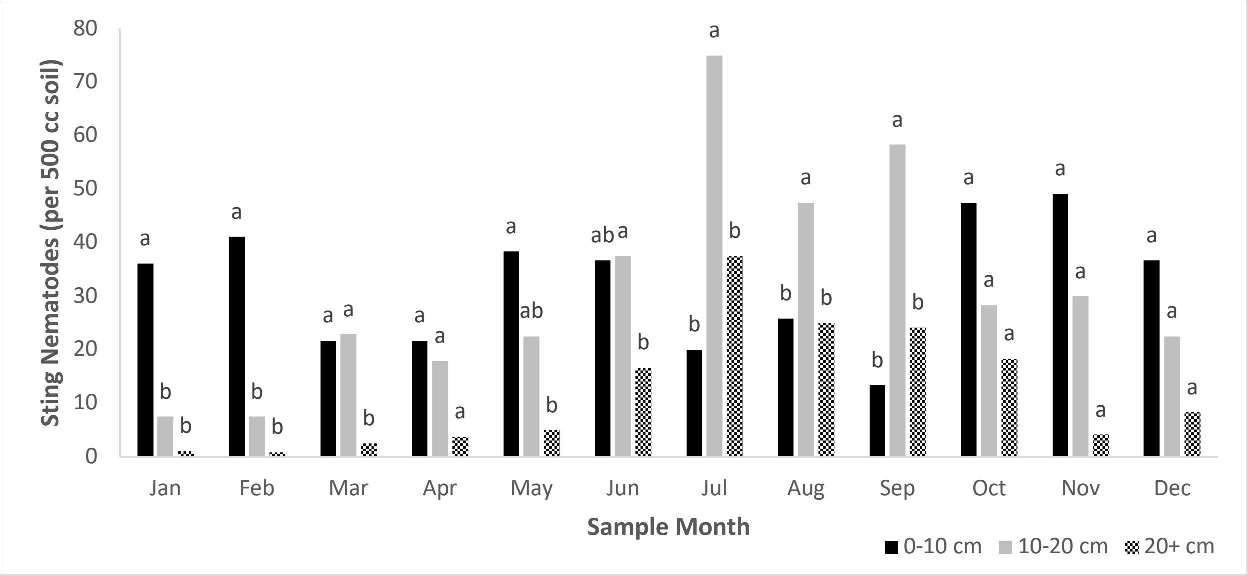

Figure 2.6. The aggregate monthly populations of sting nematode at Central Crop Research Station on maize from 2015-2017. The black bars represent the population counts in the 0-10 cm subsection. The gray bars represent the population counts in the 10-20 cm subsection. And the dotted bars represent the population counts in the 20+ cm subsection. Different letters indicate differences in means between the different depths within each month according to Tukey’s HSD (α=0.05).

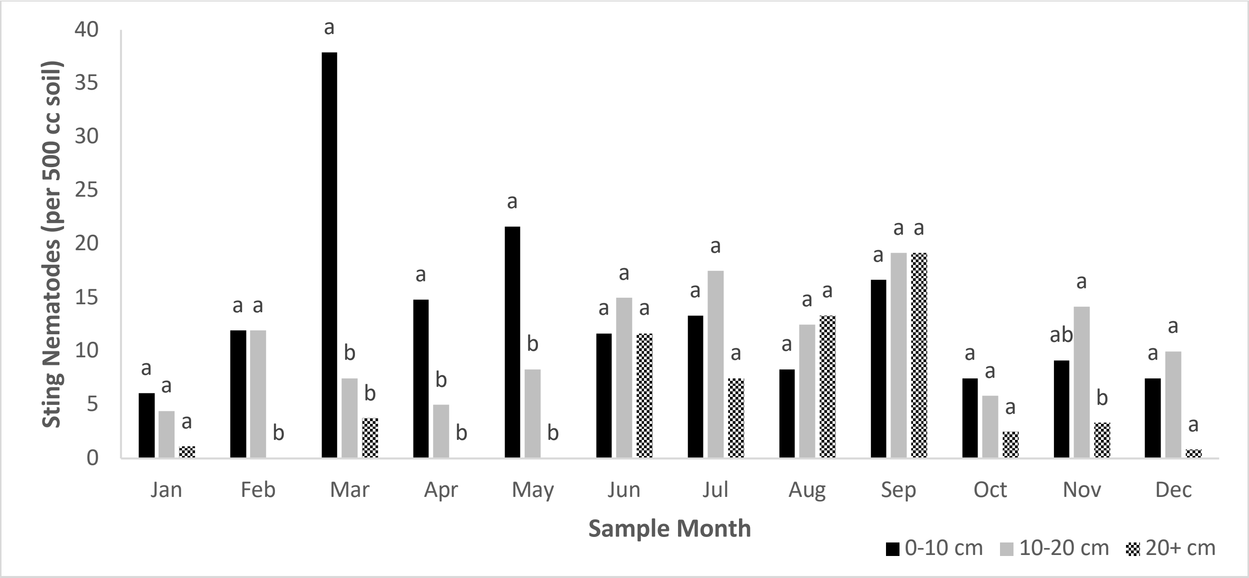

Figure 2.7. The aggregate monthly populations of sting nematode at Benvenue Country Club on hybrid bermudagrass from 2015-2017. The black bars represent the population counts in the 0-10 cm subsection. The gray bars represent the population counts in the 10-20 cm subsection. And the dotted bars represent the population counts in the 20+ cm subsection. Different letters indicate differences in means between the different depths within each month according to Tukey's HSD (α=0.05).

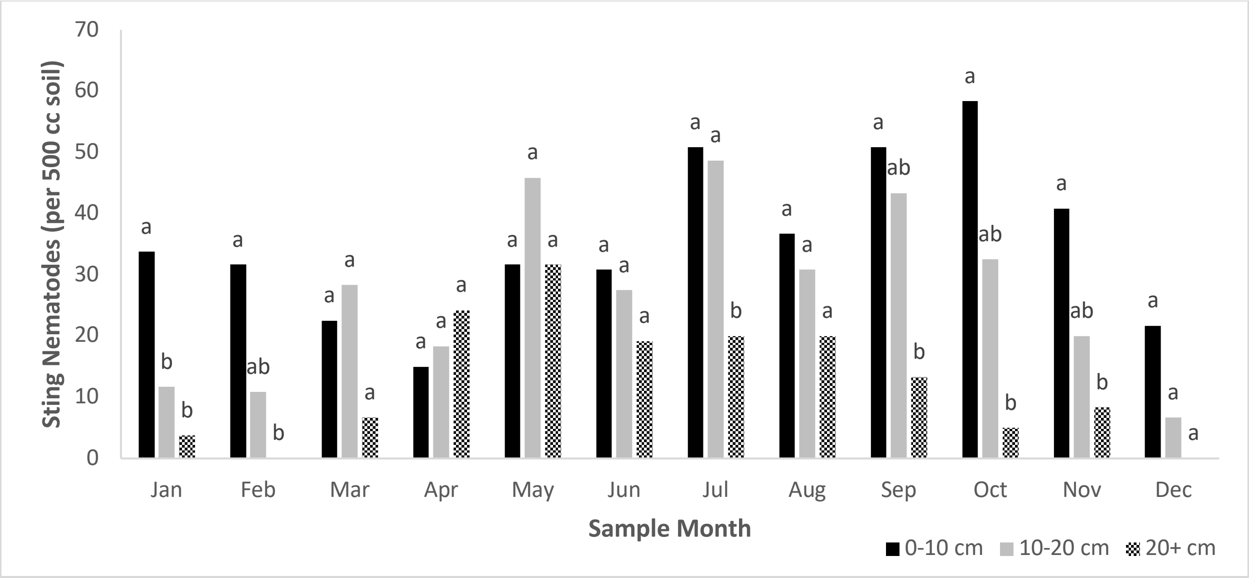

Figure 2.8. The aggregate monthly populations of sting nematode at Wilson Country Club on creeping bentgrass from 2015-2017. The black bars represent the population counts in the 0-10 cm subsection. The gray bars represent the population counts in the 10-20 cm subsection. And the dotted bars represent the population counts in the 20+ cm subsection. Different letters indicate differences in means between the different depths within each month according to Tukey's HSD (α=0.05).

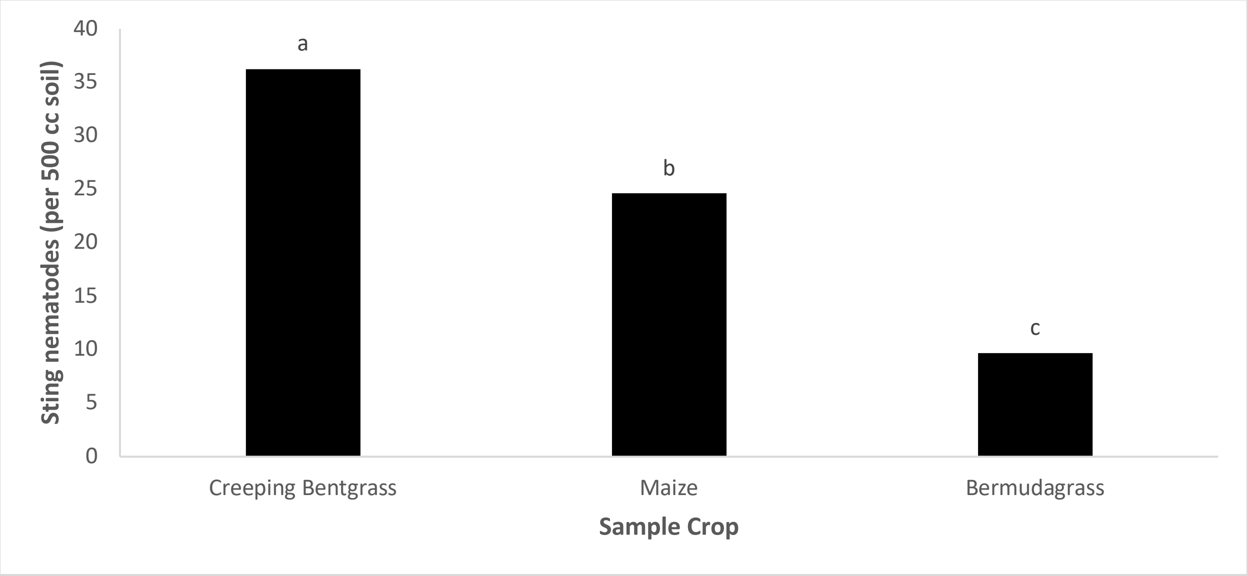

Figure 2.9. The total average monthly nematode counts over the entire study from each of the three crop types sampled. Different letters indicate differences in means between the different population counts from crop according to Tukey's HSD (α=0.05).

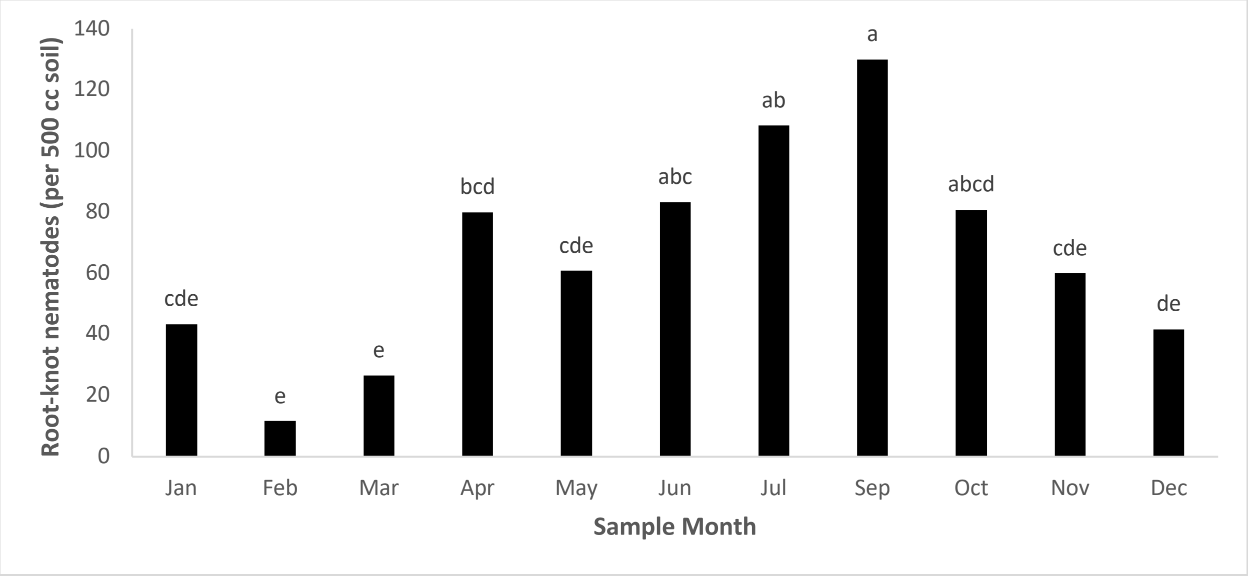

Figure 2.10. The aggregate monthly populations of root-knot nematode on hybrid bermudagrass at Sedgefield Country Club at the 10 cm depth from 2015-2017. Different letters indicate differences in means between the different population counts from each month according to Tukey's HSD (α=0.05).

CHAPTER THREE

Management of *Belonolaimus longicaudatus* on golf course putting greens using abamectin and fluopyram

ABSTRACT

Nematode management on golf course turf is difficult, and nematicides are a critical component in effectively managing and reducing the populations of these pests. A variety of nematicides have employed as part of golf course management schemes, but many of these are no longer available due to health and environmental concerns. The gradual decrease in available products culminated in the withdrawal of fenamiphos from the market voluntarily in 2007, and left many superintendents without viable options for nematode management. In the mid 2010's abamectin and fluopyram were both introduced to the turf nematicide market and were immediately incorporated into disease management programs. The objective of this study was to evaluate these nematicide products and determine how to improve efficacy through precise timing of applications and rates. Five different studies were performed at a variety of sites throughout the state of North Carolina from 2014-2018. Spray programs using abamectin and fluopyram at various rates and timings throughout the spring and early summer were developed and tested on sting (*Belonolaimus longicaudatus*) and root-knot (*Meloidogyne* spp.) nematode populations. Nematode population totals were determined at the beginning and end of each study, and turf quality was assessed throughout the duration of each study. The use of abamectin did not reduce nematode populations of either species and turf quality improvements were also not observed. Fluopyram applications significantly reduced the population totals of sting nematode in 2016 and 2017, with April and May applications being more effective than those applied in June. Over the three years of the fluopyram focused studies (2016-2018), overall turf quality was shown to

improve substantially. Root-knot nematode populations were not impacted by either nematicide treatment in any year of the studies. These studies show that fluopyram is an important addition to a nematicide spray program, and early applications can be successful at reducing sting nematode populations.

INTRODUCTION

Nematodes are significant pathogens of turfgrasses in North Carolina. These parasites feed on the plant roots, reducing the ability to uptake nutrients and water. Symptoms typically include brown or yellow patches, thinning of turf stands, and in cases of severe infestation can result in complete turfgrass death. Management of these pests is critical for maintaining and improving turfgrass health, particularly in the summer months.

Historically, management of nematode populations has primarily been achieved through the use of chemical nematicides. However, in the early 2000's the most widely used nematicide fenamiphos (Bayer CropSciences, Research Triangle Park, NC) was voluntarily withdrawn from the market (Anonymous 2002). Its official stop use date was October 2017, but sales were suspended starting in 2011. Products such as furfural (Agriguard, Cranford, NC) and *Bacillus firmus* (Bayer CropSciences, Research Triangle Park, NC) were effective, but there was still a need for more products to compliment these options (Crow and Luc 2014; Crow 2014).

Starting in the mid-2010's two new nematicides were registered for use in turfgrass including abamectin (Syngenta Crop Protection, Research Triangle Park, NC) and fluopyram (Bayer CropScience, Research Triangle Park, NC). Abamectin has been used as an insecticide/miticide, and was originally labeled for this purpose as Avid™ 0.15 EC. However, it was given special registration 24(c) for use as a nematicide in golf turf starting in 2011. It is a contact nematicide, and one study found that after a single hour of contact the nematodes did not recover (Faske and Starr 2007). However, it has a very high affinity for organic matter and requires large amounts of irrigation to move it beyond the first 2.5 cm of the soil profile (Gannon et al. 2016). This can have significant impact on its efficacy, especially on older golf course putting greens with a thicker organic layer.

Fluopyram was first registered for use in 2016 under the trade name Indemnify^{TM}. It is a succinate dehydrogenase inhibitor (SDHI) fungicide used for a variety of soilborne pathogens and is one of many fungicides that have been discovered to have potential nematicidal activity. Fluopyram was found to reduce the motility of root-knot (*Meloidogyne incognita*) nematodes (RKN) and inhibit their ability to infect roots as a nematistatic, or a product that does not directly kill nematodes (Faske and Hurd 2015). Unlike abamectin, it has a lower soil adsorption coefficient and is likely to be more mobile in soils. With these new products available on the market, research is necessary to best determine how to utilize them in an effective nematicide program for turfgrass management.

The objectives of this study were to look at the timing and rates of abamectin and fluopyram application throughout the year for management of sting (*Belonolaimus longicaudatus*) and root-knot nematodes. Field trials were conducted over a five-year period at multiple golf courses throughout central and eastern North Carolina on both warm and cool season putting greens.

MATERIALS AND METHODS

Abamectin Research Trials

Abamectin efficacy and timing trials were conducted from 2014-2015 at four golf courses throughout central North Carolina. Two courses managed creeping bentgrass (*Agrostis stolonifera* L.) putting greens: Raleigh Golf Association (RGA) in Raleigh, NC and Wilson Country Club in Wilson, NC with 'L-93' and 'Penn A-1/A-4', respectively. The two other courses managed 'Champion' bermudagrass (*Cynodon dactylon* L.) putting greens: Sedgefield Country Club in Greensboro, NC and Benvenue Country Club in Rocky Mount, NC. The courses were maintained according to the discretion of the golf course superintendents. All nematicide application to the research greens was suspended during the course of the study, but any fertility or pesticide applications that would not interfere with nematode activity were allowed to continue as needed.

The experiments were arranged in a randomized complete block design (RCBD) with 4 or 6 replications of each treatment (6 replications in 2014 and 4 in 2015). Each plot was 0.91 x 1.82 m. Experimental location was based upon previous sampling and placed in areas with the highest concentration of either sting or root-knot nematode populations, respectively. Treatments were applied using a CO2-pressurized backpack sprayer with a single nozzle boom. All treatments are listed in Table 3.1. Treatments were applied with the Revolution^{TM} (Aquatrols, Paulsboro, NJ) wetting agent to aid in movement of the product through the thatch layer and were irrigated with 0.3 cm immediately after application. The trials in 2014 were initiated on 19-March at Raleigh Golf Association and Sedgefield Country Club and on 21-March at Benvenue Country Club. The trials in 2015 were initiated on 10-March at Raleigh Golf Association. and 11-March at Benvenue Country Club and Wilson Country Club. Trials were started at Sedgefield Country Club but were ultimately suspended in May due to severe winterkill of the putting green. Soil samples were taken at the start of each trial as well as at the completion of the trial. In 2014 samples were taken on 22-July and in 2015 there were taken on 31-August. Eight 1.6-cm diameter soil cores were taken from each plot to a depth of 20 cm. The eight cores were combined into a single sample per plot and submitted to the North Carolina Department of Agriculture and Consumer Services (NCDA&CS) nematode assay lab for nematode extraction, species identification and population counts.

Turfgrass quality ratings were taken on a monthly basis throughout the study. The turf was rated for uniformity, density and color on a 1-9 scale (1=poor, 9=excellent, 6=acceptable). Normalized difference vegetation index (NDVI) ratings were also taken. All data analysis was completed using the GLIMMIX procedure using SAS 9.4 (SAS, Cary, NC), and means separation was achieved using Tukey's HSD ($\alpha=0.05$). Analysis of covariance (ANCOVA) and repeated measures analysis were also utilized.

Fluopyram Research Trials

Three experiments conducted from 2016-2018 at two courses in North Carolina; Raleigh Golf Association, Raleigh NC and Sedgefield Country Club, Greensboro, NC. The Raleigh Golf Association location was sampled for sting nematode populations, and the Sedgefield location was sampled for root-knot nematode populations. Both courses were maintained according to the golf course superintendent. Nematicide applications as well as fluopyram-based fungicides were not made to the research greens during the three years.

All experiments were arranged in a randomized complete block design (RCBD) with four replications. Plots were 0.91 x 1.82 m. and all applications were made using a CO2-pressured backpack sprayer. Trials were initiated on 16-March 2016, 12-April 2017, and 9-April 2018. Applications were made monthly and are outlined in Table 3.2. Nematode counts were taken at the start and conclusion of the study in all three years. The final soil samples were taken on 9-September 2016, 18-August 2017, and 20-July 2018. Eight soil cores were taken from each plot using a 1.6-cm diameter soil cores. Cores from each plot were combined and send off to the NCDA&CS nematode assay lab for nematode extraction, identification and counting.

Turfgrass quality ratings were taken on a monthly basis from the start of the trials. The turf was rated for uniformity, density and color on a 1-9 scale (1=poor, 9=excellent, 6=acceptable). All data analysis was completed using the GLIMMIX procedure using SAS 9.4 (SAS Institute, Cary, NC), and means separation was achieved using Tukey's HSD ($\alpha = 0.05$).

RESULTS

Abamectin Research Trials

No significantly different results were observed from either 2014 or 2015 in the abamectin research trials. In both years, there was no increase or decrease in turf quality as a result of abamectin application, regardless of timing compared to the control. There was also no difference in the final nematode counts as a result of abamectin use. Analysis of covariance (ANCOVA) using pre-count populations as the covariant and repeated measures analysis was also performed to look for trends in turf quality over time, and no differences were observed in any treatment.

Fluopyram Research Trials

The first year of fluopyram trials occurred i 2016 when the product was initially launched to the market as a nematicide. The efficacy trial showed a significant decrease in sting nematodes populations when fluopyram was a part of the treatment list. Populations dropped from 497 nematodes/500 cc soil in the control to between 25-37 nematodes/500 cc soil in the fluopyram treated trials (Figure 3.1). There was no difference in timing or rate of fluopyram treatments, and additives such as abamectin or *B. firmus* did not provide any additional boost to performance. However, there were no observed differences in overall turf quality in any of the treatments throughout the duration of the experiment.

In 2017, all fluopyram treatments applied in the spring showed significantly lower sting nematode populations than the control plots or plots treated with abamectin. The treatments with fluopyram application in April were the most successful, with the high rate of fluopyram reducing population counts to 5 nematodes/500 cc soil. Applications made in May also reduced the population significantly with nematode counts in the range of 30-45 nematodes/500 cc soil (Figure 3.2). The only application that was not significantly separated from the control was the lowest rate of fluopyram in applied in June. However, this treatment still reduced the population by approximately 33% from the control. Overall, there were no significant differences between using a high rate or a low rate, but in each of the treatments there was a slight reduction in the counts when using the higher of the two rates. Similar to 2016, there were no detectable differences in turf quality between any of the treatments.

In 2018, there was no significant difference in sting nematode populations between the applications of fluopyram compared to the control. However, initial sting populations were extremely low as a result of multiple years of nematicide applications to the same location, beginning with the abamectin trials in 2014. The overall average of all nematode counts in 2016 at RGA was 182 nematodes/500 cc soil and by 2018 the overall count in the whole trial was reduced to 15 nematodes/500 cc soil ($p<0.0001$), showing a significant decrease in the overall nematode population counts in the entire green in total. Further, overall turfgrass quality of the research area improved greatly from 2016 to 2018, increasing from a rating average of 4.6 to 5.9 in that time period ($p<0.0001$).

Trials in 2016 and 2017 at Sedgefield Country Club looking at the effects of fluopyram and abamectin on root-knot nematode did not have any significant differences in either final nematode count or turf quality (Table 3.3). Root-knot nematode populations per 500 cc of soil ranged from 56.75 to 154.5 in 2016 and 21.5 to 48.75 in 2017. However, no treatment effect was determined and neither product reduced populations significantly.

DISCUSSION

Fluopyram was found to have a significant effect on sting nematode populations at both of the recommended rates. Timing was an important factor, with sting nematode populations significantly reduced with May applications compared to fluopyram applications in July. This is likely a result of managing the population early in the season before optimum nematode growth conditions. Several studies have shown populations start to increase in mid-spring, and by reducing the population early enough we can prevent a population explosion (Mc Groary et al. 2009; Bekal and Becker 2000; Galle et al. unpublished).

We did not observe any differences in sting nematode populations between high and low application rates of fluopyram in 2016, and only saw a slightly lower sting nematode population counts with the higher application rates in 2017. We also did not see any negative effect on efficacy from a split application of the low fluopyram rate. This results provides an option for multiple applications throughout the year. Our research has shown that sting nematode populations are elevated throughout much of the fall and early winter months, and a potential fall application may be useful for managing populations (Galle et al. unpublished). Sting nematode females will produce a single egg a day under ideal conditions, and therefore populations will be slow to recover from spring applications (Huang and Becker 1999). The potential for multiple applications could be effective at quickly reducing populations by never allowing nematodes to rebound from the last nematicide application.

Further, we did not see any differences in turf quality in any of the three years of the study with fluopyram use. This is potentially a result of being located on golf courses where the turf is not managed to promote disease, unlike experimental turf plots on a research station. However, after the conclusion of the trial in 2016 it was observed that fluopyram treated plots recovered from nematode feeding damage considerably faster than the non-treated control plots (Figure 3.3). Turfgrass is very slow to exhibit nematode feeding symptoms and also can take a long time to recover (Giblin-Davis et al. 1992). While the fluopyram treatments did not reduce the stress damage in the summer, these observations show that recovery can be rapid if sting nematode populations are kept low, thus further reducing the potential for fall feeding damage when nematode populations become more active. We did attempt to investigate this observation in 2017, but the nematode damage was not severe, and differences did not develop.

Repeated applications of fluopyram to an area over several years did show a significant reduction in nematode population numbers, and in the three-year time of this study almost eliminated sting nematode populations entirely from some of the plots sampled. Along with population decreases over time, the overall quality of the putting green increased from 2016 with an average quality rating increase of 4.6 to 5.9 by 2018. This indicates a cumulative effect of fluopyram applications, and a slow reduction in the population of sting nematode resulting in significant impacts on increased overall turf quality. This reinforces our recommendations that superintendents must be patient with nematicide usage, and while the effects may not be immediate nematicide use can have long-term positive and lasting effects.

Abamectin did not reduce populations throughout any of the 5 years of the study. Similar results were found recently in Florida with only marginal reductions of sting nematode with abamectin use (Gu and Crow 2018). The soil chemistry of this product is the likely issue, and with sting nematode populations being found predominately beyond the initial thatch layer of the turfgrass it is unlikely that any of the product is reaching a majority of the nematode population. The use of irrigation and a wetting agent to help improve efficacy by moving abamectin deeper into the soil column was unsuccessful. However, higher irrigation amounts greater than 0.6 cm or application around aerification events should be investigated to improve abamectin movement.

Finally, this study found that no reduction in root-knot nematode populations was achieved with the use of fluopyram. This was unexpected, as it has been shown to be very successful on root-knot nematode species in other studies on a variety of agricultural crops (Ji et al. 2019; Jones et al. 2017). Fluopyram is a contact nematicide and is likely to be reduced in its effectiveness on endoparasitic nematode species (Faske and Hurd 2015). This reduced efficacy may have been due to the limited number of applications, and more frequency may be necessary to effectively manage root-knot nematode populations, perhaps due to their high reproductive rate throughout the year. While not the focus of this study, lance nematode (*Hoplolaimus galeatus*) is another endoparasitic nematode that was not affected by fluopyram applications in 2017 and 2018. Chemical management is a commonly ineffective for endoparasitic nematodes on turf, and more research is necessary in the future.

In summary, fluopyram was found to be an excellent nematicide on sting nematodes attacking turf. Application timing is an important aspect of management for this nematode, and early applications in April or May are likely to be the most effective at reducing nematode populations. This recent addition to the nematicide market was much needed for sting nematode management, but its fluopyram's lack of effectiveness on root-knot nematode in this study indicates the need for a multifaceted approach to turfgrass nematode management. A greater diversity of products are needed to control the wide variety of nematode species infecting turfgrass, but fluopyram is an excellent addition to a nematode management program.

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Table 3.1. Treatments and application timings for abamectin research trials in 2014 and 2015. All applications were tank-mixed with the wetting agent Revolution at 19.1 l/ha. Letters correspond to application dates: A = March, B = April, C = May, D = June, E = July, F = August, G = Sept, H = Oct, I = Nov, J = Dec.

2014			2015		
Treatment	Rate (l/ha)	Timing	Treatment	Rate (l/ha)	Timing
Revolution	19.1	A-J	Revolution	19.1	A-I
Avid	4.17	A-D	Avid	4.17	A-D
Avid	4.17	B-E	Avid	4.17	B-E
Avid	4.17	C-F	Avid	4.17	C-F
Avid	4.17	ABGH	Avid	4.17	ABHI
Avid	4.17	F-I	Avid	4.17	G-J
Avid	1.51	21 day	Avid	1.67	A-J
			Avid	0.88	14 day

Table 3.2. Treatments and application timings for fluopyram and abamectin research trials from 2016-2018. Nortica is a biological control agent comprised of *Bacillus firmus* and is in kg/ha. All treatments in 2016 were applied with the wetting agent Revolution at a rate of 19.1 l/ha. Letters correspond to application dates: A = March, B = April, C = May, D = June, E = July.

2016			2017			2018		
Treatment	Rate (l/ha)	Timing	Treatment	Rate (l/ha)	Timing	Treatment	Rate (l/ha)	Timing
Control			Control			Control		
Avid	4.17	B-E	Indemnify	0.62	B	Indemnify	0.62	A
Nortica Avid	39.3 4.17	AB CDE	Indemnify	0.62	C	Indemnify	0.62	B
Nortica Indemnify	39.3 0.62	AB CD	Indemnify	0.62	D	Indemnify	0.62	C
Nortica Indemnify	39.3 1.25	AB C	Indemnify	0.62	BC	Indemnify	1.25	A
Nortica Indemnify	39.3 1.25	AB D	Indemnify	0.62	CD	Indemnify	1.25	B
Indemnify	0.62	CD	Indemnify	1.25	B	Indemnify	1.25	C
Avid Indemnify	4.17 0.62	AB CD	Indemnify	1.25	C			
			Indemnify	1.25	D			
			Divanem Revolution	0.46 19.1	B-E B-E			

Table 3.3. Fluopyram and abamectin nematicide application program at Sedgefield Country Club for 2016 and 2017. All treatments in 2016 were applied with the wetting agent Revolution at a rate of 19.1 l/ha. Root-knot nematode populations were sampled on 9-September 2016 and 18-August 2017. Letters correspond to application dates: A = March, B = April, C = May, D = June, E = July. No significant differences were observed between population counts according to Tukey's HSD (α = 0.05).

2016				2017			
Treatment	Rate (l/ha)	Timing	Root-knot Nematode counts (per 500 cc soil)	Treatment	Rate (l/ha)	Timing	Root-knot Nematode counts (per 500 cc soil)
Control			64.5 a	Control			30 a
Avid	4.17	B-E	73.25 a	Indemnify	0.62	B	75 a
Nortica Avid	39.3 4.17	AB CDE	154.5 a	Indemnify	0.62	C	41.75 a
Nortica Indemnify	39.3 0.62	AB CD	56.75 a	Indemnify	0.62	D	33.5 a
Nortica Indemnify	39.3 1.25	AB C	95 a	Indemnify	0.62	BC	48.75 a
Nortica Indemnify	39.3 1.25	AB D	70.75 a	Indemnify	0.62	CD	22.5 a
Indemnify	0.62	CD	69.75 a	Indemnify	1.25	B	43.5 a
Avid Indemnify	4.17 0.62	AB CD	115.25 a	Indemnify	1.25	C	21.5 a
				Indemnify	1.25	D	68.75 a
				Divanem Revolution	0.46 19.1	B-E B-E	36.75 a

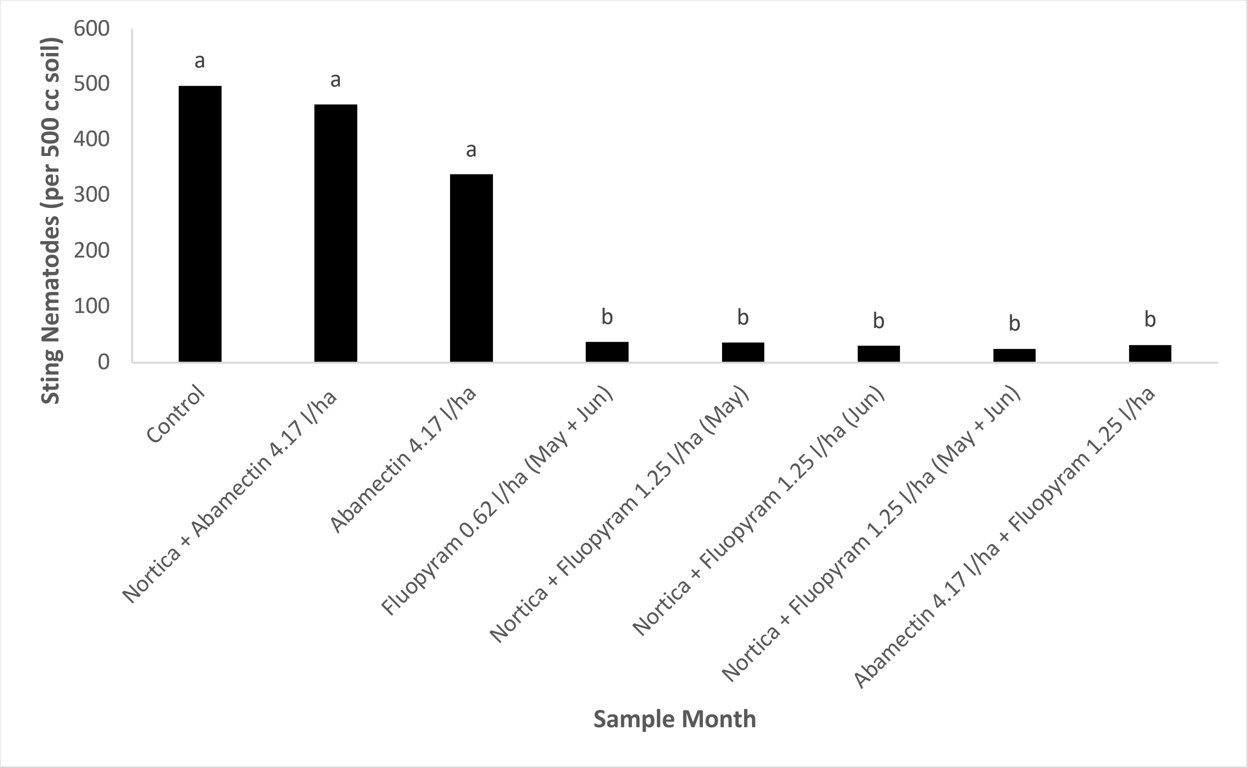

Figure 3.1. The final sting nematode count from the fluopyram and abamectin trial from 9-September 2016. Abamectin (Avid) was applied at a rate of 4.17 l/ha. Fluopyram (Indemnify) was applied at two different rates; the low rate was 0.62 l/ha and the high rate was 1.25 l/ha. Columns with similar letters are similar according to Tukey's HSD (α = 0.05).

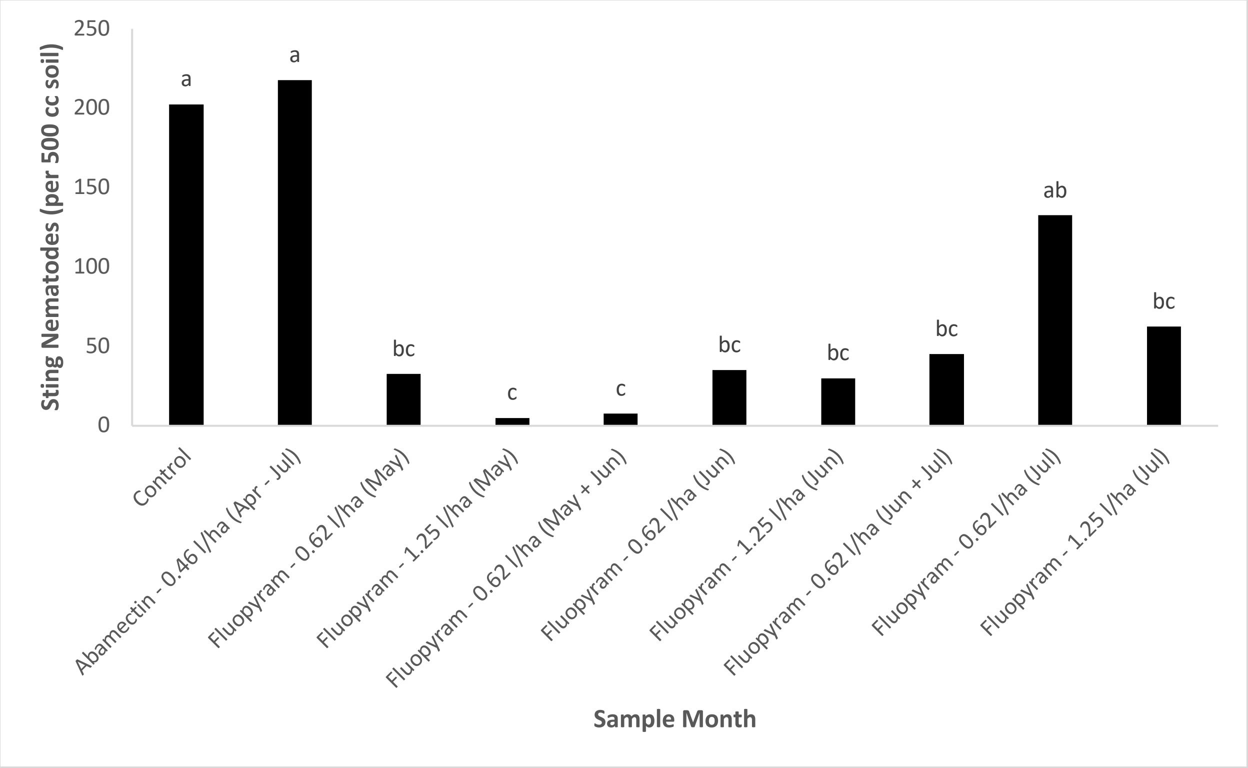

Figure 3.2. The final sting nematode count from the fluopyram trial from 18-September 2017. Abamectin (Divanem) was applied at a rate of 0.46 l/ha. Fluopyram (indemnify) was applied at two different rates; the low rate was 0.62 l/ha and the high rate was 1.25 l/ha. Columns with similar letters are similar according to Tukey's HSD ($\alpha = 0.05$).

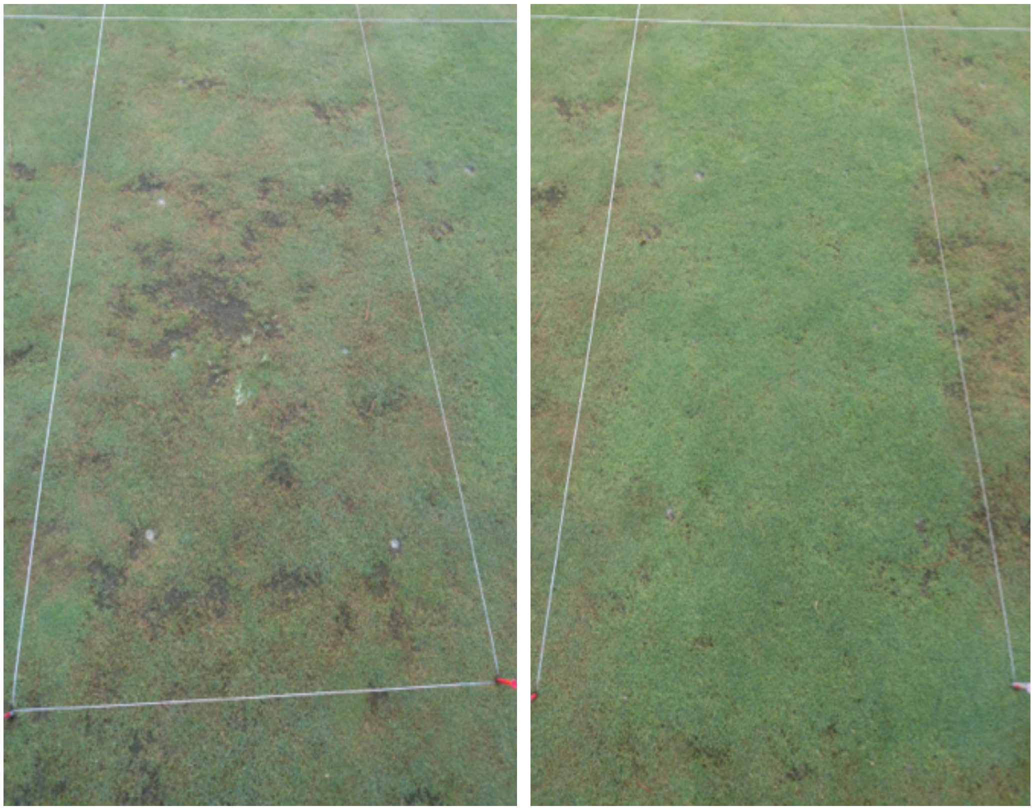

Figure 3.3. The improved overall turf quality when proper nematicide application timing was used. The image on the left was the non-treated control, and the image on the right was treated with fluopyram at the high rate in May. The photos were taken in September and highlight the recovery of the turfgrass when nematicide feeding is managed.

CHAPTER FOUR

The effects of temperature on the movement of *Belonolaimus longicaudatus*

ABSTRACT

As a transition zone state, North Carolina is a challenging location to grow turfgrass. Temperatures vary greatly throughout the year, with soil temperature ranging from 10-30°C. For golf course superintendents, regardless of turfgrass species planted for putting greens, the grass will struggle for a few months each year. To further complicate matters, those courses infested with sting nematode (*Belonolaimus longicaudatus*) will have even more issues with grass growth. *B. longicaudatus* is a highly damaging nematode and is endemic to the entire Southeastern United States. Originating out of Florida, it is widely believed to be a warm-weather nematode, although its range is slowly creeping northward. Minimal data is available on how cool temperatures affect sting nematode activity, and whether they may feed on turfgrass roots throughout the entire year in North Carolina. An in vitro study was conducted to determine the movement of sting nematode populations at temperatures ranging from 10 to 30°C. A second study was conducted looking at changes in movement as temperatures were kept constant, or raised or lowered 5°C, which can commonly occur in the fall, winter and spring months when air temperatures can vary greatly. Sting nematode moved the greatest distances at 30°C. Movement reduced as temperatures dropped, with the least movement occurring at 10°C. Movement of sting nematode dramatically increased as temperatures were increased from 10°C to 15°C. This correlates with the spring when turf plants will become more active and turf managers start applying nematicides. Overall, sting nematode is more active during the warmer summer months temperatures, but its activity at low temperatures indicates it may be a threat to turfgrass roots throughout much of the year.

INTRODUCTION

North Carolina is a state with a challenging climate, with temperatures regularly above 30°C during the summer as well as at or below freezing for extended periods of time during other times of the year. The state is located in the transition zone, meaning cool, dry winters and warm hot summers. This provides for a significant challenge for growing turfgrass on golf courses, sod farms, athletic fields and even home lawns. Predicting nematode feeding can also present a unique challenge given the varied environmental conditions of North Carolina.

Nematodes are soil borne pathogens, and feed on the roots of many plants including turfgrass. This feeding damages the roots, and some nematode species can cause severe damage (Christie et al. 1952). The damage results in an inability for the plant to cope with heat or drought stress during the summer months and can ultimately result in turf death if left unmanaged (Lucas 1982). For a golf course, this damage is very noticeable on the highly manicured putting greens and fairways which can result in decreased playability and poor aesthetics. Yet, damage can also occur on athletic fields, sod farms and home lawns.

One of the most damaging nematode species on turfgrass is sting nematode (*Belonolaimus longicaudatus* Rau 1958). This is a large nematode that can do significant damage with populations as low as 1-19 nematodes per 500 cc of soil (Clemson 2000). Sting nematode was first discovered on turfgrass in Florida in the late 1950's and has since become a problem throughout much of the southeastern United States (Rau 1958). Sting nematode is widely considered a warm weather nematode and is likely to be found more frequently in warmer climates more than cooler climates. While it has been found throughout the country, it is most problematic in warmer areas like the Southeast United States and California (Mundo-Ocampo et al. 1994).

Many factors play a significant role in nematode feeding damage, and one such factor is soil temperature. Like most plant pathogens, temperature plays a key role in growth, development and pathogenicity. For many fungal diseases, the pathogens grow and infect under very specific temperature ranges (Velasquez et al. 2018). For nematodes, these thresholds are much less defined. Traditionally for sting nematode a soil temperature of 12°C was considered the point when nematodes begin to feed thus damaging roots (Martin 2017). In Florida temperatures rarely remain at 12°C for long, and therefore sting nematode feeding is an issue year-round. However, North Carolina gets much colder in the winters with soil temperatures rarely getting above 8°C during January and February. Superintendents are not concerned with nematode feeding damage during these months, and nematicide applications are not made from late fall throughout early spring. However, little is known about feeding or even mobility of sting nematode at temperatures below 12°C.

This study used in vitro methods to determine the overall movement of sting nematode at temperatures ranging between 10 and 30°C. The first of two objectives were to evaluate the effect of temperature on mobility of sting nematode. The second objective was to examine the effect of temperature fluctuations on sting nematode movement. North Carolina can have a multitude of temperature swings throughout the fall, winter and spring where it is quite common to have soil temperatures change by as much as 5°C in a single week. This second objective focused on investigating the effects these changes have on sting nematode. Overall, this information will be useful for turf managers when determining whether sting nematode feeding pressure is an issue during the winter months.

MATERIALS AND METHODS

Nematode Inoculum Preparation

All sting nematodes for this experiment were obtained from a maize field used solely for the maintenance of nematode populations at the Central Crops Research Station in Clayton, NC. Soil samples were taken from the field and nematodes were extracted from the soil using a modified sieving-sugar centrifugation technique (Jenkins 1964). Soil was taken in 500 cc samples and stirred with water for 30 seconds. After a brief period, the solution was poured through a 100-mesh sieve placed on a 400 mesh-sieve. The solids from the 400-mesh sieve were then rinsed into a centrifuge tube and centrifuged at 4,000 rpm for 5 minutes. The supernatant was then poured off and the pellet was broken up in a 60% sucrose solution. The solution was then centrifuged at 4,000 rpm for 30 seconds, and the solution was poured through a 500-mesh sieve. The final solution of nematodes was then placed into another tube and combined with the subsequent collections of other extractions.

B. longicaudatus adults were then picked out of the solution using a root canal dental pick and placed into sterile, distilled water for 24 hours. After 24 hours, the nematodes were rinsed a second time with more sterile water. After 2 rinse periods, the nematodes were considered clean enough to move on to plating.

In vitro Nematode Temperature Assay

An experiment was initiated in winter of 2019. 100-mm diameter petri plates were filled with 25 ml of 1.5% water agar. These plates were used for the plant-less dishes. Dishes with plants were made with a 1.5% agar and were amended with Gamborg's B5 solution to provide nutrients for the plants (Huang and Becker 1997). Creeping bentgrass seeds were surface sterilized using a triple rinse of 10% bleach followed by a triple rinse of autoclaved sterile water.

The seeds were dried on autoclaved filter paper and then 3-4 seeds were placed at the center of each Gamborg's B5 amended media plate. Plants were left to germinate and grow under a light source for 7 days. At this time, non-contaminated plates were selected for inoculation with nematodes.

Inoculation of the plates with sting nematodes occurred by transferring the clean nematodes to the plates using a root canal pick. A single nematode was placed near the edge of each plate. The location of the initial placement was marked with a marker on the underside of the plate. The plates were randomly assigned to one of 5 treatment groups; 10° 15, 20, 25, and 30°C. This was to account for any potential bias of the more active nematodes being selected and plated first. Each treatment was replicated 12 times. Plates were incubated for 7 days at these temperatures. Ratings were taken every 24 hours. Nematodes were located on the plate and their location was marked. If the nematodes started to climb the walls of the plate, they were relocated, if possible, back onto the agar near the point they were found. Notes were taken if the nematodes died prematurely, if bacterial or fungal contamination occurred or if they found the plant roots and started to feed.

After 7 days, the experiment was terminated. Distance measurements were taken by measuring the distance from point to point based upon the day. Any nematode that desiccated and died by climbing the dish wall was removed from the data set. Data analysis was performed using the GLIMMIX procedure in SAS 9.4 (SAS Institute, Cary, NC) and means separation was achieved using Tukey's HSD (α=0.05). The experiment was repeated two more times in spring 2019.

In vitro Nematode Temperature Change Assay

A second experiment was initiated in winter 2019. All plates for this trial were without plant material and were made with 1.5% water agar. Inoculation occurred by transferring clean nematodes to the center of the plate using the root canal pick. This gave them a significant distance to potentially move and reduced the chance of them reaching the edge and climbing the walls. Plates were randomly assigned to one of 5 treatment groups for the first 3 days of the trial: 10, 15, 20, 25, and 30°C. Plates were marked every 24 hours for the movement of each nematode like in the previous experiment.

After 3 days, plates were randomly reassigned to one of three treatment groups for each temperature. Plates were either moved to an incubator that was 5°C colder, kept at the same temperature or moved to an incubator that was 5°C warmer (e.g. 25°C plates would either stay at 25°C, move up to 30°C or down to 20°C). The plates with an initial temperature of 10°C were either kept at 10°C or moved into a 15°C incubator. Similarly, plates at 30°C were kept at 30°C or moved into a 25°C incubator. Nematode movement was then tracked for another 3 days. Each treatment was replicated 6 times.

After 6 days total the experiment was terminated. Any nematode that desiccated and died by climbing the dish wall was removed from the data set. Data analysis was performed using the GLIMMIX procedure using SAS 9.4 (SAS Institute, Cary, NC). Means separation was achieved using Tukey's HSD (α=0.05). Only in Figure 4.5 was an α of 0.10 used. This experiment was repeated in spring 2019.

RESULTS

In vitro Nematode Temperature Assay

Of the three runs of the experiment, the presence of a turfgrass seedling only affected nematode movement in the first trial. Sting nematodes moved on average 38.7 mm in 7 days when a plant was present compared to only 12.3 mm ($p<0.0001$) without a plant (Figure 4.1). However, there was no significant draw of the nematode towards the plant. Only two out of the 312 nematodes examined remained near the plant roots, and feeding was not observed.

In all three trials, temperatures significantly altered nematode movement. Nematode movement at 15, 20, 25 and 30°C were similar and movement at 10, 15, 20, and 25°C were also similar except for in run one. In run one, 25 and 30°C were found to increase nematode movement the most. In all three runs, sting nematode movement was significantly greater at 30 than at 10°C. Overall, there was no interaction between plant and temperature, and temperature was a more significant driving factor in nematode movement distance than the presence of a plant.

In vitro Nematode Temperature Change Assay

When nematodes were moved from one temperature to another, only in the 10°C starting treatment was there significant difference. Nematodes that were kept at 10°C moved slightly less, but those that were moved up to 15°C saw an increase in overall movement as shown in Figures 4.3 and 4.4 ($p = 0.0368, 0.0065$). In the other four temperatures, when a nematode was moved down in temperature, there was a decrease in movement distance. However, the decrease was typically the same as that in the control group that was left at a consistent temperature for the entire 6 days of the experiment. The same was true of the nematodes moved up in temperature that they moved less but was not less than the original starting temperature. This was similar for both replications of the experiment.

Breaking down the data further, there was a slight negative trend when combining the data from all five temperatures into the three movement categories (Figure 4.5). Both runs of this experiment displayed this trend, however only in the second run was there a significant difference ($p<0.0524$). When nematodes as a whole were moved down 5°C, their movement dropped more (-19.24 and -32.44 mm) than those that stayed the same (-10.422 and -20.0.73). Further, when moved up by 5°C, their movement actually dropped less (-9.209 and -7.457) than those kept at an even temperature. When nematodes were submitted to decreasing temperature, nematode movement distance decreased more than by increasing temperature.

DISCUSSION

Sting nematode was found to be most active at the highest temperature of 30°C. This is consistent with reports from a variety of other crops when populations peak in the field (Bekal & Becker 2000; Kutsuwa et al. 2015). According to the North Carolina Climate Office, soil temperatures reach this temperature in hottest summer months of July, August and September. However, nematode activity was not reduced at any of the lower temperatures except for 10°C, indicating that when temperatures exceed 15°C North Carolina populations of sting nematode are actively moving in the soil and most likely feeding. North Carolina putting green soils are in in the temperature range of 15-30°C from April through October, and therefore nematode-feeding damage is likely to be a problem in most months except for December, January and February when soil temperatures are at or below 10°C. The decrease in activity does play a role in sting nematode and its distribution, but it was still slightly active at 10-15°C and does have the potential to increase its geographical distribution (Barker et al. 1969). A decade ago, sting nematode was reported in Delaware indicating sting nematode can adapt to northern climates with harsher winters, and the spread may accelerate as the global climate warms (Handoo et al. 2009).

For superintendents, the threat of consistent sting nematode feeding activity for 8 to 10 months of the year is an issue. Creeping bentgrass (*Agrostis stolonifera* L.) and ultradwarf bermudagrass (*Cynodon dactylon* x *C. transvaalensis* Burtt-Davy) are used for putting greens, and both are susceptible to nematode feeding throughout the year. For creeping bentgrass that struggles in our hot summer months, sting nematode is at its most active. Research shows that sting nematode may be below root depth during this time, however the loss of roots from earlier in the spring can predispose the grass to heat or drought stress.

Bermudagrass putting greens in North Carolina are susceptible at multiple times throughout the year. This grass enters dormancy in late fall and does not green up until April. However, soil temperatures are this time are still in an ideal range for nematode activity and feeding likely started around 15°C when the plant is not developing new shoots, but root initiation has begun to occur. Ultimately, the potential is there for the grass to be at a significant disadvantage come spring green up, and can further exacerbate winter kill issues common throughout North Carolina.

A second objective of this study was to look at nematode activity when soil temperatures fluctuate by 5°C. During much of our fall, winter and spring, the weather in North Carolina can vary greatly and soil temperatures in the top 10 cm of the turfgrass rootzone can fluctuate by as much as 2-3°C over the day. Only in the 10°C starting treatment did we measure a significant change in movement, and the change from 10 to 15°C was the only treatment that had an increase in activity compared to the consistent temperature control. This supports the common advice given to superintendents that when soil temperatures are around 15°C nematicide applications should occur to prevent sting nematode from feeding on new turfgrass roots (Martin 2017).

When looking at the aggregate data from the temperature shifts, it showed a trend that nematode activity slows less when temperatures shift up as opposed to a shift down or staying constant. Sting nematode is typically considered a warm weather nematode and should be of concern when temperatures are high (Robbins & Barker 1974). This is especially true of creeping bentgrass, where the ideal temperatures for sting nematode are the least ideal temperatures for this grass species.

This lesser decrease in overall movement as temperatures are shifted up may play a role in nematicide application. As nematode feeding activity and movement are likely to increase with warmer soil temperatures, or during the heat of the day, this may represent an ideal time to make a nematicide application. More movement by the nematode increases the chance that the nematode comes into contact with a nematicide and is more likely to kill the nematode. Currently most superintendents make applications during the early morning hours, when soil temperatures are slightly cooler. That said, this spray timing should only be used in the cooler spring and fall months. Sting nematode populations are known to move deeper during the hot summer months, and therefore while activity may increase their vertical migration to deeper depths could potentially move them outside of the range of the nematicides available on the market (Bekal & Becker 2000; McSorley & Dickson 1990).

Overall, this study showed an increase in nematode activity at higher temperatures as expected. The biggest change in movement was from 10 to 15°C and indicates that during winter months in North Carolina nematode activity is likely to be minimal, but as soon as spring starts and soil temperatures rise nematode activity will quickly become a problem and movement increases as temperature increases. Implications for management will differ by turfgrass species, but regardless sting nematode can be very active at high temperatures and is likely to make heat and drought stress to turfgrass putting greens significantly worse during the summer.

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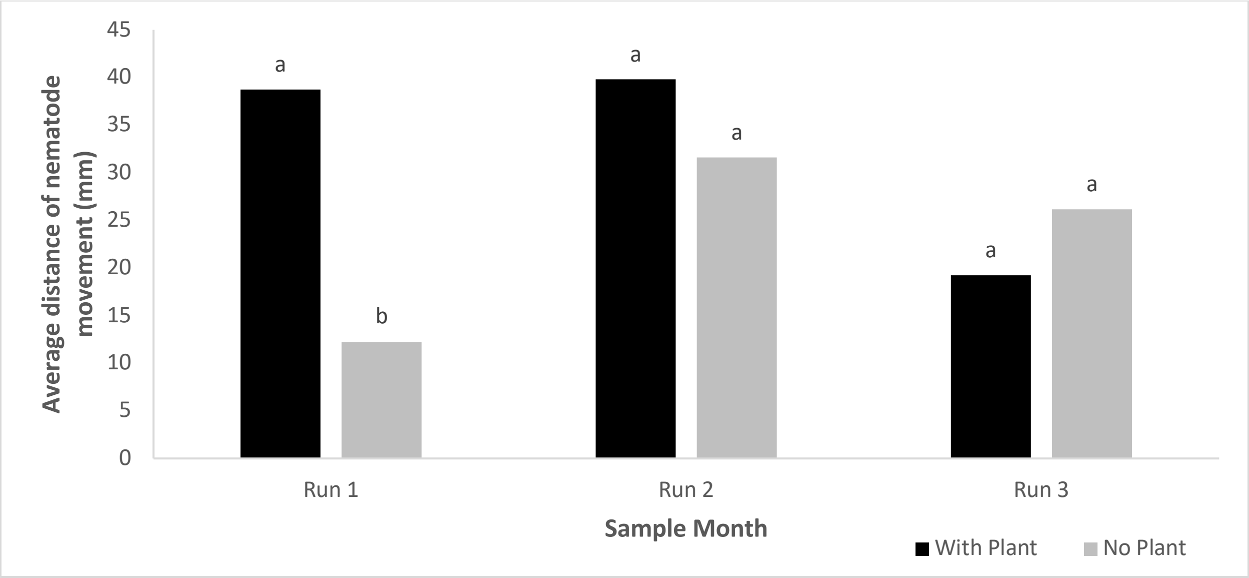

Figure 4.1. The average movement distance of sting nematode when on an agarose plate with either a creeping bentgrass seedling or no seedling. Black bars represent mean movement distance for all 5 temperatures in the presence of a seedling. Grey bars represent mean movement distance for all 5 temperatures without the presence of a seedling. Columns with the same letter were not different according to Tukey's HSD (α=0.05).

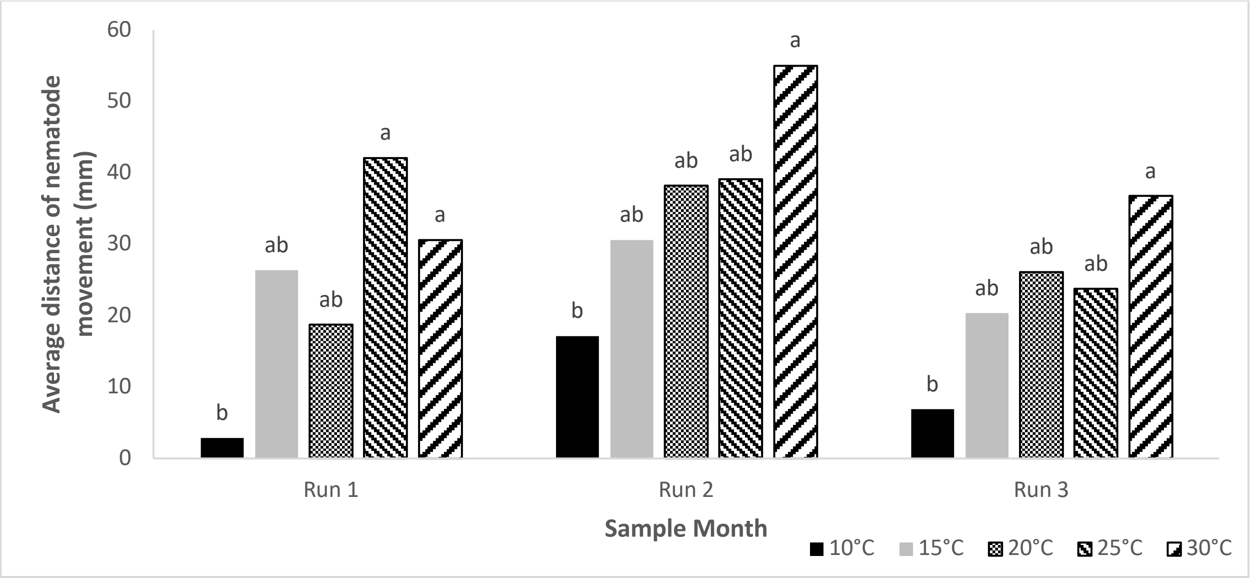

Figure 4.2. The average movement distance of sting nematode when on an agarose plate when exposed to 5 different temperatures; 10, 15, 20, 25, and 30°C. Each bar represents that nematode movement for a different temperature. Columns with the same letter were not different according to Tukey's HDS (α=0.05).

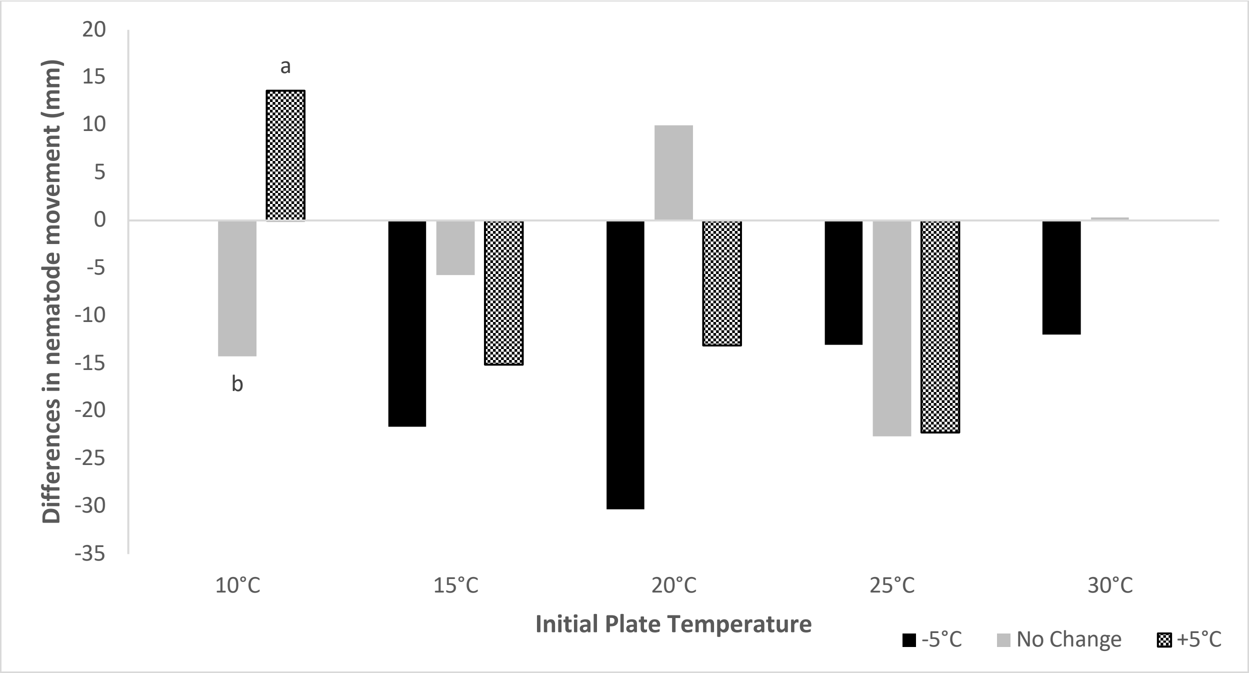

Figure 4.3. The average change in total movement distance by sting nematode from the first 3 days at a steady temperature and the second 3 days when the temperature either stayed constant or was raised or lowered by 5°C. The black bars represent nematode movement differences when temperatures were decreased by 5°C, the gray bars represent nematode movement differences when the temperature was kept constant, and the dotted bars represent nematode movement differences when the temperatures were increased by 5°C. Data shown is from the first run of this experiment. Columns with the same letter were not different according to Tukey's HSD (α=0.05).

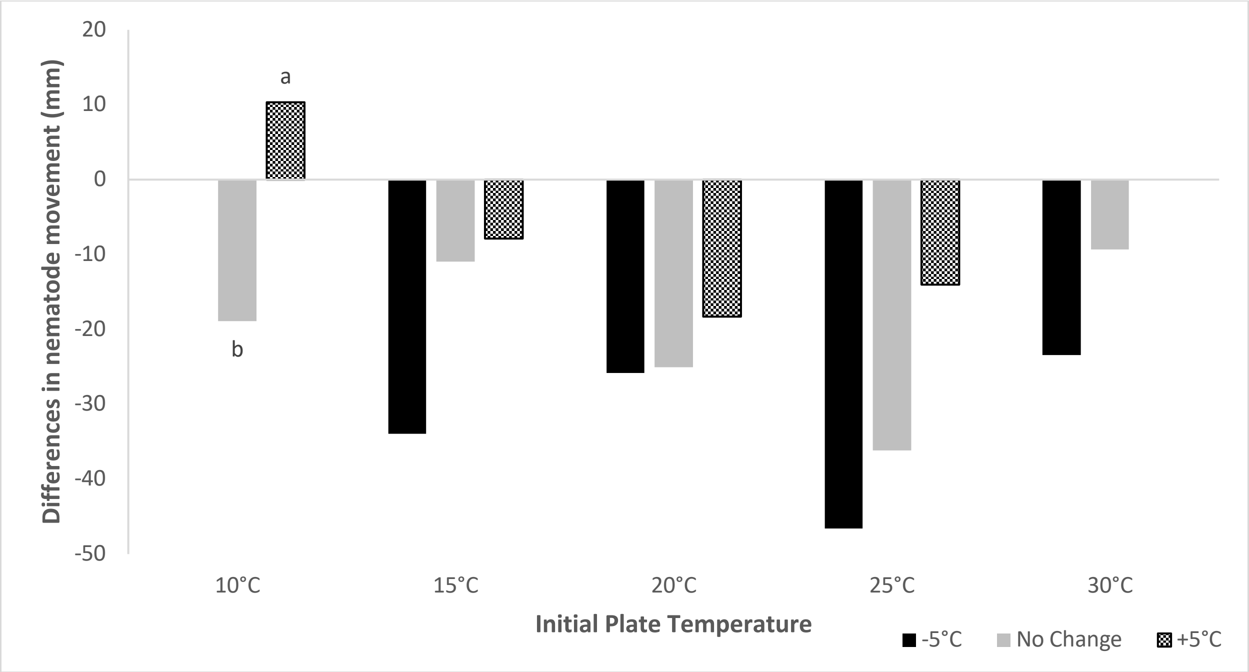

Figure 4.4. The average change in total movement distance by sting nematode from the first 3 days at a steady temperature and the second 3 days when the temperature either stayed constant or was raised or lowered by 5°C. The black bars represent nematode movement differences when temperatures were decreased by 5°C, the gray bars represent nematode movement differences when the temperature was kept constant, and the dotted bars represent nematode movement differences when the temperatures were increased by 5°C. Data shown is from the second run of this experiment. Columns with the same letter were not different according to Tukey's HSD (α=0.05).

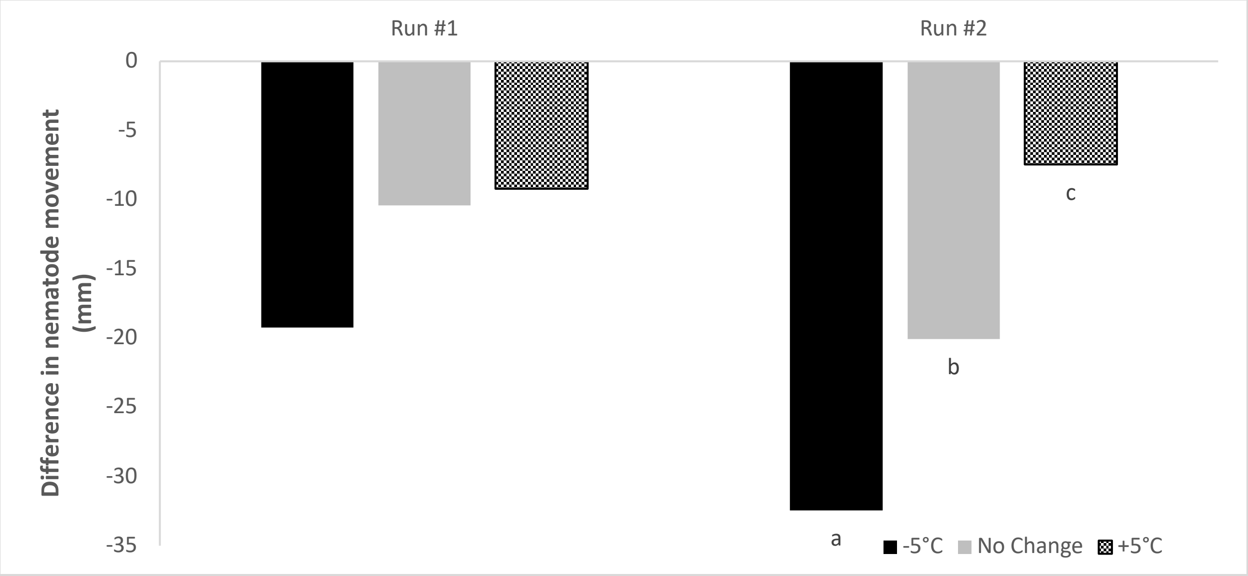

Figure 4.5. The difference in sting nematode movement when plate temperatures stayed constant or where raised or lowered by 5°C. The black bars represent mean movement distance of all five starting temperatures when the temperature was decreased by 5°C. The gray bars represent mean movement distance of all five starting temperatures when the temperature was kept constant. The dotted bars represent mean movement distance of all five starting temperatures when the temperature was increased by 5°C. Columns with the same letter were not different according to Tukey's HSD (α=0.10).

CHAPTER FIVE

Screening creeping bentgrass and ultradwarf bermudagrass for feeding tolerance by *Belonolaimus longicaudatus*

ABSTRACT

In the past decade, many golf courses have undergone significant renovations as superintendents switch their putting green surfaces from cool season creeping bentgrass (*Agrostis stolonifera* L.) to warm-season hybrid bermudagrass (*Cynodon dactylon* x *Cynodon transvaalensis* Burtt-Davy). This transition was driven by many factors, including disease susceptibility. The two species share many similar pathogens, but disease resistance has been neglected. This is especially true for nematode pathogens including sting nematode (*Belonolaimus longicaudatus*). For golf course putting greens with severe nematode infestations, information on cultivar tolerance would be helpful for superintendents in making renovation decisions. Little data is available on sting nematode tolerance of turfgrass cultivars, and so the objective of this study was to evaluate cultivars of creeping bentgrass and bermudagrass for their ability to tolerate feeding damage. Experiments were conducted in a growth chamber with 5 cultivars of each species subjected to sting nematode feeding and drought stress conditions. Turf quality was evaluated throughout the stress period. The creeping bentgrass cultivars of 'Pure Distinction' and 'Crystal Bluelinks' maintained the highest overall turf quality when subjected to nematode feeding. For bermudagrass cultivars 'MiniVerde' had the highest overall turf quality throughout the study. Creeping bentgrass was determined to maintain a higher quality when subjected to nematodes and could sustain quality when inoculated with 20 nematodes compared to only 10 for bermudagrass. For growers looking to convert green surfaces, unless the budget allows for

chemical nematicide applications, they must be selective in what species they choose to avoid the most damage from sting nematode feeding.

INTRODUCTION

The golf course segment of the turfgrass industry in North Carolina has undergone a major species shift in the past decade. Many courses have changed their putting green surface from the cool-season creeping bentgrass (*Agrostis stolonifera* L.) to warm-season ultradwarf bermudagrass (*Cynodon dactylon* x *Cynodon transvaalensis* Burtt-Davy). North Carolina is within the transition zone and has the ability to sustain both creeping bentgrass and bermudagrass, yet neither grow optimally (Patton 2012). There are been many reasons for this conversion, including better tolerance to hotter summers, better drought tolerance and a perceived thought that ultradwarf bermudagrasses would have less disease. Disease incidence and severity is as intense for bermudagrass as it is for creeping bentgrass, and many nematode species found in North Carolina thrive on both turfgrass species.

Creeping bentgrass is a cool-season grass and is grown throughout much of the central and western portions of the state. It maintains some growth throughout the entire year, and thrives during the fall, winter and spring months when nighttime temperatures are below 21°C. However, during the summer months when temperatures regularly exceed 30°C, this grass struggles to maintain rooting and canopy density necessary for ball roll (Beard 1973). Heat and drought stress are major factors and can become more severe when nematode feeding damage occurs. The weakened root system is unable to cope with these stresses, and the turf thins in patches (Lucas et al. 1974).

Bermudagrass is a warm-season grass and is grown throughout the central and eastern coastal regions of the state of North Carolina. While it generally thrives in our hot, humid summers it is mostly dormant or slow growing during the cool winters in NC (Beard 1973). This leaves the grass susceptible to nematode feeding pressure during the late fall, winter and early spring months when roots are damaged. This can result in a slower green up in the spring, and results in the grass being at a disadvantage throughout the spring and summer. It also results in the grass being more susceptible to environmental stresses, with a weakened root system that is unable to cope with stress. Between the two species, bermudagrass also has a much shallower root system than creeping bentgrass. This further results in a disadvantage in the event of environmental stress when the roots have been shortened significantly due to nematode feeding.

The most damaging nematode on turfgrass throughout the southeastern United States and North Carolina is sting nematode (*Belonolaimus longicaudatus*). This nematode can cause severe issues and is widespread throughout the eastern and central portions of the state (Zeng et al. 2012). It feeds at the root tips, and stunts plant root growth and weakens the root system (Christie et al. 1952). It is non-preferential and will feed on any types of turfgrass including creeping bentgrass and bermudagrass (Crow 2005). Feeding is most active throughout the spring when soil temperatures rise above 15°C and can continue throughout the year until temperatures drop in the fall (Smiley et al. 2007). Some of our research has shown that sting nematode is active below 15°C, but we do not know if it is feeding or having any negative impacts on the turfgrass roots below this temperature.

Nematode feeding tolerance screening of turfgrasses has been performed on *B. longicaudatus* in the past, but primarily focuses on warm-season grasses including bermudagrass and St. Augustinegrass (*Stenotaphrum secundatum* L.). It also has not focused on putting green cultivars of bermudagrass. Some cultivars have shown resilience to nematode feeding, but none showed any resistance (Pang et al. 2011; Aryal et al. 2015). Therefore, feeding tolerance and increased root production under nematode feeding has been the focus of evaluations. However, there currently are no studies focused on putting green cultivars of either creeping bentgrass or the ultradwarf bermudagrass cultivars which are the most common hosts to sting nematodes on golf courses in North Carolina.

The objectives of this study were to evaluate a variety of old and new cultivars of both creeping bentgrass and bermudagrass for nematode feeding tolerance by evaluating turfgrass quality over time. This was further tested by stressing the grasses using simulated drought stress to look at how well the cultivars could tolerate stress in the presence of nematode feeding, which is common during summer months in North Carolina.

MATERIALS AND METHODS

Nematode Inoculation Preparation

The sting nematode populations for this study were collected from a nematode infested maize field located at the Central Crop Research Station in Clayton, NC. Soil samples were taken from the field and brought back for extraction using sieving. Soil samples of 500 cc were taken and nematodes were extracted using a 100-mesh sieve placed on a 400-mesh sieve. The soil was mixed with water for 30 seconds, and after a brief pause the solution was poured through the stacked sieves. The soil and nematodes collected from the 400-mesh sieve were then rinsed into a bucket. This solution would eventually be used for identification of the plants.

To count and identify nematodes, subsamples of the solution were further processed using a modified sieving-sugar centrifugation technique (Jenkins 1964). Samples were collected into centrifuged tubes and centrifuged at 4,000 rpm for 5 minutes. The supernatant was then poured off, and the pellet was broken up and mixed with 60% sucrose solution. After being centrifuged for 30 seconds at 4,000 rpm, the solution was poured through a 500-mesh sieve. The nematodes were rinsed thoroughly with water and then poured into a small tube. Sting nematode identification and counting was then achieved using light microscopy. Calculations were then performed to determine how many nematodes were in each milliliter of solution collected from the initial extraction.

Stress Study

The study was conducted in two climate-controlled growth chambers at the North Carolina State University Phytotron facility. Creeping bentgrass seeds were obtained from Pure Seed Testing (Rolesville, NC). The cultivars used were ‘Penn A-1’, ‘Penncross’, ‘Pure Distinction’, ‘Crystal Bluelinks’ and ‘Pure Select’. Creeping bentgrass plants were initiated in a rooftop greenhouse at the Phytotron facility before being moved to the growth chambers. 30-centimeter depth conetainers were filled with 100% coarse sand and seeded at approximately 1.22 grams per square meter. The seeds were allowed to germinate and grow for 8-10 weeks in the greenhouse before being transferred to the growth chamber. At this time, they were watered twice daily with no added fertility. The plants were considered ready to inoculate when they had completely covered the surface of the conetainer and trimmed at least twice.

Five cultivars of ultradwarf bermudagrass were used for this study: ‘Champion’, ‘MiniVerde’, ‘TifEagle’, ‘Imperial’ and an experimental. The ‘Champion’ and ‘MiniVerde’ cultivars were collected from the North Carolina State University Lake Wheeler Turfgrass Research Station in Raleigh, NC. The other three cultivars were taken from research plots at TPC Sawgrass in Ponte Verde, FL. Thirty 3-cm square cores of each cultivar were taken and split into 4 small sections and transplanted into a conetainer filled with 100% coarse sand. The pots were then grown in for 4-6 weeks in the growth chambers while being watered one daily in the AM with a 1/2x Hoagland’s solution.

The creeping bentgrass cultivars and the bermudagrass cultivars were maintained in separate growth chambers in the Phytotron. The creeping bentgrass was maintained on a 14-10 hr light cycle, with a daytime temperature of 26°C and a nighttime temperature of 22°C. The bermudagrass was also on a 14-10 hr light cycle, with a daytime temperature of 30°C and a nighttime temperature of 22°C. Plants were watered once daily in the AM with a 1/2x strength Hoagland’s solution. Plants were trimmed to approximately 1/4 inch 3 times weekly.

After a 2-week conditioning period, plants were arranged into a split-plot randomized complete block design. The blocks were aligned from left to right across the chamber to account for air movement from fan placement on the left side of the growth chamber. The treatments were nematode inoculation amount: 0, 10, 20, or 40 sting nematodes per conetainer. Ten replicates of each treatment per cultivar were used. The conetainers were inoculated by pipetting the nematode solution into the root zone. A pipet tip was used to make a small hole in the canopy, and a calculated amount of the nematode solution was added. The canopy was then smoothed over help cover up the divot. At the time of inoculation, turf quality was assessed on a 1-9 scale (1=poor, 6=acceptable, 9=excellent). These ratings were conducted every week for the duration of the study.

Five weeks after inoculation with the nematodes, all water was cut off for the plants. This was to simulate drought stress and determine cultivars susceptibility to stress as a result of nematode feeding damage. At the initiation of the stress phase, 1/3 of the pots were removed. Nematodes were extracted and root quality was measured from the removed pots. Roots were also cleaned, dried, and weighed. Root quality was assessed on a 0-4 scale (0=dead, 4=healthy). During the stress phase, ratings were taken daily as were photos. Temperatures in both chambers were also changed to 26/22°C to avoid a faster dry down of bermudagrass plants. The trial was terminated when all pots were considered dry and dead, which occurred within 4-7 days.

Data was analyzed using the GLIMMIX procedure using SAS 9.4 (SAS Institute, Cary, NC). Turfgrass species were analyzed separately. Runs were analyzed separately as well. CLASS variables looked at included nematode inoculation, date (of the dry down period) and cultivar. Mean separation was achieved using Tukey's HSD ($\alpha = 0.05$).

RESULTS

Variability in tolerance of nematode feeding was much more prevalent in creeping bentgrass cultivars than bermudagrass cultivars. During the stress period, 'Pure Distinction' was able to retain the highest overall turf quality compared to the others in both runs of the experiment ($p<0.0001$) (Figure 5.1). In both runs 'Crystal Bluelinks' did not separate from 'Pure Distinction' in overall quality. 'Pure Select' and 'Penncross' were not different from 'Crystal Bluelinks' in either replicate of the study, although they were different ($p<0.0001$) from 'Pure Distinction'. 'Penn A-1' had inconsistent performance between each run. It had the second highest quality rating in the first run but had the lowest overall quality in the second run of the experiment and was different ($p<0.0001$) from all of the cultivars except for 'Penncross'.

In bermudagrass cultivars, 'MiniVerde' had the highest overall quality in both runs (Figure 5.2). In the first run, 'MiniVerde was found to maintain a higher overall quality throughout the stress period than all cultivars except for the 'Experimental' ($p=0.0007$). In the second run, 'MiniVerde' was different from the other cultivars ($p=0.0022$). 'Champion' had the lowest overall quality in both runs throughout the stress period.

Nematode inoculation did not have a significant correlation with any of the creeping bentgrass cultivars except for 'Pure Distinction'. When inoculated with 0 or 10 nematodes, 'Pure Distinction' plants were able to maintain a high quality, but when inoculated with more than 20 there was decrease in overall quality. Quality dropped from 5.7 to 3.6 steadily between 0 and 40 inoculated nematodes ($p<0.0002$). This difference only occurred in the first repeat, although there was a large difference in the second run that was not different.

When all bentgrass cultivars were grouped together by species there was a significant drop in overall turf quality when nematode populations were greater than 20 nematodes per conetainer as described in Figure 5.3 in boths runs (p<0.0027, 0.0014). This was similarly found with bermudagrass as a whole, although it was found to tolerate a lower nematode population before a decrease in quality at 10 nematodes per conetainer in both runs (Figure 5.4; p<0.0001, <0.0001).

When averaged across turfgrass species there were differences in how long a species could maintain an acceptable quality before dropping off significantly. For creeping bentgrass, quality declined slowly throughout the first 3 days of the drought period, before dropping rapidly at day 4 in both runs (Figure 5.5; p<0.0001, <0.0001). For bermudagrass, this decrease started immediately, and saw quality drop by over a point by day 2 in both runs (Figure 5.6; p<0.0001, <0.0001). Further, bermudagrass cultivars were found to die out completely at 4 days, while most of the creeping bentgrass cultivars lasted anywhere from 5 to 7 days before turning completely brown and drying out.

DISCUSSION

Variability in creeping bentgrass cultivars indicated that newer cultivars on the market maintained a higher quality during the drought stress period when exposed to nematode feeding than did older cultivars. However, this did not translate directly to nematode feeding tolerance, and in some cases the inverse was shown. At lower amounts of nematode inoculation, 'Pure Distinction' was significantly better than most in both repeats although this became skewed as nematode inoculation amounts increased above 20 nematodes. At 20 and 40 inoculated nematodes, 'Pure Distinction' did not separate out from the other four cultivars. This indicates that 'Pure Distinction' are better at mitigating damage pressure at lower amounts, but when sting nematode feeding pressures reaches above 20 nematodes there were no differences. For superintendents there are significant ramifications for this information. Through chemical management, we can often bring populations down to counts as low as 5 nematodes/500 cc soil but not completely eradicate populations (Gu & Crow 2018; Watson & Desaeger 2019). Therefore, these newer cultivars will compliment a proper nematicide program in developing a proper integrated pest management protocol.

The same was not true of bermudagrass cultivars, which only saw "MiniVerde' separate from any of the other bermudagrass cultivars tested. This was unexpected, as 'MiniVerde' is not considered one of the highest quality cultivars. Because of this and the fact that it was not dramatically different than the other five cultivars, its value as a cultivar for nematode infested putting greens is limited. Bermudagrass as a whole saw a decrease in quality starting at 10 nematodes, indicating poor overall tolerance to nematode feeding damage for the species when compared to creeping bentgrass higher tolerance. This is likely due to the deeper root system typical of creeping bentgrass compared to the shallower root system of bermudagrass. For superintendents with preexisting sting nematode populations, this can be a large problem particularly if the populations are not well managed by a nematicide program. While bermudagrass may have other advantages over creeping bentgrass in warmer coastal climates, this may be a significant drawback.

This experiment did show that creeping bentgrass was able to maintain turf quality longer than bermudagrass, which contraindicates a lot of the current research on these two grass species. Bermudagrass is known to be very drought tolerant as a C4 plant, but with nematode feeding pressure this may not be the case (Turgeon 1999). The already weaker root system of bermudagrass is not able to cope with significant root damage as sting nematode can cause, and therefore in the presence of nematode feeding is not very drought tolerant. However, creeping bentgrass has a much more robust root system, and therefore is able to mitigate root loss from feeding damage due to a high volume of roots. Fortunately, golf courses have irrigation systems to mitigate drought damage, but should be of concern in areas that are prone to extended droughts and rationing of water.

Overall, the newest cultivars of creeping bentgrass were more tolerant of low populations of nematode feeding damage, but when populations got about 20 nematodes, turf quality was reduced in all cultivars. For bermudagrass, no differences were observed. However, the differences observed between the species is important for superintendents to note when picking a new putting green surface. Those with preexisting issues or a small budget that doesn't allow for regular nematicide applications should take this into consideration, especially if renovation of greens is in their future.

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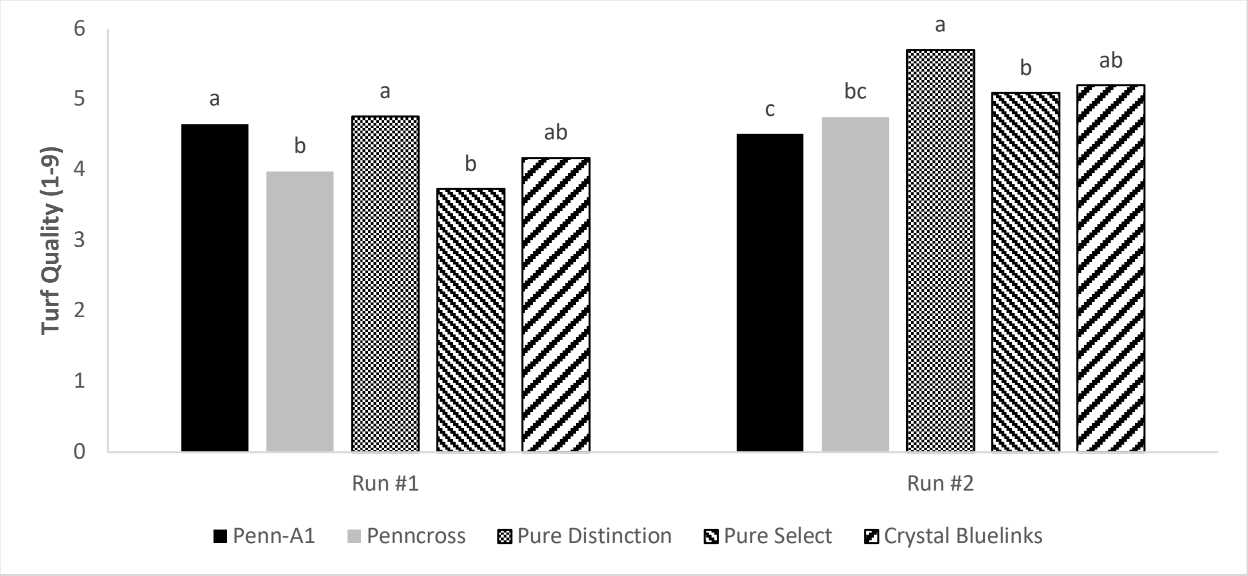

Figure 5.1. Differences in overall turf quality throughout the entire stress period of each cultivar of creeping bentgrass. The black bar represents the average turf quality rating of all 4 nematode inoculation treatments for 'Penn A-1' collected once daily for 5 days. The gray bar represents the average turf quality rating of all 4 nematode inoculation treatments for 'Penncross' collected once daily for 5 days. The dotted bar represents the average turf quality rating of all 4 nematode inoculation treatments for 'Pure Distinction' collected once daily for 5 days. The thin slashed bar represents the average turf quality rating of all 4 nematode inoculation treatments for 'Pure Select' collected once daily for 5 days. The thick slashed bar represents the average turf quality rating of all 4 nematode inoculation treatments for 'Crystal Bluelinks' collected once daily for 5 days. Both runs of the experiment are shown above. Different letters represent a difference between average turf quality among cultivars within each run according to Tukey's HSD (α=0.05).

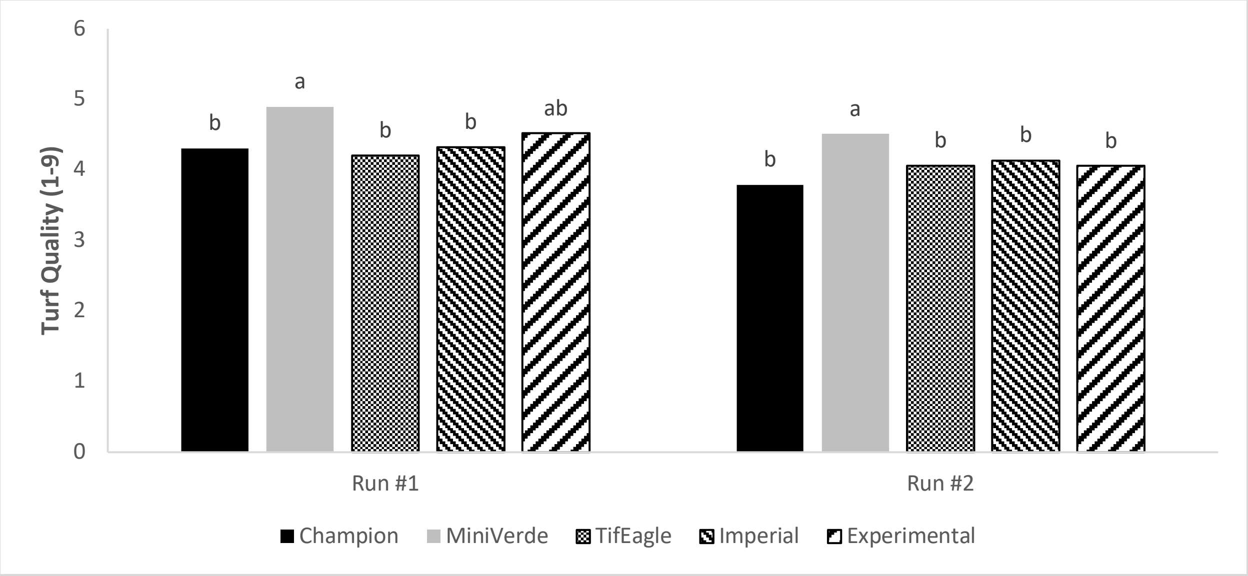

Figure 5.2. Differences in overall turf quality throughout the entire stress period of each cultivar of bermudagrass. The black bar represents the average turf quality rating of all 4 nematode inoculation treatments for 'Champion collected once daily for 4 days. The gray bar represents the average turf quality rating of all 4 nematode inoculation treatments for 'MiniVerde' collected once daily for 4 days. The dotted bar represents the average turf quality rating of all 4 nematode inoculation treatments for 'TifEagle' collected once daily for 4 days. The thin slashed bar represents the average turf quality rating of all 4 nematode inoculation treatments for 'Imperial' collected once daily for 4 days. The thick slashed bar represents the average turf quality rating of all 4 nematode inoculation treatments for the experimental cultivar collected once daily for 4 days. Both runs of the experiment are shown above. Different letters represent a difference between average turf quality among cultivars within each run according to Tukey's HSD (α=0.05).

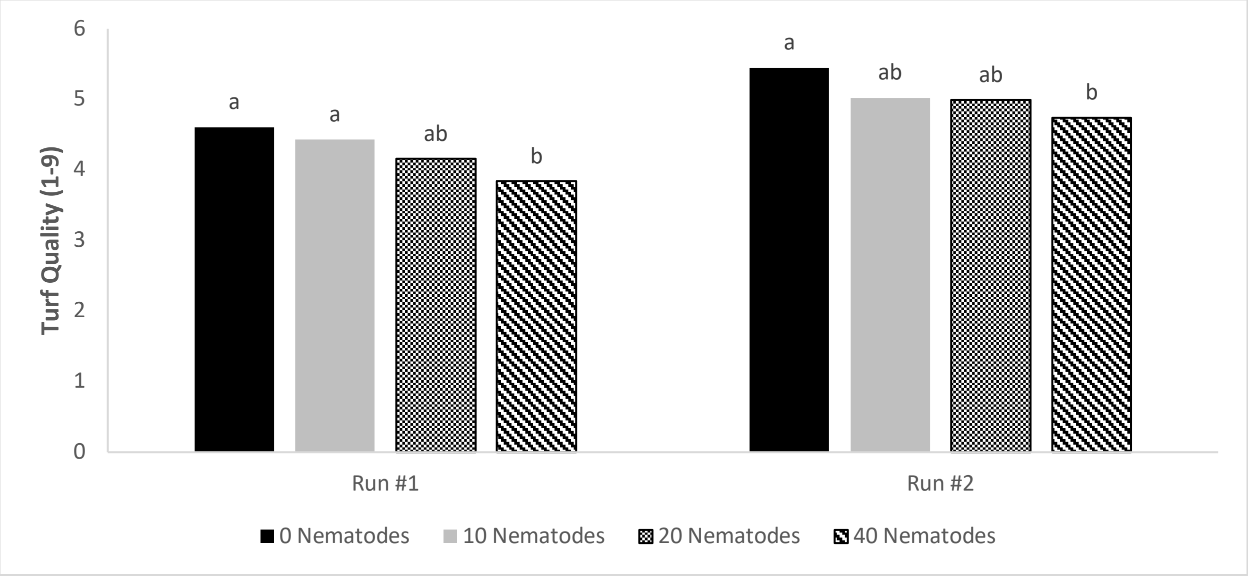

Figure 5.3. Differences in overall turf quality throughout the entire stress period of all creeping bentgrass cultivars combined as a result of sting nematode inoculation amounts. The black bar represents the overall turf quality rating for the 0 inoculated nematode treatment from one daily rating over 5 days. The gray bar represents the overall turf quality rating for the 10 inoculated nematode treatment from one daily rating over 5 days. The dotted bar represents the overall turf quality rating for the 20 inoculated nematode treatment from one daily rating over 5 days. The slashed bar represents the overall turf quality rating for the 40 inoculated nematode treatment from one daily rating over 5 days. Different letters represent a difference between average turf quality among cultivars within each replicate according to Tukey's HSD (α=0.05).

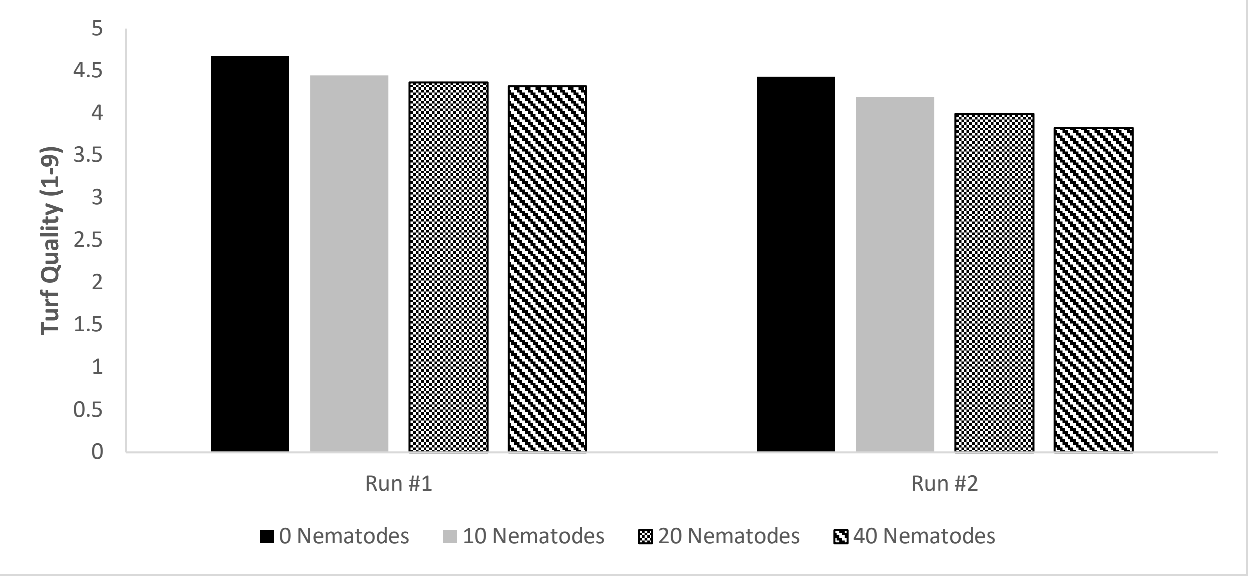

Figure 5.4. Differences in overall turf quality throughout the entire stress period of all creeping bentgrass cultivars combined as a result of sting nematode inoculation amounts. The black bar represents the overall turf quality rating for the 0 inoculated nematode treatment from one daily rating over 4 days. The gray bar represents the overall turf quality rating for the 10 inoculated nematode treatment from one daily rating over 4 days. The dotted bar represents the overall turf quality rating for the 20 inoculated nematode treatment from one daily rating over 4 days. The slashed bar represents the overall turf quality rating for the 40 inoculated nematode treatment from one daily rating over 4 days. Different letters represent a difference between average turf quality among cultivars within each replicate according to Tukey's HSD (α=0.05).

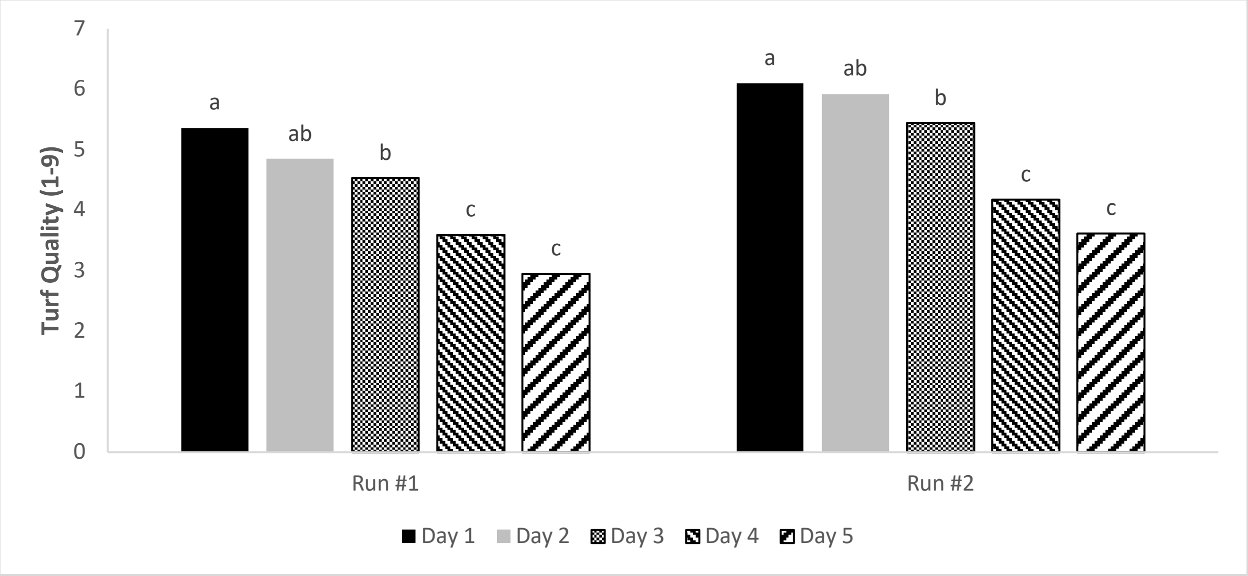

Figure 5.5. Differences in overall turf quality throughout at each day of the stress period for all creeping bentgrass cultivars combined. The black bar represents the combined turf quality ratings from all five cultivars and all four nematode inoculation amounts taken on day 1 of the simulated drought. The gray bar represents the combined turf quality ratings from all five cultivars and all four nematode inoculation amounts taken on day 2 of the simulated drought. The dotted bar represents the combined turf quality ratings from all five cultivars and all four nematode inoculation amounts taken on day 3 of the simulated drought. The thin slashed bar represents the combined turf quality ratings from all five cultivars and all four nematode inoculation amounts taken on day 4 of the simulated drought. The thick slashed bar represents the combined turf quality ratings from all five cultivars and all four nematode inoculation amounts taken on day 5 of the simulated drought. Different letters represent a difference between average turf quality among cultivars within each replicate according to Tukey's HSD (α=0.05).

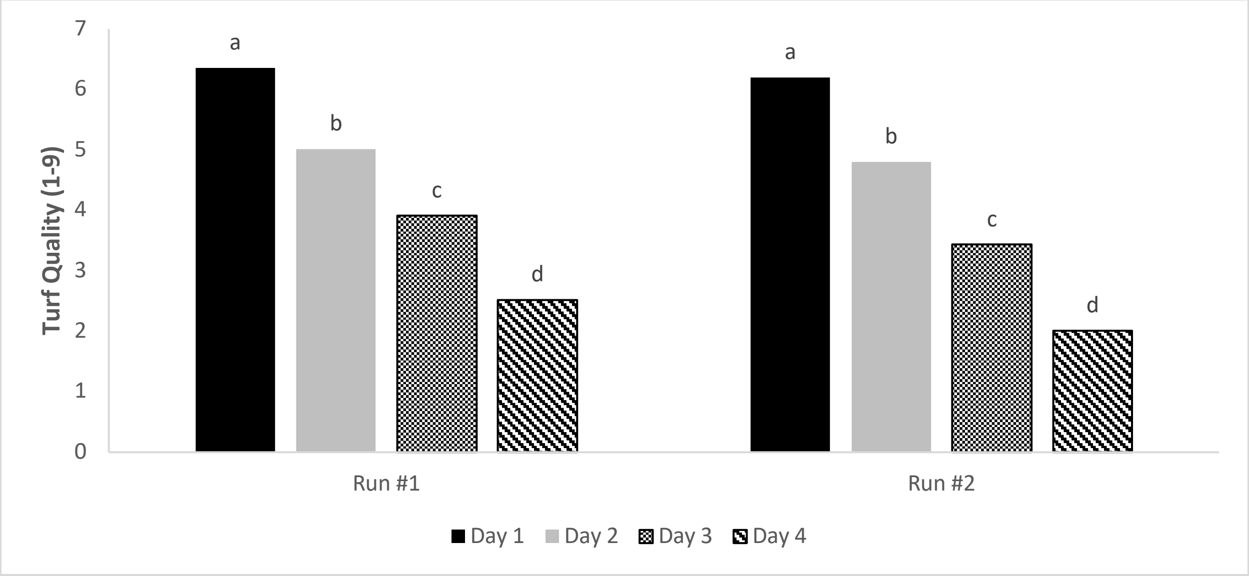

Figure 5.6. Differences in overall turf quality throughout at each day of the stress period for all creeping bentgrass cultivars combined. The black bar represents the combined turf quality ratings from all five cultivars and all four nematode inoculation amounts taken on day 1 of the simulated drought. The gray bar represents the combined turf quality ratings from all five cultivars and all four nematode inoculation amounts taken on day 2 of the simulated drought. The dotted bar represents the combined turf quality ratings from all five cultivars and all four nematode inoculation amounts taken on day 3 of the simulated drought. The thin slashed bar represents the combined turf quality ratings from all five cultivars and all four nematode inoculation amounts taken on day 4 of the simulated drought. Different letters represent a difference between average turf quality among cultivars within each replicate according to Tukey's HSD (α=0.05).

APPENDICES

Appendix A

Influence of nitrogen rate and timing, fungicide application method, and simulated rainfall after fungicide application on brown patch severity in tall fescue

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ABSTRACT

In North Carolina, tall fescue (*Festuca arundinacea* Schreb.) is widely grown throughout the mountain and piedmont regions. North Carolina is in the transition zone, which is subject to hot, humid summers that predispose tall fescue to brown patch (*Rhizoctonia solani* Kühn). Field trials were conducted over a two-year period (2015-2016) to evaluate the effects of nitrogen rate and timing, application method of a fungicide, and rainfall following fungicide application on brown patch severity on lawn height tall fescue. Seven rates of urea providing 0 to 6 lb N 1000 ft^{-2} yr^{-1} were initiated each year in March with repeat applications monthly at 1 lb N 1000 ft^{-2}. In a separate study, various timings of urea were conducted throughout the year for a total of 3 lb N 1000 ft^{-2} yr^{-1}. In 2015, no significant differences in disease severity or turfgrass quality were observed among the seven N rates. Only the application of 6 lb N 1000 ft^{-2} yr^{-1} resulted in significantly higher brown patch compared to the non-treated control in 2016. No significant differences in disease severity or turfgrass quality were observed in the timing study in both years.

Azoxystrobin was applied with a ride-on spreader/sprayer (11 gal water-carrier acre^{-1}), a commercial applicator gun (130 gal water-carrier acre^{-1}), and a research spray boom (88 gal water-carrier acre^{-1}). No differences were detected among application methods. A rainfall event of 0.5 in. was simulated with overhead irrigation 30 minutes after application of fungicides. No differences were detected among the fungicide treatments and all provided excellent control of brown patch.

INTRODUCTION

Tall fescue (*Festuca arundinacea* Schreb.) is widely planted in North Carolina in residential and commercial landscapes. Based on the most recent survey of turfgrass in NC (NCAS 1999), 47.8% of the total turfgrass acreage was planted with tall fescue. The total maintained turfgrass acreage in NC is 2.14 million acres and of that 69% of the area is in single family dwellings. Single family dwellings also accounted for 60% of the expenditures and 79% of the value of turfgrass equipment. Tall fescue is selected for this region mainly because of its adaptability to multiple soil types, adequate shade and drought tolerance, ease of establishment, and relatively low fertility requirements. Throughout much of NC, tall fescue is the only cool-season turfgrass recommended for landscape use. Despite the benefits of tall fescue, it is extremely susceptible to diseases such as brown patch (*Rhizoctonia solani* Kühn), Pythium blight (*Pythium* sp.), and gray leaf spot (*Pyricularia grisea* Sacc.) during hot, humid summers common in NC.

The turfgrass faculty at NC State University conduct yearly education programs throughout the state for landscape managers and are frequently challenged regarding current recommendations for tall fescue management. In particular, they are most concerned with the recommendation of no nitrogen fertility between March 15 and September 1 (NC State Extension 2000). This recommendation is based on research demonstrating increased brown patch severity with increasing nitrogen rate (Burpee 1995). Specifically, Burpee (1995) observed a significant increase in brown patch severity in tall fescue plots in Georgia treated with 1 lb N 1,000 ft^{-2} $month^{-1}$ when compared to plots treated with 0.5 lb N 1,000 ft^{-2} $month^{-1}$. A more recent study in Virginia determined that brown patch was most severe in tall fescue plots maintained at a height of 2.5 inches and treated with 4.5 lb N 1,000 ft^{-2} $year^{-1}$ (Cutulle et al. 2014). These studies support a recommendation of limited nitrogen use in tall fescue landscapes during the summer months. Two of the largest lawn care companies in central NC apply low rates of liquid nitrogen in conjunction with fungicides through the summer months without sacrificing exceptional brown patch management. Research conducted at NC State University in 2005 (Tredway et al.) supports combining low rates of nitrogen with fungicide applications for brown patch management. Since current nitrogen fertility recommendations for tall fescue are dated and the influence of nitrogen fertility on brown patch severity is limited, a study focused on the impacts of nitrogen rate and timing on brown patch severity was warranted.

Other common issues raised by landscape managers are the effects of application method and post-application irrigation or rainfall on fungicide efficacy for brown patch. These are excellent questions since most of the research conducted on these topics has been on dollar spot (*Clarireedia* sp.) in golf course turf. A study conducted in Kentucky on a creeping bentgrass fairway showed no difference in dollar spot control when comparing spray volumes of 1 gal vs 2 gal water^{-1} 1,000 ft^{-2} (Vincelli et al. 2004). Further support of this research was shown in Maryland on another creeping bentgrass fairway with no difference observed between a carrier volume of 1.15 gal vs 2.5 gal water^{-1} 1,000 ft^{-2} on dollar spot control (McDonald et al. 2006). Kennelly and Wolf (2009) demonstrated no differences in dollar spot control when chlorothalonil was applied in 0.5, 1, or 2 gal water^{-1} 1,000 ft^{-2} carrier volume. Similar research has not been conducted for brown patch management in tall fescue. Lawn care operators typically use a spray gun that delivers fungicides in a carrier volume of 3 gal water^{-1} 1,000 ft^{-2}. It is not known if application methods that deliver fungicides in high carrier volumes (3 gal 1,000 ft^{-2}) affect brown patch management in tall fescue. If lower carrier volumes prove effective, this would save operational costs for fungicide applications.

The average annual rainfall in Raleigh, NC is 46.58 inches with 36% occurring from May through August (US Climate Data 2019), which coincides with brown patch development and management. The effect of post-application irrigation or rainfall on fungicide efficacy in brown patch is unknown. Previous studies examining the influence of simulated rainfall on fungicide performance in dollar spot management in creeping bentgrass indicated a diminished long-term dollar spot control, yet none of the treatments were totally ineffective (Pigati et al. 2010, Inguagiato and Miele 2016). Highly effective dollar spot products such as fluazinam did not lose efficacy due to simulated rainfall (Inguagiato and Miele 2016). The influence of simulated rainfall on QoI fungicides, an extremely efficacious and widely used fungicide mode of action for brown patch, is not clear.

To address the influence of nitrogen rate and timing, fungicide application methods, and simulated rainfall immediately after fungicide application on brown patch severity, three separate studies were conducted over a two-year period. The objective of the nitrogen study was to determine the influence of nitrogen rate and timing on brown patch development in tall fescue. The objective of the application method study was to evaluate the effect of three different fungicide application methods for brown patch management. The objective of the post-application rainfall study was to examine the influence of simulated rainfall on the performance of QoI fungicides on brown patch severity.

RESEARCH SITE MANAGEMENT

During 2015 and 2016, a 2-yr study was conducted on an 11-yr old ‘Coronado’ tall fescue sward at the Lake Wheeler Turfgrass Field Laboratory in Raleigh, NC. The soil type is a Cecil sandy loam with a pH of 6.4 and was managed similar to a commercial landscape. A rotary mower was used 3 times weekly in the afternoon at a height of 3.5-inches. During the growing season, irrigation was applied up to 3 times weekly (0.25-inches/application) as needed to prevent drought stress. Granular fertilizer (25-5-12) was applied annually in the spring at 1.5 lb N 1,000 ft^{-2}to study areas except for nitrogen studies. The nitrogen studies did not receive supplemental N during the duration of the 2-yr study. No additional inputs or cultivation were conducted in these experimental areas.

INFLUENCE OF NITROGEN RATE AND TIMING EXPERIMENTAL DESIGN

Studies were arranged as a randomized complete block design with 4 replications. In both years, treatments were applied to the same plots (5 ft x 6 ft) without new randomization to ensure there were no carry-over effects of nitrogen on brown patch severity. All plots in both studies were infested with *R. solani* (AG 1-IA) on 18 May 2015 and 24 May 2016. Inoculum was grown on sterilized rye grain and 10 cc was placed at 2 points within each plot near the soil surface. Fungicides were withheld during the duration of both studies. Treatments for nitrogen rate and timing studies are presented in Table 1. Urea (46-0-0) was solubilized in a carrier volume of 88-gal water acre^{-1} and applied using a $C0_2$ powered backpack sprayer equipped with TeeJet DG8004 nozzles at 40 psi. To minimize foliar burn, all treatments were immediately irrigated with 0.1-inches after application. For the rate study, N was applied at 1 lb N 1,000 ft^{-2} at each date (Table A.1). For the timing study, all plots annually received 3 lb N 1,000 ft^{-2} divided equally across various timings (Table A.1).

Plots in both studies were monitored 5 times weekly from May to November for brown patch symptoms and changes in turf quality. Turf quality was visually assessed every two weeks from May to November using a 1-9 scale where 1 = dead, 6 = acceptable, and 9 = best. Brown patch severity was visually assessed every two weeks from June to October as a percent area (0-100) of the plot affected. Data was transformed to area under turf quality curve (AUTQC) and area under disease progress curve (AUDPC) values for turf quality and brown patch severity respectively. Data was subjected to analysis of variance using the GLIMMIX procedure in SAS 9.4 (SAS Institute, Cary, NC). Means separation was assessed using least square means.

FUNGICIDE APPLICATION METHOD EXPERIMENTAL DESIGN

The study was arranged as a randomized complete block design with 4 replications. In both years, treatments were applied to plots (10 ft x 10 ft) and were re-randomized each year. All plots were infested with *R. solani* on 18 May 2015 and 24 May 2016. Inoculum was grown on sterilized rye grain and 10 cc was placed at 3 points within each plot near the soil surface. Treatments consisted of the following application methods; CO_2 powered backpack sprayer at 88-gal water acre^{-1} carrier volume, PermaGreen Triumph spreader/sprayer (PermaGreen^{TM} Supreme Inc., Valparaiso, IN) equipped with 2 Spraying Systems Turbo Floodjet^{TM} nozzles at 11-gal water acre^{-1} carrier volume, and LESCO Trugreen (SiteOne, Roswell, GA) spray gun at 130-gal water acre^{-1} carrier volume. All application methods applied 17 oz acre^{-1} of Heritage WG (50% azoxystrobin) 21 May, 17 Jun, 16 Jul in 2015 and 18 May, 16 Jun, 14 Jul in 2016. The CO_2 method was applied as described above. For the PermaGreen method, a single pass was made in each plot in low gear at approximately 3.5 mph. For the LESCO method, the same experienced applicator from Eastern Turf Maintenance (Garner, NC) applied the fungicide using their standard method and equipment for lawncare applications. The applicator made one pass through each plot with a sweeping side to side motion uniformly.

Plots were monitored daily from May to August for brown patch symptoms and changes in turf quality. Turf quality and brown patch severity were visually assessed as described above on 16 Jun, 7 Jul, 14 Jul, 10 Aug in 2015 and on 14 Jun, 12 Jul in 2016. Data was transformed to area under turf quality curve (AUTQC) and area under disease progress curve (AUDPC) values for turf quality and brown patch severity respectively in 2015 only. Data was subjected to analysis of variance using the GLIMMIX procedure in SAS 9.4 (SAS Institute, Cary, NC). Means separation was assessed using least square means.

SIMULATED RAINFALL EXPERIMENTAL DESIGN

Study was arranged as a randomized complete block design with 4 replications. In both years, treatments were applied to plots (5 ft x 6 ft) and were re-randomized each year. All plots were infested with R. solani on 18 May 2015 and 24 May 2016. Inoculum was grown on sterilized rye grain and 10 cc was placed at 2 points within each plot near the soil surface. Fungicides evaluated were Insignia SC (30 fl oz acre^{-1}), Fame 480 (16 fl oz acre^{-1}), Heritage WG (8.7 and 17.4 oz acre^{-1}), and Heritage G (174 lbs acre^{-1}). Fungicide treatments were applied in a carrier volume of 88-gal water acre^{-1} using a $C0_2$ powered backpack sprayer equipped with TeeJet DG8004 nozzles at 40 psi. The granular fungicide was applied using the shaker jar method. Within 15 min of application, 0.5-inches of irrigation was applied to the test area to simulate rainfall. Treatments were applied on 20 May, 17 Jun, 15 Jul in 2015 and 17 May, 15 Jun, 13 Jul in 2016.

Plots were monitored daily from May to August for brown patch symptoms and changes in turf quality. Turf quality and brown patch severity were visually assessed every two weeks from May to August using the methods described above. Data was transformed to area under turf quality curve (AUTQC) and area under disease progress curve (AUDPC) values for turf quality and brown patch severity respectively. For turf quality and brown patch severity, 4 rating dates were used to calculate AUTQC and AUDPC values. Data was subjected to analysis of variance using the GLIMMIX procedure in SAS 9.4 (SAS Institute, Cary, NC). Means separation was assessed using least square means.

INFLUENCE OF NITROGEN RATE AND TIMING ON BROWN PATCH SEVERITY

Brown patch symptoms were observed during both years in both studies in mid-June and persisted through mid to late August. For the N rate study, disease severity was greatest on 3 Aug 2015 and 15 Aug 2016 with disease severity peaking at 31% and 49% respectively. For the N timing study, disease severity was greatest on 3 Aug 2015 (26%) and 6 Jul 2016 (33%). Within both studies, brown patch was uniformly distributed in both years. Although disease severity was uniform, differences in disease progress between years required that years to be analyzed separately. In 2015, differences in brown patch severity among N rates were not detected (Table A.2). In 2016, brown patch severity in the highest N rate (6 lb N 1,000 ft^{-2}) was significantly greater than the non-treated control and 1,2, and 3 lb N 1,000 ft^{-2} (Table A.2). In both years of the N rate study no differences were detected in turfgrass quality (Table A.2). No differences in brown patch severity or turfgrass quality were observed with N timings in both years (Table A.3).

INFLUENCE OF FUNGICIDE APPLICATION METHOD ON BROWN PATCH SEVERITY

Brown patch symptoms were observed during both years in mid-June and persisted through mid to late August. Disease severity was greatest on 7 Jul 2015 and 12 Jul 2016 with disease severity peaking at 41% and 48% respectively. Although disease severity was fairly uniform, differences in disease progress required years to be analyzed separately. Despite extreme disease pressure, all methods provided exceptional suppression of brown patch in both years and there were no differences among the methods (Table A.4). All methods provided acceptable turfgrass quality in both years when compared to the non-treated control (Table A.4).

INFLUENCE OF POST-APPLICATION IRRIGATION ON FUNGICIDE EFFICACY

Brown patch symptoms were observed during both years in mid-June and persisted through mid to late August. Disease severity was greatest on 14 Jul 2015 and 12 Jul 2016 with disease severity peaking at 14% and 18% respectively. Although disease severity was fairly uniform, differences in disease progress required years to be analyzed separately. All fungicide treatments suppressed brown patch when compared to the non-treated control in both years (Fig. A.1A and B). There were no differences in disease severity among treatments in both years. Turf quality in fungicide treatments was significantly better than the non-treated control in 2015 (Fig. A.1C). Insignia, Heritage (17.4 oz acre^{-1}), and Heritage G were the only treatments with significantly better turf quality than the non-treated control in 2016 (Fig. A.1D).

CONCLUSIONS

Over a two-year period, nitrogen rates ≤5 lb N 1000 ft^{-2} year^{-1} did not increase brown patch severity. The current recommendation for tall fescue in North Carolina is 3-4 lb N 1000 ft^{-2} year^{-1} (NC State Extension 2000), which in our study did not result in an increase brown patch severity. Increasing N rate to 5 lb 1000 ft^{-2} year^{-1} also did not increase brown patch severity, yet N rates as high as 5-6 lb 1000 ft^{-2} year^{-1} are likely not practical. Applications of N during the summer months did not influence brown patch severity in this study. Supplemental N fertility outside of the current recommendation will not promote brown patch as long as the total N year^{-1} does not reach or exceed 6 lb. The influence of supplemental N during the summer months on turfgrass quality in conjunction with a fungicide program remains unclear. It is likely that a 6-month gap in N applications as per the current recommendation is insufficient for optimal tall fescue health. Light and frequent N applications during the summer months are commonplace for other cool-season turfgrasses in golf course and athletic field management because these grasses need fertility to help mitigate the stresses often incurred during this time frame for recovery and survival (Ryan et al., 2011).

Application method with azoxystrobin did not influence brown patch suppression. As long as lawn care operators use a highly efficacious product such as azoxystrobin, application method will not alter brown patch control. The control observed with the ride-on spreader/sprayer that deployed azoxystrobin in a carrier volume of only 0.25 gal water 1,000 ft^{-2} was excellent and unexpected. This illustrates the importance of proper fungicide selection rather than application method when managing brown patch in tall fescue. To be clear, the results with the spreader/sprayer unit in this study cannot be translated to other fungicides or diseases. Further studies are required before this type of application method can be recommended for general tall fescue management. The low carrier volumes did not reduce brown patch suppression. A lower application volume could potentially help lawn care operators expand the number of clients visited during a day.

Simulated rainfall up to 0.5 inches within 15 minutes of fungicide application did not alter performance of products tested in this study. All products tested were QoI fungicides, but varied in topical mode of action or formulation. Insignia, a localized penetrant, performed as well as Heritage and Fame, both are acropetal penetrants. This was unexpected as we hypothesized that the performance of a localized penetrant like Insignia would be hindered with simulated rainfall, yet this was not observed. Studies with dollar spot demonstrated a loss of efficacy after simulated rainfall with iprodione, a localized penetrant (Inguagiato and Miele 2016). The difference between these studies could be the inherent activity of Insignia against brown patch and iprodione for dollar spot. Rainfall events shortly after fungicide application should not comprise control, however control may be lost during events such as severe thunderstorms, hurricanes, etc. Studies simulating rainfall at 1 inch or higher were not feasible at our research station due to pump output limitations.

This work demonstrates that tall fescue lawns can be fertilized during the summer months at modest N rates without affecting brown patch severity. When an appropriate fungicide such as Heritage was used for brown patch management, the method of fungicide delivery did not affect brown patch suppression. Simulated rainfall up to 0.5 inches within 15 minutes of fungicide application did not compromise fungicide efficacy. These results will allow lawn care operators to be more efficient with brown patch management in tall fescue, since fungicide reapplications following ≤0.5 inches rain will not be required.

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Table A.1. Rate and timing of urea (46-0-0) for evaluation on brown patch of tall fescue.

Nitrogen Rate Study		Nitrogen Timing Study	
Treatment Timing	lb N 1,000 ft^{-2} year^{-1}	Treatment Timing	lb N 1,000 ft^{-2} year^{-1}
Mar	1	Mar/Sep/Nov	3
Mar/Apr	2	Mar/Apr/Sep	3
Mar/Apr/May	3	Mar/Apr/May	3
Mar/Apr/May/Jun	4	Apr	3
Mar/Apr/May/Jun/Jul	5	Mar/Apr/May/Sep	3
Mar/Apr/May/Jun/Jul/Aug	6	M/A/M/J/J/A/S/O/N	3
Non-treated control	0	Jun/Jul/Aug	3
		Sept/Oct/Nov	3
		Sep	3
		Non-treated control	0

Table A.2. Effect of urea (46-0-0) on brown patch severity and turf quality.

		2015		2016	
Treatment Timing	lb N^{-1} $year^{-1}$	Turf quality[†]	Brown patch severity[‡]	Turf quality	Brown patch severity
Mar	1	885.1 a[§]	1730.7 a	597.8 a	1562.3 b
Mar/Apr	2	861.9 a	1753.7 a	613.2 a	1624.8 b
Mar/Apr/May	3	896.6 a	1427.9 a	625.6 a	1479.6 b
Mar/Apr/May/Jun	4	902.3 a	1546.4 a	603.6 a	2094.0 ab
Mar/Apr/May/Jun/Jul	5	868.4 a	1836.9 a	601.4 a	1931.1 ab
Mar/Apr/May/Jun/Jul/ Aug	6	893.9 a	1568.6 a	600.5 a	2614.8 a
Non-treated control	0	892.0 a	1300.0 a	620.0 a	1135.5 b

[†] AUTQC is Area Under Turf Quality Curve for rating dates between Mar 31 – Sep 18, 2015 and Apr 22 – Aug 15, 2016.

[‡] AUDPC is Area Under Disease Progress Curve for rating dates between Mar 31 – Sep 18, 2015 and Apr 22 – Aug 15, 2016.

[§] Means within columns followed by the same letter are not significantly different according to Fisher's protected LSD test (P=0.05).

Table A.3. Effect of urea (46-0-0) timing on brown patch severity and turf quality.

		2015		2016	
Treatment Timing	lb N^{-1} $year^{-1}$	Turf quality†	Brown patch severity‡	Turf quality	Brown patch severity
Mar/Sep/Nov	3	902.7 a§	1613.5 a	623.1 a	1366.5 a
Mar/Apr/Sep	3	907.4 a	1550.4 a	631.3 a	1600.0 a
Mar/Apr/May	3	892.4 a	1885.2 a	622.2 a	1388.0 a
Apr	3	895.9 a	1559.2 a	635.5 a	1486.0 a
Mar/Apr/May/Sep	3	894.2 a	1687.4 a	612.2 a	1674.5 a
M/A/M/J/J/A/S/O/N	3	917.9 a	1242.0 a	620.7 a	1890.0 a
Jun/Jul/Aug	3	880.7 a	1305.1 a	619.7 a	1485.0 a
Sept/Oct/Nov	3	894.7 a	1386.0 a	615.0 a	1451.5 a
Sep	3	903.3 a	1357.6 a	618.6 a	1473.5 a
Non-treated control	0	902.8 a	1459.0 a	632.5 a	1190.0 a

†AUTQC is Area Under Turf Quality Curve for rating dates between Mar 31 - Sep 18, 2015 and Apr 22 - Aug 15, 2016.

‡ AUDPC is Area Under Disease Progress Curve for rating dates between Mar 31 - Sep 18, 2015 and Apr 22 - Aug 15, 2016.

§ Means within columns followed by the same letter are not significantly different according to Fisher's protected LSD test (P=0.05).

Table A.4. Influence of application method on brown patch management in tall fescue.

		2015		12 Jul 2016	
Application Method	WCV[†]	Turf quality[‡]	Brown patch severity[¶]	Turf quality[§]	Brown patch severity
Spreader/Sprayer	11	386.1 a[#]	18.6 a	7.1 a	0.0 b
Commercial Spray Wand	130	394.2 a	0.0 a	6.8 a	5.5 b
Research Backpack	88	398.9 a	0.0 a	7.0 a	0.0 b
Non-treated control		283.6 b	1811.6 b	4.5 b	48.3 a

[†] WCV is water-carrier volume in gallons per acre.

[‡] AUTQC is Area Under Turf Quality Curve for rating dates between Mar 31 - Sep 18, 2015 and Apr 22 - Aug 15, 2016.

[¶] AUDPC is Area Under Disease Progress Curve for rating dates between Mar 31 - Sep 18, 2015 and Apr 22 - Aug 15, 2016.

[§] Turf quality was evaluated using a 1 to 9 scale (9=best, 6=acceptable) based on color, density, and uniformity.

[#] Means within columns followed by the same letter are not significantly different according to Fisher's protected LSD test (P=0.05).

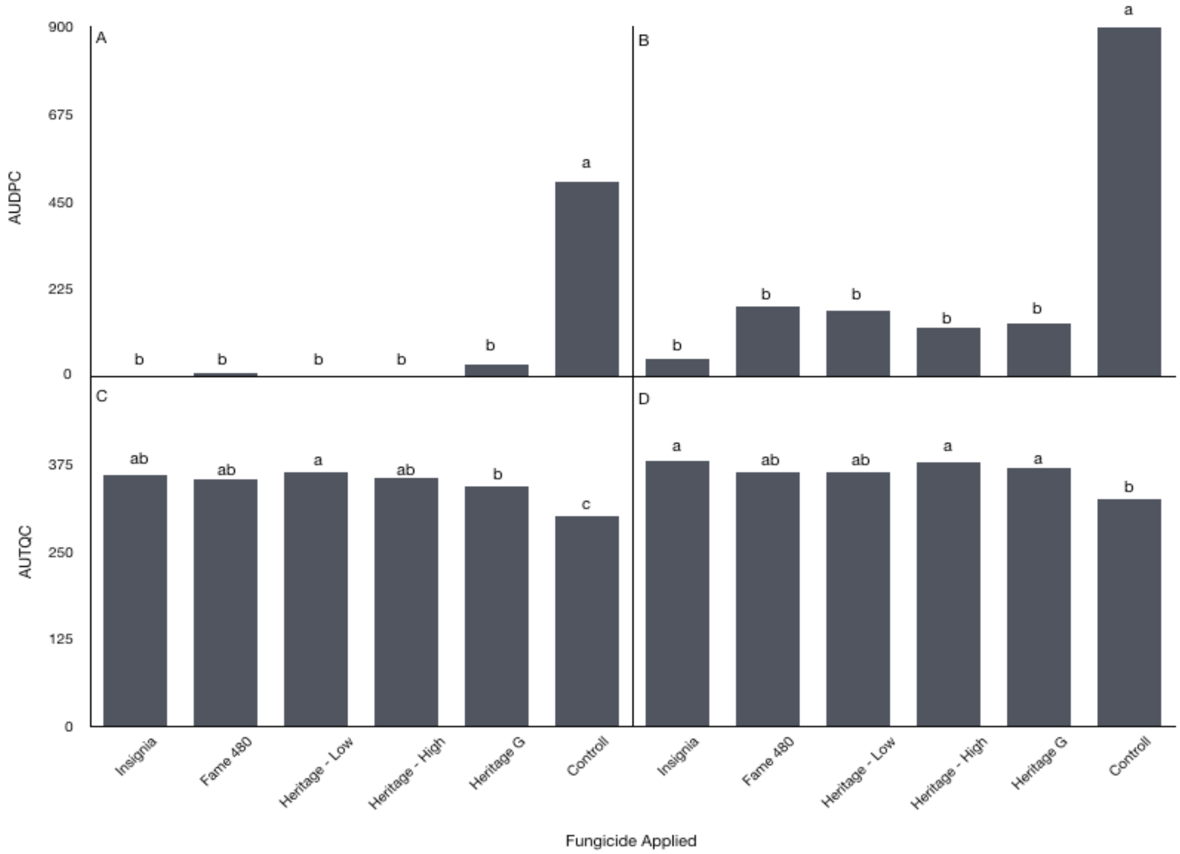

Figure A.1. Influence of simulated rainfall on fungicide efficacy on brown patch in tall fescue. A). Bars represent area under disease progress curve values as affected by simulated rainfall in 2015. B). Bars represent area under disease progress curve values as affected by simulated rainfall in 2016. C). Bars represent area under turf quality curve values as affected by simulated rainfall in 2015. D). Bars represent area under turf quality curve values as affected by simulated rainfall in 2016. Bars with the same letter within each quadrant are not significantly different according to Fisher's protected LSD test (P=0.05).