Integrated management strategies for plant-parasitic nematodes on warm-season turfgrass using plant growth-promoting rhizobacteria, chemical nematicides, and remote sensing technology

by

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Abstract

Plant-parasitic nematodes are a major pathogen of turfgrass throughout the United States, yet management strategies rely almost entirely on a limited number of chemical nematicides. The overall objective of this research was to gain a deeper understanding of how plant-parasitic nematode population dynamics are impacted by seasonal changes in Alabama, and to evaluate multiple strategies for managing these nematodes in turfgrass when population density reaches damaging levels. Management practices evaluated in these studies include evaluating a new chemical nematicide for its ability to reduce nematode population density, using PGPR to suppress nematode population density while also promoting root growth, and combining remote sensing technology with chemical nematicides to help standardize rating assessments of plant-parasitic nematode infested turfgrass.

Chapter I is a detailed review of literature related to turfgrass and how plant-parasitic nematodes influence its growth and development. This review gives details on the importance of turfgrass from an economic perspective, as well as provides information on its biology. An in-depth analysis is also provided on the numerous genera of plant-parasitic nematodes that can impact turfgrass, including the differences in turfgrass host range, population density levels, and damage potential. Current and potential management strategies for plant-parasitic nematodes are also discussed.

In Chapter II, a survey was conducted of six highly maintained bermudagrass sites in Alabama. Monthly or bimonthly sampling was conducted at each site over 2018 and 2019 to identify which plant-parasitic nematode genera were present, and if there are any seasonal differences in population density. Over both years, seven plant-parasitic nematode genera were

identified: *Belonolaimus, Helicotylenchus, Hemicycliophora, Hoplolaimus, Meloidogyne, Mesocriconema (sensu lato),* and *Tylenchorhynchus* (*sensu lato*). Of these seven genera identified, only two were ever found at potentially damaging levels: *Belonolaimus* and *Meloidogyne*. Interestingly, highest population density of *Belonolaimus* was found in April and October, and conversely, highest population density of *Meloidogyne* was found during midsummer (June through September). These results indicate that nematode genera are influenced by seasonality in turfgrass. This data also reinforces the importance for Alabama turfgrass managers to sample for nematodes throughout the year, and not rely on one sample date for management decisions.

In Chapter III, the chemical nematicide reklemel was evaluated for its efficacy as a potential option for plant-parasitic nematode management on turfgrass. This product was screened against *B. longicaudatus* and *M. incognita* on bermudagrass in greenhouse, microplot, and field settings. In the greenhouse, reklemel significantly reduced *B. longicaudatus* population density compared to the untreated control in both evaluation trials, and significantly reduced *M. incognita* population density in one of the two evaluation trials. In the microplot setting, reklemel was effective at lowering population density against both *B. longicaudatus* and *M. incognita* in all trials during the 2018 and 2019 growing seasons. Reklemel also led to an improvement of visual turfgrass quality and NDVI ratings compared to the untreated plots. A negative correlation was also observed between both visual turfgrass ratings and NDVI with nematode population density at multiple sample dates, showing that as reklemel reduced nematode population density, turfgrass quality improved. In the field setting, the higest rate of reklemel was most effective at lowering the population density of both *B. longicaudatus* and *M. incognita*, but no significant

differences in visual quality or NDVI ratings were ever observed. Overall, reklemel shows promise as a chemical nematicide for plant-parasitic nematode management on turfgrass.

The primary research objective for Chapter IV was to evaluate the ability of plant growth-promoting rhizobacteria (PGPR) for their nematicidal ability against *M. incognita*, while potentially also promoting bermudagrass root growth. In this study, 104 PGPR strains were evaluated for their ability to manage *M. incognita in vitro. In vitro* mortality of *M. incognita* ranged from 0.9 to 98.9%, and ten individual PGPR strains and one three-strain blend were advanced to greenhouse and microplot screening. In a greenhouse, seven of the eleven PGPR treatments significantly lowered *M. incognita* population density compared to the untreated control, with a couple strains also promoting root growth. In a microplot evaluation, five of the eleven PGPR treatments significantly reduced *M. incognita* population density. Between the greenhouse and microplot trials, three PGPR strains significantly reduced *M. incognita* population density compared to the untreated control. These were *Stenotrophomonas rhizophila* and two strains of *Bacillus aryahbatti*. Overall, these results indicate that multiple PGPR strains evaluated have the potential to reduce *M. incognita* population density on infected turfgrass.

Finally, in Chapter V, remote sensing technology was evaluated for the abilty to track the plant health of plant-parasitic nematode infested turfgrass in combination with chemical nematicides. For this study, the chemical nematicides abamectin, fluensulfone, fluopyram, and furfural were evaluated over two years in microplot trials for their ability to reduce both *B. longicaudatus* and *M. incognita* on bermudagrass. During these trials, visual turfgrass quality ratings were taken as well as NDVI and NDRE values. In both years of data, visual turfgrass quality, NDVI and NDRE were found to be strongly correlated with plant-parasitic nematode population density: as plant-parasitic nematode population density declined, turfgrass vigor

ratings improved. This study was also taken to a golf course infested with multiple genera of plant-parasitic nematodes in 2019. In this study, the nematicides abamectin, fluensulfone, and fluopyram were evaluated for their ability to reduce plant-parasitic nematode density, as well as their ability to impact visual turfgrass quality, NDVI, and NDRE. Similarly to the microplot evaluations, as nematode population density declined, all evaluation parameters improved. These results indicate that using NDVI and NDRE data in conjunction with visual turfgrass quality ratings provides a strong foundation for capturing the ability of currently available chemical nematicides to manage plant-parasitic nematodes on turfgrass.

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List of abbreviations

CFU	Colony forming unit
DAT	Days after treatment
IPM	Integrated pest management
NDRE	Normalized difference red edge index
NDVI	Normalized difference vegetative index
NIR	Near infrared
PCR	Polymerase chain reaction
PGPR	Plant growth-promoting rhizobacteria
PPN	Plant-parasitic nematode
RKN	Root-knot nematode
SEM	Standard error of the mean
TSA	Tryptic soy agar
UAS	Unmanned aerial system
UAV	Unmanned aerial vehicle

Chapter I: Review of literature

Rationale of the study

Plant-parasitic nematodes are widely considered a major pest of highly maintained turfgrass, yet there are few research groups actively working to understand management strategies for them. The most recent survey report on plant-parasitic nematodes in Alabama dates back to 2001, and an updated temporal analysis would help provide an idea of what the current issues our turf managers are facing related to nematode management (Sikora et al. 2001). With recent losses of the industry standard organophospate nematicide over the past 10 years, multiple new nematicides have entered the market, and little data is present showing their effectiveness in turfgrass and how they can be included in modern integrated pest management systems (Giannakou et al. 2007; Crow 2014). Biological control offers a potentially strong addition to chemical nematicides, yet little research has been conducted on their efficacy in turfgrass, and previous reports show that their effectiveness can be inconsistent (Pal and McSpadden, 2006). Finding a consistent biological control option would be a major step forward for plant-parasitic nematode management on turfgrass. The use of Unmanned Aerial Systems (UAV) have been widely used for nutrient analysis, fungal disease suppression, and weed management, but there are few examples of evaluating and implementing this technology into nematode management on turfgrass (Caturegli et al. 2017; Sykes et al. 2017; Xiang and Tian, 2011).

The overall goal of this study was to gain an updated understanding of the state of nematode management on turfgrass in Alabama, as well as evaluate potential new chemical and biological options for nematode control in turfgrass. This study also evaluated the ability of UAV equipped with multispectral cameras for their ability to track plant vigor in nematode

infested turfgrass with and without applications of nematicides. The specific objectives of this study were: i) to track multiple turfgrass locations for a temporal analysis of plant-parasitic nematodes over two growing seasons; ii) to evaluate the ability of fluazaindolizine for its ability to manage *Meloidogyne incognita* (root-knot nematode, RKN) and *Beloinolaimus longicaudatus* (sting nematode) as a chemical nematicide in turfgrass; iii) evaluate multiple PGPR strains through *in vitro*, greenhouse, and microplot studies for their ability to manage *M. incognita* on turfgrass; and iv) evaluate the ability of a UAV equipped with a multispectral camera to track plant-parasitic nematode symptom expression over a growing season.

Turfgrass: importance and biology

Turfgrass has become a fully integrated staple in our world, providing not just an aesthetic benefit, but also a practical benefit by reducing water runoff, heat dissipation, and soil erosion (Beard and Green, 1994). Turfgrass is an economically relevant crop in the United States, with a total estimated revenue at over $62 billion, and an employment impact of over 800,000 jobs (Haydu et al. 2005). From a geographic standpoint, homeowner turfgrass lawns cover an estimated 163,812 km^2 of land, which is over three times higher than total land area of irrigated corn (43,000 km^2) at the time of the study (Milesi et al. 2005).

The professional turfgrass industry can be divided into four basic groups: golf courses, contracted services (professional lawn care and irrigation services), sod production, and institutional facilities (Breuninger et al. 2013). The golf industry, which on its own makes up 44% of the total turfgrass industry, has a generated impact of $33.2 billion nationwide (Haydu et al. 2008). In the state of Alabama alone, it has been estimated that there are approximately 250 golf facilities, with an estimated revenue of $808.1 million, providing an added 20,000 jobs in the state (SRI International, 2010). While not being as large as the impact of golf courses,

contracted services still have a substantial impact on the United States, with an estimated total in sales of $23.7 billion in 2009 for professional lawn care, and turfgrass irrigation installation and maintenance generating an estimated revenue of $5.1 billion in 2010 (Breuninger et al. 2013). Sod production and institutional facilities are also significant revenue sources for the turfgrass industry in the United States, with an estimated 1,739 sod farms in the U.S. in 2017 (USDA, 2017). The turfgrass industry also has a strong economic impact on multiple types of institutional facilities including state and national parks, public and private universities, highway roadsides, airports, and government facilities (Breuninger et al. 2013).

Turfgrass in the United States can be broken down into geographic regions based upon weather and climactic conditions that determine optimal growing conditions. These two groups are defined as cool-season and warm-season grass species. Cool-season grasses are best adapted for temperature ranges from 65 to 75°F (18 to 24°C), and warm-season grasses are best adapted to temperatures ranging from 80 to 95°F (27 to 35°C) (Christians, 2011a). These grasses primarily differ based upon their photosynthetic systems. Cool-season grasses are known as C_3 grasses, meaning that they begin carbohydrate production with a three-carbon compound, whereas warm-season grasses are C_4 grasses, and begin carbohydrate production with a four-carbon compound (Jones, 1985). While the primary difference between these two grasses is their geographical range for optimal growth, there are also physiological factors that can differentiate these grasses.

With the southeastern United States categorized as a warm, humid region, it is best suited for C_4 grasses. These grasses have a higher growth rate from late spring to early fall, and go dormant when soil temperatures drop below 50°F (10°C) (Snyder et al. 2008). Examples of grasses grown in the southeast include bermudagrass (*Cynondon* spp. Rich.), zoysiagrass (*Zoysia*

spp. Willd.), St. Augustinegrass (*Stenotaphrum secundatum* [Walt.] Kuntze), bahiagrass (*Paspalum notatum* Flugge.), centipedegrass (*Eremochloa ophiuroides* [Munro] Hack.), buffalograss (*Buchloe dactyloides* [Nutt.] Engelm.), and carpetgrass (*Axonopus fissifolius* [Raddi] Kuhlm). These are all favorable grasses for the southeast because they are fast growers, highly heat tolerant, and produce deep root systems (Watson and Dallwitz, 1992).

Of the warm-season grasses, bermudagrass (*Cynodon* spp.) is the most widely used grass in the southern United States, and is found commonly between the latitudes of 45°N and 45°S (Taliaferro, 1995). It originated in Africa and was introduced into the United States during the mid-1700s as a forage grass (Hanson et al. 1969). Most bermudagrasses used for sod, golf-courses, and other high maintenance areas are interspecific hybrids that are a cross between common bermudagrass (*Cynodon dactylon* (L.) Pers.) and African bermudagrass (*Cynodon transvaalensis* Burt-Davy). In the southern United States, hybrid bermudagrass occupies more golf course acreage than any other grass (Lyman et al. 2007). Some bermudagrass hybrids commonly used in the southeast include 'Champion', 'Tifway', 'TifTuf', 'TifEagle', 'Princess 77' and 'FloraDwarf' (Beard and Sifers, 1996; Taliaferro and McMaugh, 1993). 'Tifway' is one of the first hybrids developed, and was released in 1960 by Dr. G. Burton from the Georgia Agricultural Experiment Station in Tifton, Georgia, the origin of the prefix 'Tif' (Christians, 2011b). Because of the significance and importance bermudagrass has in the southeast, a majority of the work in this study was performed on hybrid bermudagrass.

Plant-parasitic nematodes: overview and common species in the southeast

Plant-parasitic nematodes are microscopic unsegmented roundworms that live in the soil and feed on plant roots (Hussey, 1989). All plant-parasitic nematodes have a small structure referred to as a stylet, which is used to penetrate the plant tissue and feed on the host (Hussey,

1989). Plant-parasitic nematodes can be divided into three groups based upon feeding type: 1) ectoparasites that remain outside of the roots and use the stylet to feed on epidermal cells, 2) migratory endoparasites that enter and migrate within the root, thus feeding on multiple locations, and 3) sedentary endoparasites that enter the root as a vermiform juvenile and go through dimorphic molts before the female becomes fully sedentary (Hussey, 1989). Turfgrass in the southeast can be parasitized by both ectoparasites and endoparasites, and previous surveys in multiple states have frequently found these nematodes at or above damaging levels in turfgrass systems (Crow, 2005; Sikora et al. 2001). In Alabama alone, at least 10 genera of plant-parasitic nematodes have been recovered in routine assays from turfgrass soil (Mullen, 1998), and in a recent survey in Florida, over 80% of sampled golf courses were infested with plant-parasitic nematodes at potentially damaging levels (Crow, 2005; Aryal et al. 2017).

Meloidogyne spp. are classified as sedentary endoparasitic nematodes, and are historically recognized by the knots or galls that form on the roots of the host plant as the nematode feeds (Hunt and Handoo, 2009). The root-knot nematode was first reported in 1855 by Reverend Miles Joseph Berkeley when he observed galls on cucumber roots in a greenhouse that he eventually associated with the root-knot nematode (Hunt and Handoo, 2009). On a global scale, *Meloidogyne* spp. is considered one of the most devastating plant-parasitic nematodes, with a broad host range and wide geographic distribution. While total impact of *Meloidogyne* can be hard to assess, estimates have equated approximately 14.6% of crop loss in tropical and sub-tropical climates, and 8.8% in developing countries to the root-knot nematode (Nicol et al. 2011). *Meloidogyne* spp. reproduce at high levels in light and sandy soils, and tend to decline in population density in heavier soil types that have high percentages of silt and clay (Robinson et. al. 1987; Starr et. al. 1993).

With a majority of highly maintained turfgrass installed on a soil profile with a significant sand concentration, this group of turfgrass has a favorable environment for *Meloidogyne* spp. reproduction. Overall, multiple species of *Meloidogyne* have been reported at damaging levels on turfgrass. These include *M. marylandi* (Jepson and Golden), *M. naasi* (Franklin), *M. minor* (Karssen et al.), *M. graminicola* (Golden and Birchfield), *M. incognita* (Kofoid and White) Chitwood, and *M. graminis* (Sledge and Golden) Whitehead, with *M. graminis* being the most frequently found species in southeastern turfgrass (Crow, 2019; Zeng et al. 2012; McClure et al. 2012). While there are multiple species present, symptomology is similar across all the *Meloidogyne* species. Galling associated with *Meloidogyne* on turfgrass tends to be much less pronounced than on other hosts, and can be easy to miss (Crow, 2019). In turfgrass, above ground symptoms can look similar across most genera of plant-parasitic nematodes, which typically consists of overall plant decline and stunting, chlorosis, and potential necrosis. These symptoms tend to occur in irregular shaped patches randomly scattered over the infested area.

Hoplolaimus galeatus (Cobb), commonly known as the lance nematode, was described by N.A. Cobb, and originally named *Nemochus galeatus*. It was renamed *Hoplolaimus galeatus* in 1935 by Thorne. These nematodes are large, can reach up to 1.5 mm in length, and are classified as semi-endoparasitic nematodes (Orton Williams, 1973). While not being as damaging on as large of a scale as *Meloidogyne,* this nematode has a very wide host range, and can reproduce at damaging levels in a wide range of soil types (Kirkpatrick et al. 2017). In the United States, *H. galeatus* has been reported across the entire east coast (New England to Florida), all along the Mississippi River, and in Colorado, California, and Texas (Crow and Bammer, 2015; Zeng et al. 2012). *Hoplolaimus galeatus* is strongly pathogenic to Bermudagrass

and St. Augustine grass, and symptoms look similar to other nematodes, including chlorosis leading to eventual necrosis and stunted plants, often in patchy irregular areas (Crow, 2005b). Because *H. galeatus* is a semi-endoparasitic nematode, they are hard to control with chemical nematicides (Crow and Brammer, 2015).

Belonolaimus longicaudatus (Rau), commonly known as the sting nematode, is widely considered one of the most damaging plant-parasitic nematodes on turfgrass in the southeast United States. Sting nematode has a very wide host range, ranging from horticultural to agronomic crops (Abu-Gharbieh and Perry, 1970; Kutsuwa et al. 2015). It traditionally has been found in coastal regions of the southeastern United States, and favors soil that has at least 80-90% sand and less than 10% clay (Robbins and Barker, 1974; Crow and Han, 2005). With a majority of golf course putting greens built on a significant sand profile for improved drainage, sting nematode can be extremely detrimental in the golf course industry (Martin, 2017a). *Belonolaimus longicaudatus* is classified as a migratory ectoparasite, and – like the large *H. galeatus* - reach lengths as long as 2-3 mm (Crow and Han, 2005). Unique to sting nematodes, feeding typically occurs on the root tips of turf, thus inhibiting root growth and development, leading to reductions in the root biomass, water uptake, and nutrient absorption. Symptoms of this feeding are similar to other nematodes, including wilting, chlorosis, and thinning of turf in irregularly shaped patches (Crow and Han, 2005). Because this nematode has a migratory ectoparasitic feeding nature, the nematode can feed on multiple locations of the root system, thus causing damage to turfgrass quality at very low numbers (as low as 10 nematodes per $100cm^3$ soil) (Shaver et al. 2017).

Helicotylenchus spp. are commonly called spiral nematodes, because their body often is seen curled up in the shape of a spiral. The spiral nematode is broadly considered an

ectoparasitic nematode, however some species have been observed in the southeastern United States with slightly different feeding patterns. *Helicotylenchus pseudorobustus* (Steiner) Golden has a semi-endoparasitic feeding nature, penetrating the host root with its anterior body region (Vovlas and Larizza, 1994). The nematode typically feeds on cortical cells, inserting its stylet into the epidermis and cortical cells to ingest cellular contents (Vovlas and Inserra, 1985). Overall, this nematode has a very wide host range, and can be found on almost all agronomic and horticultural crops, but is rarely considered an important pest on most hosts (Pang et al. 2011). However, *H. pseudorobustus* and *H. paxilli* (Yuen) have been shown to have an impact on turfgrass at high population densities (Pang et al. 2011). Overall, symptoms of spiral nematodes on turfgrass are not as significant or severe as other nematodes such as root-knot or sting nematodes, but have been shown to reduce the root system (Pang et al. 2011). Symptoms are typically expressed in small chlorotic patches that are unevenly distributed throughout an infested area, and rarely move past a yellowing discoloration of the turfgrass (Crow, 2017).

Mesocriconema spp. (*sensu lato*), or ring nematodes, are small ectoparasitic nematodes that are found throughout the United States. Ring nematodes have a very wide host range, and are commonly found on a wide variety of turfgrasses and perennial plants including peaches, apples, and walnuts (Crow, 2005; Zehr et al. 1990). Ring nematodes are commonly found in most turfgrass soil samples, but population density at or above 500 nematodes per 100 cm^3 of soil can be associated with turfgrass showing symptoms of discolored roots with small lesions and above ground chlorosis of the leaf tissue (Kirkpatrick et al. 2017).

Geographic and host range impact on plant-parasitic nematodes

Plant-parasitic nematode management varies by geographic location in the United States. For example, *B. longicaudatus* is a primary pest of turfgrass in the southern United States, but

not found as frequently and at as high of population density in other regions of the country (Crow and Han, 2005). As previously mentioned, *B. longicaudatus* requires a minimum 80% sand concentration in the soil profile, making the southern coastal plains an ideal habitat. *Belonolaimus longicaudatus* also does not survive as well over winter in frozen soils, limiting its impact in areas that have colder winters such as the northeastern United States (Martin, 2017a). Host range of plant-parasitic nematodes also vary depending on turfgrass species (Table 1.1). For example, *B. longicaudatus* has the ability to damage all warm-season and cool-season grasses, and while *Meloidogyne* spp. can reproduce on all warm-season grasses except for bahiagrass and centipedegrass, the only cool-season grass that *Meloidogyne* spp. reproduces on is creeping bentgrass (Dernoeden, 2002; Martin, 2017b). *Hoplolaimus galeatus*, similarly to *Meloidogyne* spp., only can reproduce on the cool-season creeping bentgrass, but varies in that bahiagrass and other warm-season grasses act as a host. Since these host ranges differ, it is important for turfgrass managers to know these differences so they can anticipate what plant-parastic nematodes have a higher chance of becoming an issue. These host ranges can also influence a manager's decision on a golf course or athletic field when selecting a grass type for establishing.

Table 1.1: Plant-parasitic nematodes host ranges across cool- and warm-season grasses in the United States.[†]

		Sting	Ring	Root-knot	Lance	Spiral
Common Name	Scientific Name	*Belonolaimus longicaudatus*	*Mesocriconema* spp.	*Meloidogyne* spp.	*Hoplolaimus galeatus*	*Helicotylenchus* spp.
Cool-season grasses						
Creeping Bentgrass	*Agrostis stolonifera*	+[‡]	+	+	+	+
Kentucky Bluegrass	*Poa pratensis*	+	-	-	-	+
Perennial Ryegrass	*Lolium perenne*	+	-	-	-	+
Tall Fescue	*Festuca arundinacea*	+	-	-	-	+
Warm-season Grasses						
Bahiagrass	*Paspalum notatum*	+	+	-	+	+
Bermudagrass	*Cynodon* spp.	+	+	+	+	+
Centipedegrass	*Eremochloa ophiuroides*	+	+	-	-	+
Seashore Paspalum	*Paspalum vaginatum*	+	-	+	+	+
St. Augustine grass	*Stenotaphrum secundatum*	+	+	+	+	+
Zoysiagrass	*Zoysia* spp.	+	+	+	+	+

[†]Host ranges summarized from Dernoeden, P.H., 2002 and Martin, B. 2017b, meaning nematode can reproduce on turfgrass species and increase population density.

[‡](+/-) designates if the grass type is a host (+) or nonhost (-) of the nematode in the designated column.

Plant-parasitic nematode management in the southern United States

Cultural control

In most agricultural cropping systems, cultural practices including crop rotation, leaving the field fallow, timing of crop planting, destruction of plant roots, or cover crops can be implemented for managing plant-parasitic nematodes (Trivedi and Barker, 1986; Muller and Gooch, 1982; Allison, 1956; Oostenbrink, 1972; Nusbaum and Ferris, 1973). However, most of the practices listed above are not options for turfgrass. Being an extreme monoculture system, cultural practices for nematode management in turfgrass can be challenging.

For turfgrass, plant-parasitic nematodes can significantly influence nutrient status and fertility management. By feeding on plant roots, a high population density of plant-parasitic nematodes inhibits the ability of the turfgrass to extract nutrients from the soil, making them less efficient at nitrogen uptake. This causes nitrogen to become more likely to leach, and leads to more expressed nutrient deficiencies (Luc et al. 2006, 2007; Koppenhofer et al. 2013). Current fertility recommendations for plant-parasitic nematode infested turf include splitting nitrogen applications into more frequent applications, but not increasing total cumulative nitrogen output through the season (Crow et al. 2005). Mowing height can have an impact on nematode-infested turfgrass, but is not considered to be a major factor. However, research has shown that raising mowing height can improve turf quality by improving turf tolerance to plant-parasitic nematodes (Giblin-Davis et al. 1991; Settle et al. 2006; Crow, 2014). Deep and infrequent irrigation of turfgrass can also promote a deeper root system, thus providing an improved tolerance to plant-parasitic nematode population density compared to frequent shallow irrigation (Nelson, 1995).

Chemical control

Chemical control through nematicide use for turfgrass has undergone a substantial change over the past 15 years. Since its release in 1973, fenamiphos (Nemacure, Bayer CropScience, St. Louis, MO) was the most commonly used nematicide for turfgrass in the United States, and was widely considered the standard practice until production was stopped in 2007, due to environmental and human health concerns (Anonymous, 2002; Crow, 2005). Fenamiphos was shown to provide strong nematode control with a long residual against a majority of plant-parasitic nematodes, making it very successful in turfgrass nematode management (Opperman and Chang, 1991). Thus, after its removal from the market, many turfgrass managers were left with limited options for nematode management.

One of the main chemical methods for nematode management used after fenamiphos was the fumigant 1,3-dichloroproene, or 1,3-D (Curfew Soil Fumigant, Dow Agrosciences, Indianapolis, IN). 1,3-D can also be a useful nematode management tool as a post planting injection. In a study by Crow et al. (2003), five of ten experiments showed that 46.8 liters/ha of 1,3-D was effective in reducing *B. longicaudatus* population density compared to untreated plots up to one month after treatment, and nematode suppression generally lasted up to two months. Another study showned that 1,3-D in a nematode infested turfgrass under drought conditions can maintain as high as 40% higher turf quality and 27% less leaf wilting compared to other treatments (Trenholm et al. 2005).

Recently, newer chemical nematicide options have become available for management in turfgrass. One of these options is abamectin (Divanem, Syngenta Crop Protection, Greensboro, NC). *In vitro* research has shown that abamectin has nematicidal effects, especially against *Rotylenchulus reniformis* (Linford and Oliveira) and *M. incognita*. A study by Faske and Starr

(2006) calculated LD_{50} values of 1.56 µg/ml and 32.9 µg/ml for *M. incognita* and *R. reniformis* respectively based on two hour *in vitro* exposure. In turfgrass, abamectin significantly reduced population density of *H. galeatus* and *Tylenchorynchus dubius* (Buetschli) Filipjev on turfgrass compared to untreated controls in greenhouse studies (Blackburn et al. 1996). A more recent study by Aryal et al. (2016) found that including abamectin in a scheduled calendar-based IPM program helped significantly reduce nematode population density compared to both 1,3-D only and an untreated control.

Another nematicide recently introduced to the turfgrass industry is the granular formulation of fluensulfone (Nimitz Pro G, Adama, Pasadena, TX). Fluensulfone received initial EPA registration in 2014, and was labelled for turf starting in 2017 (Castillo et al. 2018). Fluensulfone has a mode of action distinct from any other nematicide, and has been proven effective against a wide range of nematodes (Oka, 2014; Kearn et al. 2014). Oka et al. (2012) demonstrated that in peppers infested with *M. incognita*, fluensulfone reduced galling index by 80% and nematode egg numbers by 73-82% compared to an untreated control. A study in England demonstrated that fluensulfone at 6 kg ai/ha significantly reduced *Globodera pallida* (Stone) juvenile counts in a potato field compared to the untreated control (Norshie et al. 2016). On turfgrass in the southeast, fluensulfone showed success for lowering *Meloidogyne* spp. population density compared to untreated plots by applying low rates (either four applications of 67 kg of product/ha or three applications of 90 kg of product/ha) on a monthly basis (Crow et al. 2017).

Fluopyram is a succinace dehydrogenase inhibitor (SDHI) fungicide that also has nematicidal properties. Research trials conducted in 2014 and 2015 by Lawrence et al. showed that fluopyram + imidacloprid (Velum Total, Bayer CropScience, St. Louis, MO) statistically

lowered both *M. incognita* and *R. reniformis* nematode population density in cotton compared to an untreated control. Velum Total was registered in 2015 for nematode management in both cotton and peanut. Further studies have shown that fluopyram causes paralysis of *M. incognita* and *R. reniformis*, and 2 hour exposure EC_{50} values of 5.18 and 12.99 µg/ml were calculated for each, respectively (Faske and Hurd, 2015). Following this discovery, fluopyram was released for use on turfgrass under the trade name Indemnify (Bayer CropScience, Research Triangle Park, NC), and sold as of late 2016 (Martin, 2017b). Research trials at the University of Florida found that fluopyram can provide reductions of plant-parasitic nematodes for up to 6 to 8 months, thus providing positive turf responses (Crow et al. 2017). In California, research trials conducted on *Poa annua* (L.) golf course greens have shown that one to two applications of fluopyram a year led to season-long control of *Anguina pacificae* (Cid del Prado Vera and Maggenti) (Baird et al. 2017).

Biological control

Biological control offers a different management strategy compared to chemical control, and there are many examples of biological control agents that provide various levels of suppression of plant-parasitic nematodes. Recently, multiple studies have reported the potential of naturally growing plant-growth promoting rhizobacteria (PGPR) for nematode suppression. Certain rhizosphere colonizing bacteria provide significant increases in root growth development, as well as significant reductions in severity and incidence in a wide variety of diseases (Kloepper, 1993; Kloepper et al. 2004). Diseases and pests managed by PGPR related to turfgrass specifically include fall armyworm on bermudagrass, gray leaf spot in ryegrass, and brown patch on creeping bentgrass (Coy et al. 2017; Viji et al. 2003; Suzuki et al. 2004). Research has shown that similar bacteria can provide suppression of nematodes in multiple

cropping systems in addition to turfgrass. A majority of these PGPR belong to the genus *Bacillus.* Xiang et al. (2017a) found that multiple strains of *Bacillus* spp. reduced cyst numbers of *H. glycines* on soybean in greenhouse, microplot, and field trials. This group also identified multiple *Bacillus* spp. that provided a reduction in *M. incognita* nematode population density in greenhouse, microplot, and field settings on cotton (Xiang et al. 2017b).

In turfgrass, plant and microbe interactions primarily refer to the relationship of fungi and cool-season grasses, with over 80 examples of turf-type cultivars that have these fungal endophytes incorporated into their growth and development (Meyer et al. 2013). However, Coy et al. (2019) recently inoculated bermudagrass with multiple strains of *Bacillus* spp., and were able to reisolate these same strains up to 12 weeks after inoculation, as well as prove that these strains can reduce oviposition of fall armyworm on bermudagrass (Coy et al. 2017). On a commercial level, *Bacillus* spp. has been investigated for nematicidal activity, with *Bacillus firmus* strain I-1582 (Nortica 5WG; Bayer CropScience, St. Louis, MO) currently on the market for plant-parasitic nematode management on turfgrass. Research out of Florida showed that this strain can reduce *B. longicaudatus* population density, but more commonly promotes an increase in root biomass in nematode infested turf (Crow, 2014). This data showed that *B. firmus* strain I-1582 can be implemented as an effective preventative management tool for *B. longicaudatus* on bermudagrass, primarily as an early season treatment.

Unmanned aerial systems: an overview

UAS, or Unmanned Aerial Systems, are known by many different names and acronyms. These include UAV (Unmanned Aerial Vehicle), aerial robot, or simply drone. The terms drone and UAV are the most commonly used terms, being mostly interchangeable. The term UAS is the widely accepted all-encompassing term for the technology, and was officially adopted by the

US Department of Defense (DDD), and the Civil Aviation Authority (CAA) of the UK (Colomina and Molina, 2014). Motorized Unmanned Aerial Systems (UAS) for scientific research can be traced back as far as the late 1970s, but due to the initial heavy weight, lack of GPS and autopilots, poor image quality capabilities, and United States governmental limitations, there were very few practical applications (Hogan et. al. 2017). Most of this early technology was used and improved by military needs, and few applications to the academic world were explored. However, there were early examples of UAS use beyond the military, including research involving crop dusting practices in Japan, and meteorological studies in Australia (Colomina and Molina, 2014).

Over the past 10 to 15 years, there has been a significant boom in rise of UAS related uses in both commercial and academic settings. The number of times that the term "UAS" was cited in 2005 in peer-reviewed journals was 544, compared to 1708 in 2013, just 8 years later (Colomina and Molina, 2014). There are several factors that can be tied to this change. These include decreases in price, consumer demand, improved technology related to battery life, GPS integration, customizable apps for smartphones and tablets, and improved flight longevity and ease of use related to cameras and sensors (Hogan et. al. 2017). Sensor technology has vastly improved in conjunction with UAS technology, with dozens of lightweight visible-spectrum and multispectral cameras available. The pixel quality of these cameras can now allow for quick high resolution and reliable data that can be captured and analyzed in a single day, making this technology quick, efficient, and accurate (Whitehead and Hugenholtz, 2014).

Sensors and imagery analysis for UAS

Drone technology has widely been accepted into agricultural use, with a large majority of this related to crop stress level monitoring. There are many different camera types mounted on

drones for scouting, each with their own specific pros and cons. The first camera and most commonly found camera is the RGB camera. The RGB camera captures red, green, and blue light, and is commonly referred to as a visual light camera since it captures the three primary colors of visible light. This is the standard camera type used for basic photography. The visible light spectrum has a small range of nanometers that fall in this window, ranging from approximately 380 to 700 nanometers (NASA, 2010). This range goes from violet to red light respectively. The RGB camera is the most common camera mounted on a drone, making it heavily utilized in the agriculture sector. RGB cameras are often relatively inexpensive, easily accessible, and easy to use. The implementation of a drone using a RGB camera has a low learning curve, and can be quickly used for field analysis. Research groups have integrated up to four or five different cameras on to the drone for field evaluations (Grenzdorffer et al. 2012). Other groups have reported using RGB cameras to measure distances based upon time-of flight correlated with imagery collected (Kohoutek and Eisenbeiss, 2012). With the rapid increase in smartphone imagery technology, research has also began using Samsung Galaxy S and S2 smartphones on drones to produce quick imagery of construction zones in South Korea (Yun et al. 2012).

While there is work being conducted with RGB imagery, these cameras only provide a small fraction of the light spectrum that is being reflected. One of the primary sensors used for large scale agricultural research is the Near-infrared (NIR) sensor. This sensor captures reflectance in the infrared light region, which ranges from 700 nm to 2500 nm, and typically allows for crop stresses to be seen sooner than with a standard RGB camera (Mills, 2017). Most NIR cameras focus on the lower portion of the IR spectrum, typically 700-1100 nm, which tends to more accurately correlate to plant stress (Al-Amoodi et al. 2004). The NIR spectrum has

traditionally been used for agricultural research, with implementations ranging from tracking soil moisture, soil nutrient levels, plant fertility levels, and produce ripeness (Garcia-Sanchez, et al. 2017). The NIR spectrum has also recently been used for pathogen and insect detection as well (Falade et al. 2017; Wang et al. 2011). NIR sensors largely use the Normalized Difference Vegetation Index (NDVI) to correlate levels of plant health. This is done by mathematically comparing red and NIR light signals to differentiate plant health. Vegetation that is actively growing and producing more energy for photosynthesis tends to absorb the most red light but reflect near infrared light, while diseased, stressed, or dead vegetation reflects more red light and less near infrared light. Based upon these reflection values, a formula is used to assign numerical values that correlate to plant health based upon these reflectance rates. The formula used for this is as follows: NDVI = (NIR – Red)/(NIR + Red). When this formula is applied to a NIR image, a range from -1 to 1 is given for each pixel, relating to how green the image is. A value of "1" correlates to more vegetative green growth than "0" does. Many of these NIR sensors capture various wavelengths of the IR spectrum, ranging from the red edge (680 nm to 730 nm), to complete IR sensors, capturing the entire 700-2500 nm range. There are other indices that can be used, including the photochemical reflectance index (PRI) and the stress index (SI), but NDVI is the most commonly used index for plant reflectance evaluations (Nansen and Elliot, 2016). Typically, many of these sensors are packaged into multispectral cameras. Multispectral cameras contain multiple sensors (usually no more than 5 in total) that can be captured simultaneously during a drone flight for immediate analysis and comparison. An example of such a camera for agriculture analysis is the RedEdge-M (MicaSense Inc., Seattle, WA). This camera captures five different spectral bands: blue, green, red, near-IR, and red edge, allowing various spectral reflectance values to be captured at one time.

Integrating UAS into agriculture and turfgrass

There are many applicable uses for this technology in modern agricultural and turfgrass systems. A group from Finland has been screening various multispectral cameras mounted on UAS looking at various parameters related to crop health. These studies have been conducted on wheat and barley - primarily focused on fertility or seeding rates. While some research has correlated imagery data to various seeding and nitrogen rate applications, the chances of variability are high, and weather patterns such as cloud coverage can play a major role in levels of correlation found (Honkavaara et al. 2013). Another group in France found similar results in small wheat plots when scouting with both standard RGB and multispectral cameras. While they were able to correlate imagery analysis with various wheat varieties and fertility rates, they found high levels of variability from flight to flight, demonstrating the importance of numerous flights under similar weather conditions (Lelong et al. 2008). A more recent study out of Italy evaluated multispectral cameras across three warm season cultivars of turfgrass for tracking nitrogen levels. The group evaluated both a RGB camera (Canon S100) and a multispectral camera (Tetracam ADCMicro) on a bermudagrass, zoysiagrass, and seashore paspalum. Findings here showed that both RGB and multispectral imagery showed significant differences for each cultivar at varying rates of fertility, and that these cameras had potential for detecting differences in fertility levels of turfgrass (Caturegli et al. 2017).

In the United States, many research groups are starting to use UAS for assisting with a wide array of pest management programs. In California, examples include scouting strawberries to study outbreaks of spider mite and tracking water stress in almond orchards and onion operations (Hogan et al. 2017). A group in Florida has been tracking post emergence weed applications on cucumbers over multiple years at the same field site (Fletcher, 2017).

Researchers at Washington State University have conducted trials on a wide range of crops including grapes, apples, cherries, wheat and potatoes. The primary research is related to irrigation techniques, where drone gathered imagery data is a good indicator of crop vigor and canopy stress (McCollough, 2017). Kalischuk et al. (2019) also implemented a UAV-assisted scouting program for foliar disease in watermelon, and were able to increase their ability to identify disease foci and problematic areas at a 20% higher rate than conventional scouting practices.

While not extensively used, UAS are beginning to be implemented into plant-parasitic nematode management programs. Nutter et al. (2002) was able to show a benefit in using aerial image analysis for *H. glycines* scouting in soybean by demonstrating the level of variation that can occur in spectral reflectance as a response to nematode infection. Bajwa et al. (2017) also successfully correlated disease ratings associated with *H. glycines* and *Fusarium solani* f. sp. *glycines* (sudden death syndrome, Akoi et al.) and multiple vegetation indices calculated with a remote sensor in soybeans. Joalland et al. (2018) were also able to correlate multiple spectral indices with yields of tolerant and susceptible sugar beets to *Heterodera schachtii* (beet cyst nematode, Schmidt). A research group in Brazil recently also found that red, red edge, and near infrared spectral ranges were significant for discrimination of healthy coffee plants and coffee plants infected with *Meloidogyne* spp. at or above damage thresholds at an accuracy rate of 78% (Martins et al. 2017). While there are more and more research groups focusing efforts on implementing UAS and remote sensing into plant-parasitic nematode research, minimal research related to using this technology on turfgrass has been explored.

Conclusion

Plant-parasitic nematodes are a major problem on turfgrass, and the current primary means of management rely almost entirely on the use of a limited number of chemical nematicides. While chemical nematicides are an effective option for nematode management, this reliance is seldom a good strategy for an effective integrated pest management program. By conducting and combining studies on plant-parasitic nematode ecology, biological control, chemical control, and remote sensing, this project aims to offer multiple solutions for improving current recommendations for plant-parasitic nematode management in turfgrass.

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Chapter II: Temporal distribution of plant-parasitic nematodes on select hybrid bermudagrass sites in Alabama

Abstract

Plant-parasitic nematodes are a major pest of hybrid bermudagrass (*Cynodon dactylon* x *C. transvaalensis*) in the southern United States. In this study, six bermudagrass locations in Alabama were selected for monthly or bimonthly sampling of plant-parasitic nematodes throughout 2018 and 2019. Five plant-parasitic genera were recovered in 2018: *Belonolaimus, Helicotylenchus, Hoplolaimus, Mesocriconema (sensu lato)* and *Meloidogyne*. Only *Belonolaimus* was recovered at action thresholds that may warrant the use of a nematicide. *Belonolaimus* was recovered at highest levels in April and October. In 2019, seven genera were recovered from these locations: *Belonolaimus, Helicotylenchus, Hemicycliophora, Hoplolaimus, Meloidogyne, Mesocriconema* (*sensu lato*), and *Tylenchorhynchus* (*sensu lato*). Of these genera, *Belonolaimus* and *Meloidogyne* were found at a population density that may require a nematicide application. Again, highest population density for *Belonolaimus* was found in April and October. However, *Meloidogyne* population density peaked during midsummer (June through September). These results indicate that nematode genera population density vary based upon climate season, and demonstrates a need for Alabama turfgrass managers to sample for nematodes throughout the year, and not rely on one sample date for management decisions.

Introduction

The turfgrass industry is extremely economically relevant to the state of Alabama. Alabama is third highest in number of sod farms in the United States (behind Florida and Texas) at an estimated 89 operating farms (USDA, 2017). In 2010, there were also approximately 250 golf facilities in the state with an estimated total revenue of greater than $808 million, providing an added 20,000 jobs to the state (SRI International, 2010). In the southern United States, hybrid bermudagrass (*Cynodon dactylon* x *C. transvaalensis* (L.) Pers.) is the primary grass used for putting greens on golf courses. The warm and humid weather of this temperate to subtropical region makes it an ideal location for bermudagrass growth and production.

Plant-parasitic nematodes are one of the main pest issues face by turfgrass managers in the southern United States. Nematodes cause issues by feeding on the root system of the turfgrass plant, leading to chlorosis, wilting, and thinning of the turf canopy (Crow and Han, 2005). The most significant damage occurs when plant-parasitic nematodes reach high population density in the soil, and excessive feeding leads to a reduction in root biomass, water uptake, and nutrient absorption (Luc et al. 2006; White and Dickens, 1984). Plant-parasitic nematodes are common in the southern United States. A recent survey in Florida found that plant-parasitic nematodes infested over 80% of sampled golf courses at potentially damaging levels (Crow, 2005a; Aryal et al. 2017). In a survey of 111 golf courses throughout North and South Carolina, Zeng et al. (2012) found a wide diversity of plant-parasitic nematodes, with over 24 unique nematode species, belonging to 19 genera and 11 families.

In the state of Alabama, previous studies have found a wide range of plant-parasitic nematode genera present on golf courses. Mullen (1998) reported recovering the genera *Belonolaimus, Helicotylenchus, Hemicycliophora, Hoplolaimus, Meloidogyne, Mesocriconema,*

Paratrichodorus, Pratylenchus, Tylenchorhynchus, and *Xiphinema.* A more recent study by Sikora et al. (2001) identified 9 plant-parasitic genera in Alabama on hybrid bermudagrass, with four of these genera (*Helicotylenchus, Hoplolaimus, Hemicycliophora,* and *Belonolaimus*) reported on golf courses at or above threshold levels that may require a nematicide application. While these previous studies provided important insight into the specific plant-parasitic nematodes are present in Alabama, it has been almost 20 years since the last nematode survey, and detailed information on modern distribution and population levels of plant-parasitic nematodes in Alabama are lacking.

Fenamiphos (Nemacur; Bayer CropScience, St. Louis, MO), a previous chemical standard for nematode management on turfgrass, production was halted in 2007 (Keigwin, 2014). This led to the introduction of multiple new nematicides to the turfgrass market. These nematicides include abamectin (Divanem; Syngenta Crop Protection, Greensboro, NC), fluopyram (Indemnify; Bayer CropScience, St. Louis, MO), and fluensulfone (Nimitz Pro G; Adama, Pasadena, Texas). Recent studies have shown each of these products to have success against a wide range of plant-parasitic nematodes (Crow et al. 2017). However, no nematode survey has been conducted in Alabama since this change in nematicide chemistry for nematode management on turfgrass.

Knowing that plant-parasitic nematodes are a significant pest of turfgrass in Alabama, six bermudagrass locations were selected for monthly or bimonthly (every other month) sampling of plant-parasitic nematodes. The primary objective of this study was to determine which plant-parasitic nematodes are present in Alabama, and if seasonal climate has any impact on population density of each nematode genera.

Materials and methods

Soil samples were collected from five golf courses in Alabama and the Auburn University Turfgrass Research Unit over the 2018 and 2019 growing season. All locations consisted of hybrid bermudagrass. All golf courses sampled requested to have their name redacted from the study for privacy reasons, thus locations are reported with the county names in which the golf course is located for anonymity. One course was located in Shelby County, two were located in Barbour County, and two were located in Lee County, Alabama. For each golf course, one green with a known history of plant-parasitic nematodes was chosen for sampling, and was repeatedly sampled at each sample interval. If the golf course did not report a previous issue with plant-parasitic nematodes, the green sampled was selected at random. For each green, 10 soil cores (2.22 cm-diam x 10 cm-deep) were taken at roughly equal intervals in a zigzag pattern across the green and composited. Samples were collected from April to October of both 2018 and 2019. In 2018, two locations were sampled monthly, three locations were sampled bimonthly (every other month), and one location was sampled monthly starting in August through October. In 2019, three locations were sampled monthly, and three locations were sampled bimonthly.

Nematode soil samples for each location were thoroughly mixed and a 100-cm^3 soil subsample was processed to determine plant-parasitic nematode population density. Nematodes were extracted by gravity sieving followed by sucrose centrifugation following the methodology of Jenkins (1964). Nematodes were confirmed and enumerated via a Nikon TSX 100 inverted microscope at 40-x magnification, and morphologically identified to genus (Mai and Lyon, 1975; Eisenback, 2002). Nematode population density for each genus was also compared to action thresholds (minimum level of each plant-parasitic nematode genus possible to justify nematicide

treatment) used by the Alabama Cooperative Extension System. These levels were as follows: *Mesocriconema* (*sensu lato*) = 500, *Helicotylenchulus* = 300, *Hoplolaimus* = 60, *Meloidogyne* = 80, *Tylenchorhynchus* (*sensu lato*) = 1,000, *Hemicyliophora* = 80, *Belonolaimus* = 10, *Paratrichodorus* = 100, per 100 cm^3 of soil (Sikora et al. 2001).

Results

2018

In 2018, five genera of plant-parasitic nematodes were recovered from the locations sampled. These include *Belonolaimus longicaudatus* (sting nematode), *Hoplolaimus* spp. (lance nematode), *Meloidogyne* spp. (root-knot nematode), *Mesocriconema* spp. (ring nematode) (*sensu lato*), and *Helicotylenchus* spp. (spiral nematode). Nematode genera occurrence across all locations ranged from *Hoplolaimus* spp. (found on 17% of locations) to *Mesocriconema* spp. (*sensu lato*) (found on 100% of locations) (Table 1). However, neither of these nematodes were ever at action threshold levels. *Belonolaimus longicaudatus* was found above threshold levels in three samples, and was the only nematode that reached this level (Table 1). This high population density was found at Lee County, Golf Course 1 in April (Figure 2.1B) and at Barbour County, Golf Course 2 in April and October (Figure 2.1E).

2019

In 2019, seven plant-parasitic nematode genera were identified from the same six turfgrass locations. These included *B. longicaudatus, Helicotylenchus* spp., *Hemicycliophora* spp., *Hoplolaimus* spp., *Meloidogyne* spp., *Mesocriconema* spp. (ring nematode) (*sensu lato*) and *Tylenchorhynchus* spp. (*sensu lato*) (Table 2.2). *Mesocriconema* spp. (*sensu lato*) was again identified at all locations sampled, with 97% of soil samples throughout 2019 having this

nematode (Table 2.2). However, no samples were ever above threshold levels. *Belonolaimus longicaudatus* was identified on 67% of the locations sampled, with 52% of soil samples in 2019 (Table 2.2). There were four soil samples from 2019 with above threshold levels for *B. longicaudatus*: Lee County Golf Course 1 in April (Figure 2.2B), Barbour County Golf Course 2 in April and October (Figure 2.2E), and the Shelby County Golf Course in October (Figure 2.2F). *Meloidogyne* spp. was recovered from 83% of locations sampled in 2019, and 88% of total soil samples (Table 2.2). Of these samples, five individual soil samples had *Meloidogyne* spp. above threshold levels. These samples were Barbour County Golf Course 1 in June and August (Figure 2.2D), Barbour County Golf Course 2 in August (Figure 2.2E), and the Shelby County Golf Course in July and August (Figure 2.2F).

Discussion

This study confirms previous reports of plant-parasitic genera found on turfgrass in the southern United States (Crow, 2005b; Sikora et al. 2001; Zeng et al. 2012). *Mesocriconema* spp. (*sensu lato*) was the most commonly found nematode in both years, showing up in all locations sampled regardless of sampling date. However, this nematode was never found at levels above action thresholds. *Helicotylenchus* spp. was only found at 33% of sampled locations in 2018, but that rose drastically in 2019 to 67%, though none of the samples containing *Helicotylenchus* spp. were ever at damaging levels.

Belonolaimus longicaudatus was found most often in both years above action thresholds, with 10% (3 out of 30) of samples above threshold in 2018, and 12% above threshold in 2019 (4 out of 33). *Belonolaimus longicaudatus* has been reported to cause significant damage to hybrid bermudagrass throughout the southern United States (Laughlin and Williams, 1971; Luc et al.

2006). The locations with above thresholds of *B. longicaudatus* were Lee County Golf Course 1 and Barbour Golf Course 2. After receiving the initial April report with above threshold levels, Lee County Golf Course 1 implemented a yearlong nematicide program, and successfully managed *B. longicaudatus* population density throughout 2018. Barbour County Golf Course 2 did not implement a nematode management program, and while *B. longicaudatus* population dropped below treatment thresholds during the summer, population density rose above threshold levels in October. This trend repeated in 2019. Lee County Golf Course 1 and Barbour County Golf Course 2 both had above threshold levels of *B. lonigicaudatus* in April of 2019. Lee County Golf Course 1 implemented a nematicide program, and Barbour County Golf Course 2 did not. This, again, lead to lowering nematode population density below action thresholds later in the by Lee County Golf Course 1. Barbour County Golf Course 2, however, saw initial decline in population density during the peak of summer, but a rise in population density as temperatures cooled in the fall.

The other plant-parasitic nematode found above the action threshold was *Meloidogyne* spp. *Meloidogyne* spp. was present in 83% of sampled locations at some point during the growing season in 2018 and 2019, and 70% of total soil samples had *Meloidogyne* spp. present in 2018. However, none of the 2018 samples were above action threshold. In 2019, 88% of soil samples confirmed *Meloidogyne* spp. presence. Five of these 2019 samples (5 out of 33) also found *Meloidogyne* spp. population density at or above treatment thresholds. Similar to *B. longicaudatus, Meloidogyne* spp. historically is known to be a major pest of hybrid bermudagrass (Christie et al. 1954; Crow, 2005b; Ye et al. 2015). Two locations saw *Meloidogyne* spp. at action threshold levels: Barbour County Golf Course 1 and Shelby County Golf Course 2. Neither of these locations applied a nematicide in 2019, and interestingly, the

populations fluctuated in a similar fashion at both locations. *Meloidogyne* spp. population density peaked for both locations during the middle of summer (June through September). This was interesting, because it was inverse from the trend observed by *B. longicaudatus* during this study. In fact, at the Shelby County Golf Course, as the *Meloidogyne* spp. population density declined from above threshold levels to below threshold levels from September to October, the *B. longicaudatus* population density increased from below threshold levels to above threshold levels. Previous studies have shown that the optimal temperature for *B. longicaudatus* is 30°C, so seeing population density decline as temperatures exceed this are unsurprising (Smart and Nguyen, 1991).

This study is relevant for plant-parasitic nematode management on hybrid bermudagrass, because it emphasizes the importance of season long nematode sampling. This is especially true for highly maintained bermudagrass with a history of multiple genera previously reported at high population density. These results are similar to studies conducted in Florida, where McGroary et al. (2009) found that while *B. longicaudatus* population density can be highly variable based on numerous factors, population density tended to peak from March to May. Bekal and Becker (2000) found that in a temperate region of California *B. longicaudatus* population density consistently increased in early spring as grass exited dormancy and began to grow, and declined rapidly shortly after. They also found one location where *B. longicaudatus* population density consistently peaked during October. For *Meloidogyne* spp. in Alabama, highest population density occurred primarily in late summer around August. Laughlin and Williams (1971) found *Meloidogyne* spp. population density highest in May on bermudagrass in Virginia. Starr et al. (2007) found highest population density of *M. marylandi* in a nematicide trial during March in Texas, which has a semi-arid climate. Westerdahl and Harivandi (2007) found *Meloidogyne* spp.

population density was highest in September and November in central coastal California. Morris et al. (2013) reported *M. minor* on bentgrass in Ireland population density was highest from June through August. Overall, *Meloidogyne* spp. peak population density is largely dependent on geographic location, as various times throughout the year have been reported for highest population density.

While this study largely confirms previous reports that population dynamics can vary significantly based on seasonal timing of nematode sampling, this is the first report of seasonal population variability on turfgrass in Alabama. It is extremely important for a turfgrass manager to understand the importance of consistent nematode sampling, and relying on only one sample date for yearlong nematode management can lead to a misinformed decision. This research focused on six turfgrass sites in Alabama, and found plant-parasitic nematode populations that warranted a nematicide application on half of the locations. Sikora et al. estimated in 2001 that less than 10% of golf courses in Alabama test for nematodes on a consistent basis. This number has certainly increased in recent years, but it is still important to help turfgrass managers understand the importance of consistent nematode sampling in spring, summer, and fall seasons.

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Table 2.1: Frequency of occurrence of plant-parasitic nematodes in hybrid bermudagrass soil samples in central and southern Alabama, 2018

Scientific Name	Common Name	Locations with this nematode (%)[x]	Samples with this nematode (%)[y]	Samples above threshold levels[z]
Belonolaimus longicaudatus	Sting nematode	50	23	3
Helicotylenchus spp.	Spiral nematode	33	37	0
Hoplolaimus spp.	Lance nematode	17	10	0
Meloidogyne spp.	Root-knot nematode	83	70	0
Mesocriconema spp.	Ring nematode	100	87	0

[†]Percentage of turfgrass locations with at least one nematode identified during the 2018 growing season. Percentage based upon 6 bermudagrass locations.

[‡]Percentage based on a total of 30 bermudagrass soil samples.

[§]Minimum levels of nematodes that can indicate need for nematicide application: *Mesocriconema* = 500, *Belonolaimus* = 10, *Helicotylenchus* = 300, *Hoplolaimus* = 60, *Meloidogyne* = 80 nematodes per 100 cm^3 of soil (Sikora et al. 2001).

Table 2.2: Frequency of occurrence of plant-parasitic nematodes in hybrid bermudagrass soil samples in central and southern Alabama, 2019

Nematode genus	Common Name	Locations with this nematode (%)[x]	Samples with this nematode (%)[y]	Samples above threshold levels[z]
Belonolaimus longicaudatus	Sting nematode	67	52	4
Helicotylenchus spp.	Spiral nematode	67	67	0
Hemicycliophora spp.	Sheath nematode	17	9	0
Hoplolaimus spp.	Lance nematode	17	18	0
Meloidogyne spp.	Root-knot nematode	83	88	5
Mesocriconema spp.	Ring nematode	100	97	0
Tylenchorhynchus spp.	Stunt nematode	17	6	0

[†]Percentage of turfgrass locations with at least one nematode identified during the 2019 growing season. Percentage based upon 6 bermudagrass locations.

[‡]Percentage based on a total of 33 bermudagrass soil samples.

[§]Minimum levels of nematodes that can indicate need for nematicide application: *Mesocriconema* = 500, *Belonolaimus* = 10, *Helicotylenchus* = 300, *Hoplolaimus* = 60, *Meloidogyne* = 80 nematodes per 100 cm^3 of soil (Sikora et al. 2001).

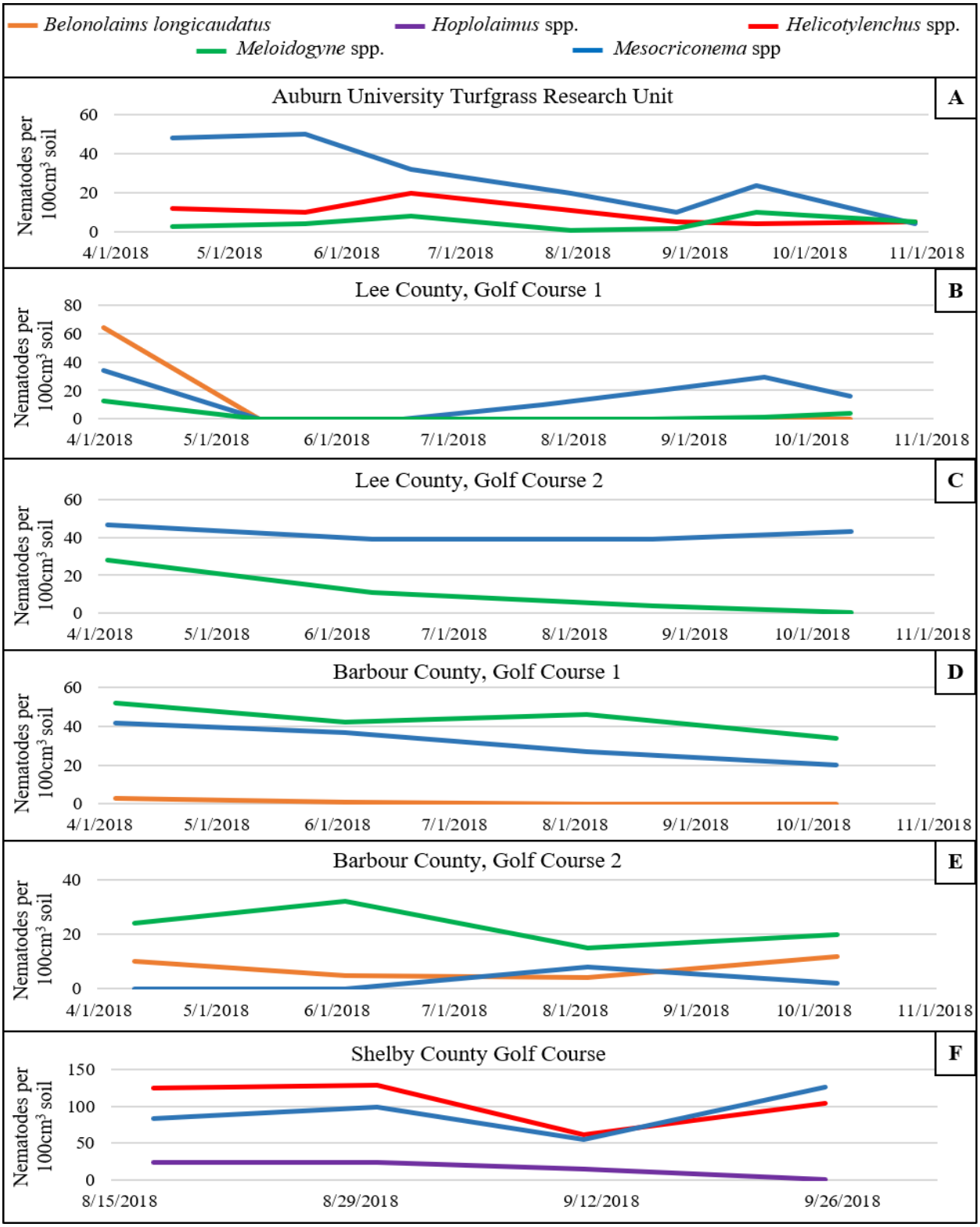

Figure 2.1. Plant-parasitic nematode population density for the Auburn University Turfgrass Research Unit (A), Lee County Golf Course 1 (B), Lee County Golf Course 2 (C), Barbour County Golf Course 1 (D), Barbour County Golf Course 2 (E), and Shelby County Golf Course (F). Nematode population density is reported as per 100 cm^3 of soil in 2018.

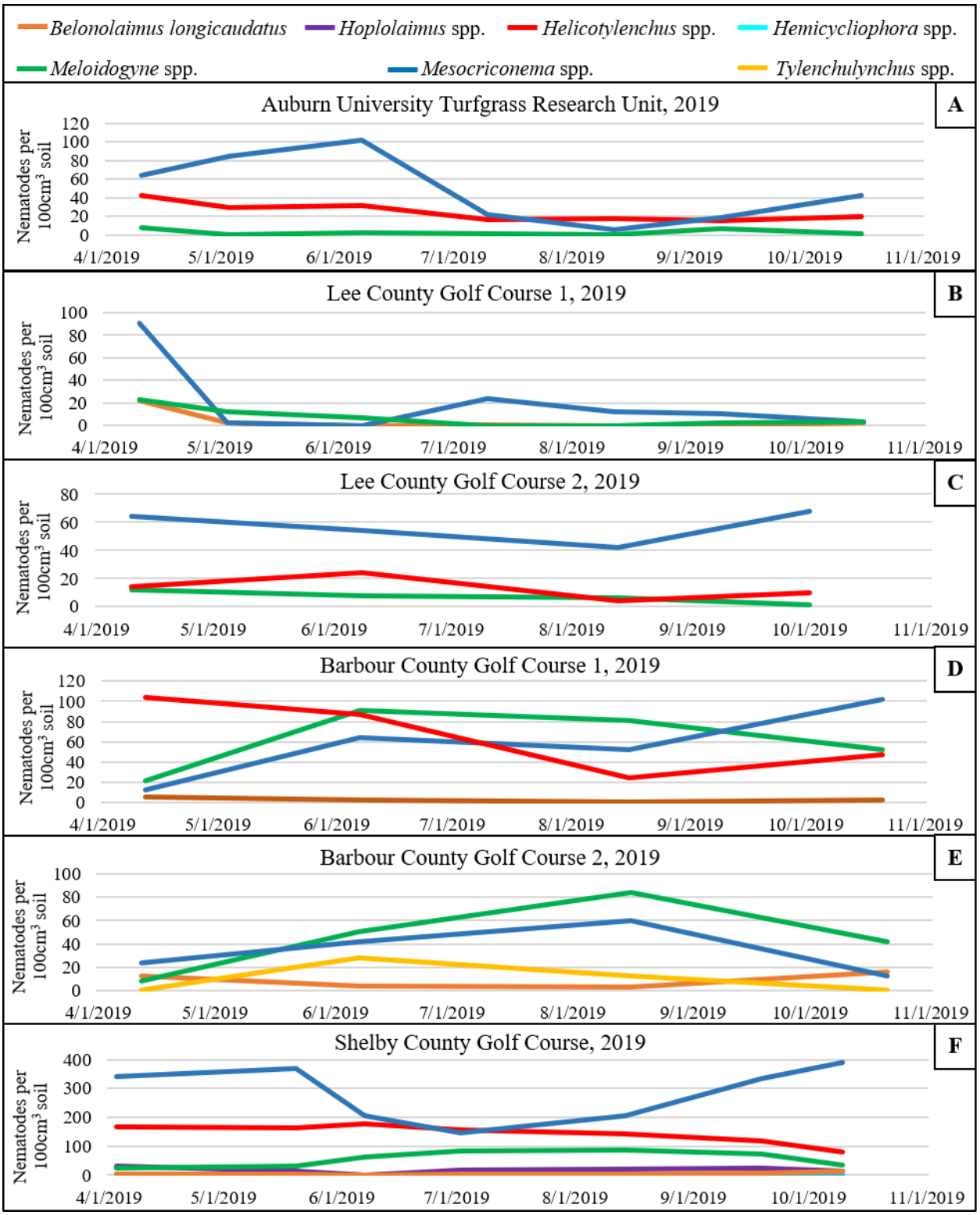

Figure 2.2. Plant-parasitic nematode population density for the Auburn University Turfgrass Research Unit (A), Lee County Golf Course 1 (B), Lee County Golf Course 2 (C), Barbour County Golf Course 1 (D), Barbour County Golf Course 2 (E), and Shelby County Golf Course (F). Nematode population density is reported as per 100 cm^3 of soil in 2019.

Chapter III: Evaluation of a new chemical nematicide, reklemel (fluazaindolizine), for plant-parasitic nematode management in warm-season turfgrass

Abstract

Plant-parasitic nematodes are a major pest of turfgrass in the United States, yet there are few options for successful management. Most current management strategies rely on the use of a limited number of chemical nematicides, so finding a new management option for nematode suppression would be extremely valuable for turfgrass managers. The goal of this study was to evaluate a new nematicide, reklemel (fluazaindolizine), for its ability to reduce plant-parasitic nematode population density and improve turfgrass quality. Separate research trials were conducted on bermudagrass infested with *Belonolaimus longicaudatus* and *Meloidogyne incognita* in greenhouse, microplot, and field settings over 2018 and 2019. Both greenhouse evaluations demonstrated multiple rates of reklemel reduced *B. longicaudatus* population density, and one of the two *M. incognita* trials showed multiple rates of reklemel reduce nematode population density. Reklemel was also effective at reducing population density of both *B. longicaudatus* and *M. incognita* in microplot settings for both 2018 and 2019, and a significant improvement in turf quality was observed for both visual turfgrass ratings and NDVI. Field trials demonstrated a significant reduction for both *B. longicaudatus* and *M. incognita* population density by multiple rates of reklemel, but no significant differences in turf quality ratings were observed. Overall, reklemel shows promise as a chemical nematicide for plant-parasitic nematode management on turfgrass.

Introduction

Turfgrass is commonly grown throughout the United States for a wide range of uses, including golf courses, pastures, homeowner lawns, sod production, and institutional facilities (Breuninger et al. 2013). Economically, turfgrass has been estimated to have a total revenue of over $62 billion dollars, and geographically cover over 160,000 km^2 of land (Haydu et al. 2005; Milesi et al. 2005). In the southeastern United States, bermudagrass (*Cynodon* spp.) is the most commonly grown perennial warm-season turfgrass (Taliaferro, 1995). The warm and humid weather of the region makes this the ideal region geographically for bermudagrass growth and production.

One of the major pest issues for bermudagrass management in the southeast are plant-parasitic nematodes. A 2005 survey of golf courses in Florida found that over 80% of courses sampled were infested with plant-parasitic nematodes at potentially damaging levels (Crow, 2005). In Alabama, at least 10 genera of plant-parasitic nematodes have been recovered in routine assays from Alabama turfgrass, with many of these being found at damaging levels (Mullen, 1998; Sikora et al. 2001). Zeng et al. (2012) identified over 24 unique plant-parasitic nematode species on over 111 golf courses throughout North and South Carolina. Nematode damage occurs as they feed on the root system, leading to wilting, chlorosis, and thinning of the turf often in irregularly shaped patches (Crow and Han, 2005). This feeding inhibits root growth and development, leading to potential reductions in root biomass, water uptake, and nutrient absorption. With the high potential for significant damage to turf by plant-parasitic nematodes, timely management is extremely important, especially on highly maintained turfgrass. The primary strategy for nematode management is through a limited number of chemical nematicides.

Since its registration in 1973, fenamiphos (Nemacur; Bayer CropScience, St. Louis, MO) has dominated the turfgrass industry as the most frequently used nematicide (Keigwin, 2014). However, production of this product was halted in 2007 and it is currently not available for use. With this ban, turfgrass nematode management has shifted to newer and safer non-fumigant nematicides. One such example is abamectin (Divanem, Syngenta Crop Protection, Greensboro, NC). Abamectin has been shown to be effective against plant-parasitic nematodes on turf in the southeast (Blackburn et al. 1996; Crow, 2014). Fluensulfone (Nimitz Pro G, Adama, Pasadena, TX), a nematicide labelled for turfgrass in 2017, has also shown promise against plant-parasitic nematodes in turfgrass (Crow et al. 2017). A third commonly used chemical nematicide that was released for use on turfgrass starting in late 2016 is fluopyram (Indemnify, Bayer CropScience, Research Triangle Park, NC). Research trials conducted to evaluate fluopyram's efficacy as a turfgrass nematicide have shown promise with a long residual of control (Crow et al. 2017; Baird et al. 2017). While each of these nematicides have been proven to provide benefit to plant-parasitic nematode management in turfgrass, recent research has also shown that relying too heavily on one nematicide has the potential to hurt soil health (Waldo et al. 2019). Thus, finding new chemical nematicides to add to an integrated pest management program is always a valuable addition for turfgrass plant-parasitic nematode management.

One potentially useful chemical nematicide for turfgrass is reklemel (fluazaindolizine), a novel sulfonamide recently discovered to have nematicidal properties (Corteva Agriscience, Indianapolis, IN) (Lahm et al. 2017; Thoden and Wiles, 2019). Assessments of reklemel *in vitro* have shown its ability to significantly reduce motility and activity of *Meloidogyne incognita* juveniles compared to untreated juveniles, and greenhouse assays of reklemel on tomato have lowered *M. incognita's* reproductive factor (Rf = final population density/initial population

density) (Wram and Zasada, 2019). Reklemel has also been shown to be effective as a nematicide treatment over a multi-year field study on *M. incognita* infested carrots (Becker et al. 2019). Hajihassani et al. (2019) found that reklemel was effective in reducing the root gall index and *M. incognita* population density, as well as provide a consistent yield increase compared to an untreated control in cucumber trials. Reklemel has also demonstrated efficacy at reducing *M. incognita* root gall index, eggs per gram of root, and nematode reproductive factor in tomato compared to an untreated control (de Oliveira Silva et al. 2019).

With such a limited number of chemical nematicide options available for plant-parasitic nematode management on turfgrass, reklemel would be a beneficial addition for nematode management. Thus, the ability of reklemel to reduce both *M. incognita* and *Belonolaimus longicaudatus* population density on bermudagrass was evaluated. The overall objective of this study was to evaluate the potential of reklemel as a chemical nematicide for turfgrass in greenhouse, microplot, and field settings. The determination of reklemels efficacy as a turfgrass nematicide was evaluated by both its ability to reduce plant-parasitic nematode population density, and its ability to improve overall turf health.

Materials and methods

Nematicide Treatments

Three rates of reklemel were evaluated for their ability to reduce both *M. incognita* and *B. longicaudatus* population density throughout the study. Fluopyram (Indemnify; Bayer CropScience, St. Louis, MO) was included as a chemical control, and an additional treatment of tap water was used as a negative control. Treatments for the experiments were (1) 4 applications of a “low” rate of reklemel at 2.2 liters of produce per hectare at approximately 0, 4, 8, and 12 weeks after trial initiation, (2) 2 applications of a “medium” rate of reklemel at 4.5 liters of

product per hectare (weeks 0 and 8), (3) 1 application of a "high" rate of reklemel at 9 liters of product per hectare (week 0), (4) 2 applications of fluopyram at 0.63 liters of product per hectare (weeks 0 and 8), (5) and an untreated tap water control.

Greenhouse Evaluations

Two separate greenhouse trials were conducted in 2018 and repeated in 2019 at the Plant Science Research Center (PSRC) at Auburn University, Auburn, AL. One trial evaluated the efficacy of reklemel on *B. longicaudatus*, and the other *M. incognita*. Five-hundred cm^3 polystyrene pots were filled with 100% sand. Each pot was then seeded with 1 gram of 'Princess 77' bermudagrass seed. Pots were watered daily as needed to allow for germination, and given a total of six weeks for root establishment before trial initiation. Pots were also fertilized every 14 days using 24-8-16 (N-P_2O_5-K_2O) at a rate of 0.5 kg N per 100 m^2 per growing month and trimmed once a week to a height of 2.54 cm. Lighting was supplied via 1,000-watt halide bulbs producing 110,000 lumens for 14 hours per day and temperatures in the greenhouse ranged from 24 to 35°C. Nematicide treatments were applied as a foliar spray via a handheld spray bottle, and treatments were diluted so that two sprays from the bottle was the calibrated rate.

Greenhouse nematode inoculum

Meloidogyne incognita race 3, originally isolated from an infested field at the Plant Breeding Unit (PBU) at E.V. Smith Research Center of Auburn University and maintained on corn "Mycogen 2H723" (Corteva AgriScience, Indianapolis, IN) in 500-cm^3 polystyrene pots in the greenhouse, was used as inoculum in the first experiment (Groover et al. 2019). To obtain the nematode population, the eggs were extracted from the corn roots following a modified version of the methodology of Hussey and Barker (1973). The root mass was placed in a 0.625% NaOCl solution and shaken for 4 minutes at 1 g-force on a Barnstead Lab Line Max Q 5000 E Class

shaker (Conquer Scientific, San Diego, CA). Roots were scrubbed by hand, and the eggs were collected on a 25-µm pore sieve and washed into a 50 mL centrifuge tube. The contents were centrifuged at 427 g-forces for 1 minute in a 1.14 specific gravity sucrose solution based on Jenkins (1964) methodology. Eggs, now located in the supernatant of the sucrose solution, were recollected on a 25-µm pore sieve, rinsed with water to remove sucrose from eggs, and their presence confirmed via a Nikon TSX 100 inverted microscope at 40-x magnification. The eggs were placed in a modified Baermann funnel (Castillo et al. 2013) on a slide warmer (Model 77; Marshall Scientific, Brentwood, NH) and incubated at 31°C for 5 to 7 days to obtain second-stage juveniles (J2). The J2 were collected on a 25-µm pore sieve, transferred to 1.5 mL micro-centrifuge tubes, centrifuged at 5,000 g for 1 minute, rinsed with sterile distilled water, and centrifuged again at 5,000 g for 1 minute. The J2 solution was adjusted to 1,000 J2 per 1 mL of water, and 2 mL of solution containing 2,000 J2 were pipetted into each pot.

For the other greenhouse experiment, *B. longicaudatus*, maintained on 'Princess 77' bermudagrass in 500 cm^3 polystyrene pots, was used as inoculum. To obtain the nematode population, total soil from each pot was collected on a 25-µm pore sieve and nematodes were extracted using the modified sucrose centrifugal flotation technique as described above. The final *B. longicaudatus* population was collected on a 25-µm pore sieve and transferred to a 1.5 mL micro-centrifuge tube, centrifuged at 5,000 g for 1 minute, rinsed with sterile distilled water, and centrifuged again at 5,000 g for 1 minute. The nematode suspension was then adjusted to 20 nematodes per 1 mL of water, and 2 mL of solution containing 40 *B. longicaudatus* were pipetted into each pot.

Greenhouse data collection

All experiments were arranged in a randomized complete block design (RCBD) with five replications. Turfgrass vigor was calculated using the National Turfgrass Evaluation Program (NTEP) guidelines (Parsons et al. 2015). Visual ratings consisted of a 1-9 rating scale, where 1 was very poor quality turf, 6 was minimal acceptable turf quality, and 9 was exceptional turf quality (Morris, 2004; Morris and Shearman, 2014). NDVI measurements using a Greenseeker Handheld Crop Sensor (Trimble Inc, Sunnyvale, CA) were taken on a weekly basis for each trial in both years. Experiments were harvested 84 days after the first nematicide applications were made. Nematode samples from each pot were collected at the completion of the trial. Soil collected from each pot was collected on a 25-µm pore sieve was then used to calculate final nematode populations for both *M. incognita* and *B. longicaudatus* trials using the modified centrifugal flotation technique as previously described (Jenkins, 1964). Extracted nematodes were enumerated at 40-x magnification using an inverted TS100 Nikon microscope and quantified as total nematodes per pot and as nematodes per gram of root fresh weight (RFW).

Microplot evaluations

The same treatments used in the greenhouse experiments was also used for trials conducted in a microplot setting. Microplot trials were conducted in two separate years (2018 and 2019) at the PSRC in Auburn, AL. All plots were arranged in a RCBD with five replications. For these trials, 26.5-liter plastic tree pots were used as microplots. Pots were nested one on top of the other with a brick in between to limit root growth by air pruning. The nested pot design was buried in the ground with one inch of the pot above the soil surface. Microplots were then filled with 100% medium-coarse sand (0.25-1.0 mm). ‘Tifway’ hybrid bermudagrass sod was established in each plot and given 10 weeks for root establishment. At the end of the 10-week

period, *M. incognita* eggs were inoculated at a rate of 50,000 eggs per pot on a weekly basis for 4 weeks to build up nematode population density in half of the microplots. The remaining microplots received an inoculation rate of 100 *B. longicaudatus* nematodes per pot on a weekly basis for 4 weeks. After the 4-week inoculation period, a 100-cm^3 soil sample was taken from each plot to confirm *M. incognita* or *B. longicaudatus* presence. Treatments were applied as a foliar spray via a handheld spray bottle, and treatments were diluted so that two sprays from the bottle was the calibrated rate for each treatment per microplot. Treatment regimen was identical in application rates and timing as greenhouse experiments. Each microplot received water at 30 mL/min by an automated drip irrigation system adjusted throughout the season to run for 30 minutes twice a day every other day. Grass was trimmed twice a week to a height of 2.5 cm.

Microplot data collection

Visual turfgrass ratings and NDVI ratings with the handheld sensor were taken throughout the trial starting at trial initiation followed by 7-day increments. Nematode population density was determined at three time points during the trial in 2018 (June 29, August 24, and September 28), and four time points in 2019 (July 22, August 19, September 23, and October 21). Nematode extraction and population density determination was performed as previously described.

Field evaluations

In 2018, a field trial evaluating reklemel as a turfgrass nematicide for *Belonolaimus longicaudatus* was conducted on common bermudagrass grown under fairway conditions at the Auburn University Gulf Coast Research and Extension Center (GCREC) in Fairhope, AL after confirming *B. longicaudatus* presence. The treatments for both greenhouse and microplot trials

was also used for the field evaluation. Individual plots were 1.5 meters by 3 meters, with a 0.6-meter border between adjacent plots. The trial was set up as a randomized complete block design with five replications. Each treatment was mixed with water to a total volume of one gallon and sprayed on the plots with a CO_2-powered backpack sprayer (R&D Sprayers, Bellspray, Inc.; Opelousas, LA).

Two field trials were conducted in 2019 for evaluating rekelmel as a turfgrass nematicide. Trials were conducted at GCREC in the same location as 2018 for *Belonolaimus longicaudatus* evaluations, and an adjacent location with a confirmed population density of *Meloidogyne* spp. The treatment list remained the same as previous studies for both nematode trials.

Field data collection

Data collection was the same for both years. Visual turfgrass ratings and NDVI values with a handheld sensor were taken at the start of the experiment, followed by approximately two-week intervals throughout the trial as previously described. Along with these measurements, field trials were mapped with a DJI Phantom 4 Professional (SZ DJI Technology Co.; Nanshan District, Shenzhen, China). This drone was equipped with a MicaSense RedEdge-M (MicaSense, Inc.; Seattle, WA). Image processing was performed with Pix4Dmapper (Pix4D; Prilly, Switzerland), and image analysis was carried out with ArcMap (Esri; Redlands, California), providing NDVI and NDRE ratings for each plot. Nematode samples were taken at three time points in 2018 (August 7, September 4, and October 2) and four time points in 2019 (July 25, August 22, September 24, and October 23), approximately at four-week intervals. Nematode samples consisted of seven 2.22-cm-diameter x 10-cm-deep cores from each plot. Samples were thoroughly mixed, and nematodes were extracted from a $100cm^3$ subsample and enumerated as previously described.

Statistical analysis

Data collected from greenhouse, microplot, and field evaluations were analyzed using the PROC GLIMMIX procedure (SAS version 9.4; SAS Institute, Cary, NC). Dependent variables included *B. longicaudatus* per 100 cm^3 soil, *M. incognita* per 100 cm^3 soil, visual turf quality, and NDVI. The fixed effect was nematicide treatment, and random effects included replication, test repeat, and location. Studen panels were generated to determine the normality of residuals. For greenhouse and microplot evaluations, LS-means were compared between treatments by Tukey's multiple range test for each evaluation date ($P \leq 0.05$). For the microplot evaluation, visual turfgrass quality and NDVI ratings were compared statistically by linear correlation at each nematode sample date ($P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$).

Results

Greenhouse trials

All nematicide treatments significantly reduced *M. incognita* population density compared to the untreated control in 2018 greenhouse evaluations ($P \leq 0.05$) (Figure 3.1). However, despite seeing numerical reductions by all nematicide treatments in 2019, there were no significant differences between treatments (data not shown). In the *B. longicaudatus* greenhouse evaluations, all treatments significantly reduced nematode population density compared to the untreated control in 2018 ($P \leq 0.05$) (Figure 3.2). Fluopyram also significantly lowered nematode population density compared to the low (2.2 liters/hectare) and medium (4.4 liters/hectare) rates of reklemel, but was statistically similar to the high rate of reklemel (9 liters/hectare) ($P \leq 0.05$). In 2019, the medium and high rates of reklemel as well as the

fluopyram control significantly reduced *B. longicaudatus* population density compared to the untreated control ($P \leq 0.05$) (Figure 3.3).

Microplot trials

In the 2018 *M. incognita* microplot evaluations, all nematicide treatments significantly reduced population density compared to the untreated control at both the August and September evaluation date ($P \leq 0.05$) (Figure 3.4A). For visual turfgrass quality, seven of the ten evaluation dates saw significant improvement by at least one nematicide treatment compared to the untreated control, with the medium rate of reklemel having the numerically highest visual turfgrass rating at eight of the ten evaluation dates ($P \leq 0.05$) (Figure 3.4B). Six of the nine NDVI evaluation dates also saw a significant improvement by at least one nematicide treatment compared to the untreated control, with fluopyram having the numerically largest NDVI value at five of the nine evaluation dates ($P \leq 0.05$) (Figure 3.4C).

In 2019, multiple nematicide treatments significantly impacted *M. incognita* population density in the microplot trial. The high rate of reklemel and the fluopyram treatment significantly reduced *M. incognita* population density compared to the untreated control at the August, September, and October sample dates, and the medium rate of reklemel significantly lowered population density at the October sample date ($P \leq 0.05$) (Figure 3.5A). Of the fifteen turfgrass visual quality evaluation dates, significant differences between treatments were observed at nine dates, with the fluopyram treatment consistently having the highest numerical visual turfgrass quality ($P \leq 0.05$) (Figure 3.5B). This was closely followed by the medium and high rates of reklemel, that had signifincalty improved visual turfgrass quality at eight evaluation dates compared to the untreated control ($P \leq 0.05$). For the NDVI evaluations, significant differences

between treatments were observed at all evaluation dates, with fluopyram having the numerically largest value throughout the entire trial ($P \leq 0.05$) (Figure 3.5C).

Belonolaimus longicaudatus population density was significantly reduced by all nematicides in the 2018 microplot trial at both evaluation dates after trial initiation ($P \leq 0.05$) (Figure 3.6A). Visual turfgrass quality was also significantly improved by at least one nematicide treatment at nine of the ten evaluation dates, with the largest improvement in visual quality in the later sample dates by fluopyram, the medium rate of reklemel, and the high rate of reklemel ($P \leq 0.05$) (Figure 3.6B). NDVI was significantly improved by all nematicide treatments at all except one evaluation date in the 2018 *B. longicaudatus* microplot trial ($P \leq 0.05$) (Figure 3.6C). In the 2019 *B. longicaudatus* microplot trial, nematode population density was significantly reduced by all nematicides at the August, September, and October sample dates ($P \leq 0.05$) (Figure 3.7A). This led to a significant increase in visual turf quality by all treatments compared to the untreated control at ten of the fifteen evaluation dates, and a significant increase in NDVI by all treatments at eleven of the fifteen evaluation dates ($P \leq 0.05$) (Figure 3.6B,C).

Significant linear correlations were observed in the microplot trials throughout 2018 and 2019 for both *M. incognita* and *B. longicaudatus* population density when compared to both visual turfgrass quality and NDVI (Table 3.1). In general, as nematode population density declined, both visual turfgrass quality and NDVI values increased. For *M. incognita* evaluations, one of the three nematode sample dates was significantly correlated with visual quality ($P \leq 0.001$) and NDVI ($P \leq 0.01$) in 2018, and all four nematode sample dates in 2019 ($P \leq 0.05$). For *B. longicaudatus* evaluations, two of the three sample dates had a significant correlation with both visual quality and NDVI in 2018 ($P \leq 0.01$), and all four nematode sample dates in 2019 ($P \leq 0.001$).

Field trials

For both 2018 and 2019 field evaluations, nematicide treatments had a significant impact on plant-parasitic nematode population density (Figure 3.8). However, no visual symptoms were ever observed in the trial, thus no significant differences for visual ratings, handheld NDVI, or drone NDVI and NDRE values were reported over both years (data not shown). In 2018, the high rate of reklemel and fluopyram both significantly reduced *B. longicaudatus* population density at the September and October sample dates, and the medium rate of reklemel significantly reduced population density at the October sample date ($P \leq 0.05$). In both the *B. longicaudatus* and *M. incognita* 2019 field trials, three of the four nematicide treatments significantly reduced nematode population density compared to the untreated control at the August evaluation date, and all nematicides significantly reduced population density at the September and October evaluation dates ($P \leq 0.05$) (Figure 3.8B,C).

Discussion

The results from both year's greenhouse, microplot, and field evaluations of reklemel indicate that there is a strong potential for plant-parasitic nematode management in turfgrass. Plant-parasitic nematode population density was significantly lowered when compared to an untreated control in all three settings. In the microplot trials, both visual turfgrass quality ratings and NDVI were significantly improved by multiple rates of reklemel. Thus, with a reduction in both *B. longicaudatus* and *M. incognita* population density, and a general improvement in plant health, both efficacy criteria for reklemel evaluation were met.

Greenhouse evaluations showed strong nematicidal activity for reklemel in both *B. longicaudatus* and *M. inocgnita* trials. A numerical rate response was also observed, with higher

rates of reklemel leading to lower final population density in all trials. While a significant reduction in *M. incognita* population density was not observed compared to the untreated control in the 2019 trial, no final population density for any of the evaluated reklemel rates were at levels that traditionally are of concern for a turfgrass manager in the southeastern United States (Sikora et al. 2001).

The results of the microplot trials also showed nematicidal activity against both *M. incognita* and *B. longicaudatus.* Unlike the greenhouse trials, a rate response was not consistently observed. In fact, in the 2018 *M. incognita* trial, while all treatments lowered population density below high risk levels, the monthly application of the low rate of reklemel (2.2 L/ha) led to the lowest population density at the end of the trial of the three reklemel rates evaluated. This was not the case in 2019, as the highest rate of reklemel applied at the start of the trial resulted in the largest reduction in *M. incognita* population density. In the 2018 *B. lonigcaudatus* trial, two applications of the medium rate (4.5 L/ha) led to the lowest population density, with the high rate (9 L/ha) treatment still having nematodes at above threshold levels. However, the 2019 *B. longicaudatus* trial showed a dose response trend, where the larger the initial application the larger the numerical reduction in nematode population density. While turf quality and NDVI were significantly improved by reklemel applications compared to the untreated control, there were rarely any evaluation dates where differences between the three rates were observed. Over all the microplot data, two rates of 4.5 L/ha of reklemel and one application of 9 L/ha of reklemel led to the most consistent improvement of turfgrass vigor compared to the untreated control, showing that these higher initial rates of the product may be the best option for plant-parasitic nematode management on turfgrass.

Field trials showed strong reductions of both *B. longicaudatus* and *M. incognita* population density by reklemel. While all nematicide rates and applications led to nematode population density reduction, the medium and high rates of reklemel as well as fluopyram most consistently lowered population density across all trials. However, despite confirming strong population density for both *M. incognita* and *B. longicaudatus* at the field sites for these trials, no visual symptoms were ever observed. Thus, while the higher rates of reklemel again had strong impacts on nematode density, no conclusions can be made on the effect reklemel had on plant vigor in the field setting.

This research confirms previous studies that have shown a similar efficacy of reklemel to *M. incognita* as a nematicide for reducing population density on crops including cucumber, carrot, and tomato (Becker et al. 2019; Hajihassani et al. 2019; de Oliveira Silva et al. 2019). However, while *M. incognita* is pathogenic to bermudagrass, it is not traditionally the main *Meloidogyne* species identified on turfgrass in the southeast (Crow, 2019; Ye et al. 2015; Zeng et al. 2012). For this research, *M. incognita* was used for trials because all research plots were artificially inoculated to obtain infested turfgrass, and *M. incognita* was available at high enough population density for successful inoculation. Moving forward, field locations with species more commonly found on turfgrass need to also be evaluated (*M. graminis* and *M. marylandi*) to confirm the findings of this study.

To our knowledge, this is the first published study on reklemel's efficacy as a turfgrass nematicide. These findings indicate that while each rate evaluated was successful at lowering both *M. incognita* and *B. longicaudatus* population density, higher initial application rates of reklemel were more consistent at both lowering nematode population density and improving plant health quality. While these results show promise for this product, more studies need to be

conducted. Overall, reklemel appears to have strong potential for including in a turfgrass nematode integrated management program.

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Table 3.1. Pearson correlation coefficients[†] resulting from linear correlation of *Meloidogyne incognita* and *Belonolaimus longicaudatus* population density in microplot bermudagrass with visual turfgrass quality and NDVI values in Auburn, AL for 2018 and 2019.

	2018		
	June 29	Aug 24	Sept 28
	Meloidogyne incognita population density		
Visual Quality[‡]	0.05	0.05	-0.77***
NDVI[§]	N/A	-0.24	-0.52**
	Belonolaimus longicaudatus population density		
Visual Quality	-0.08	-0.64***	-0.60**
NDVI	N/A	-0.62***	-0.54**

	2019			
	July 22	Aug 19	Sept 23	Oct 21
	Meloidogyne incognita population density			
Visual Quality	-0.78***	-0.60**	-0.38*	-0.38*
NDVI	-0.77***	-0.49*	-0.51**	-0.47*
	Belonolaimus longicaudatus population density			
Visual Quality	-0.73***	-0.78***	-0.75***	-0.75***
NDVI	-0.63***	-0.79***	-0.81***	-0.73***

[†]*, **, *** tests of linear correlation between variables were not significant or were significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively.

[‡]Visual quality ratings were assigned on a 1 to 9 scale, where 1 = poorest turf quality, 6 = minimally acceptable turf quality, and 9 = exceptional turf quality.

[§]NDVI = (840 nm reflectance – 668 nm reflectance) ÷ (840 nm reflectance – 668 nm reflectance) collected using a MicaSense RedEdge-M (MicaSense, Inc.; Seattle, WA) sensor.

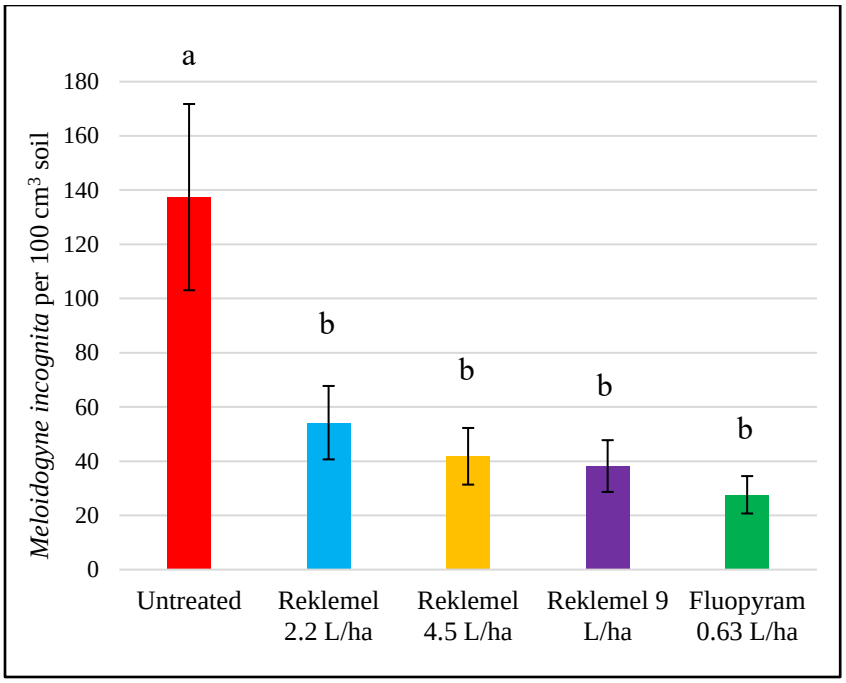

Figure 3.1. 2018 *Meloidogyne incognita* population density in greenhouse evaluations 84 days after treatment (DAT). Rates were applied as follows: Reklemel 2.2 L/ha at weeks 0, 4, 8, 12; 4.5 L/ha at weeks 0, 8; 9 L/ha at week 0; fluopyram 0.63 L/ha at weeks 0, 8. Means of bars with the same letter above them are not significantly different (Tukey-Kramer, $P \leq 0.05$).

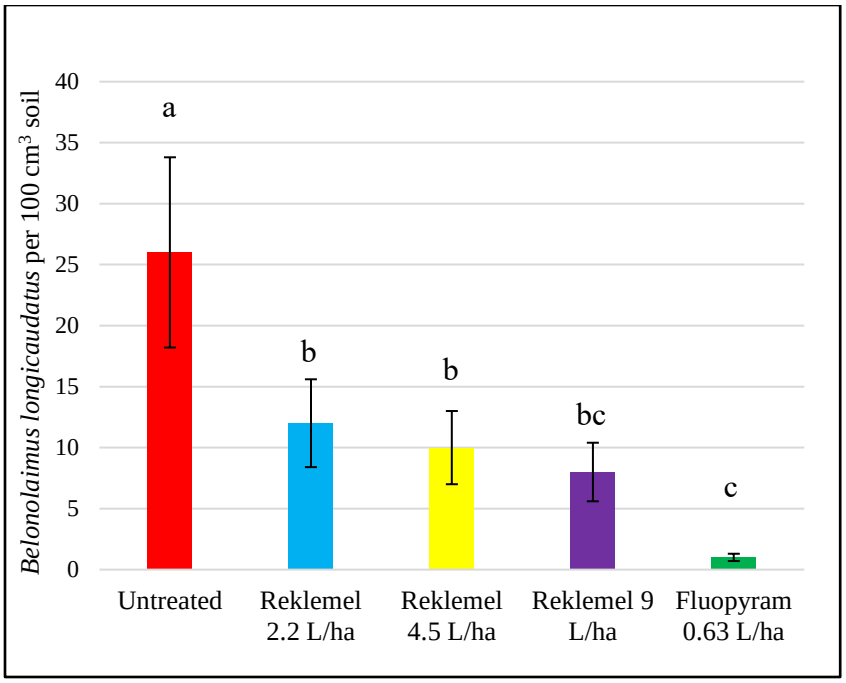

Figure 3.2. 2018 *Belonolaimus longicaudatus* population density in greenhouse evaluations 84 days after treatment (DAT). Rates were applied as follows: Reklemel 2.2 L/ha at weeks 0, 4, 8, 12; 4.5 L/ha at weeks 0, 8; 9 L/ha at week 0; fluopyram 0.63 L/ha at weeks 0, 8. Means of bars with the same letter above them are not significantly different (Tukey-Kramer, $P \leq 0.05$).

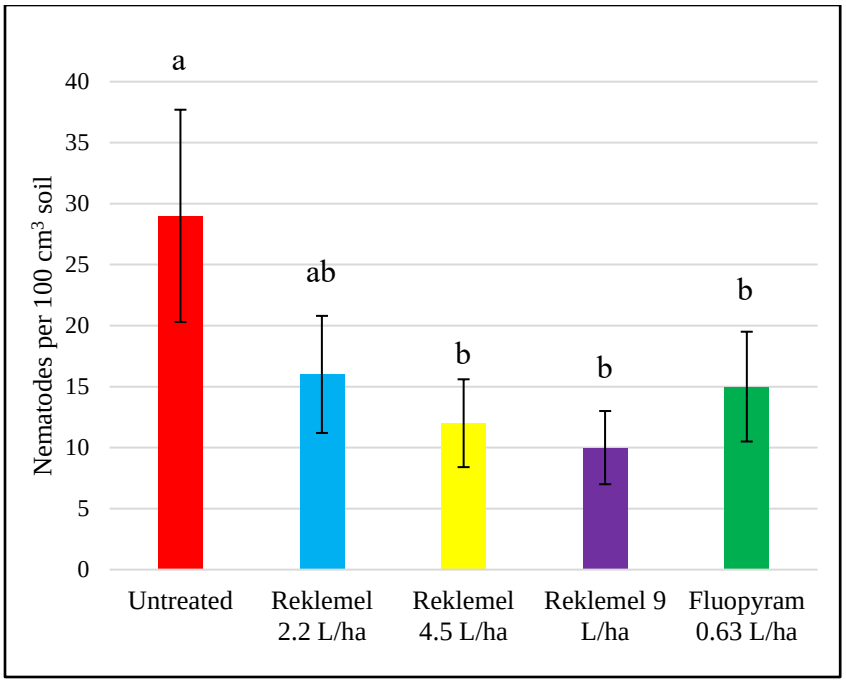

Figure 3.3. 2019 *Belonolaimus longicaudatus* population density in Auburn, AL greenhouse evaluations 84 days after treatment (DAT). Nematicide rates were applied as follows: Reklemel 2.2 L/ha at weeks 0, 4, 8, 12; 4.5 L/ha at weeks 0, 8; 9 L/ha at week 0; fluopyram 0.63 L/ha at weeks 0, 8. Means of bars with the same letter above them are not significantly different (Tukey-Kramer, $P \leq 0.05$).

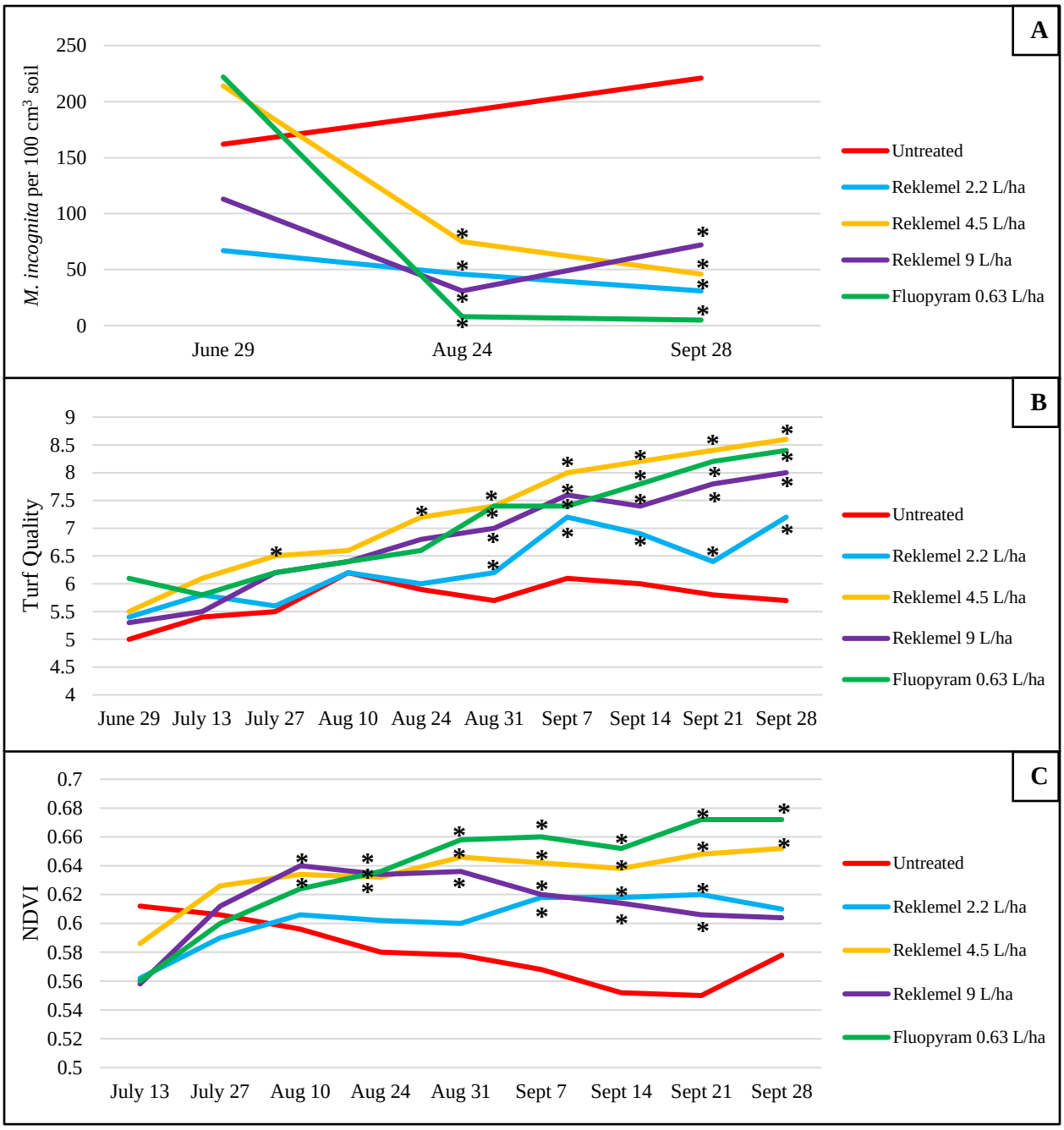

Figure 3.4. *Meloidogyne incognita* population density (A), visual turfgrass quality (B), and NDVI values (C) as affected by nematicide applications through 2018 bermudagrass microplot evaluations in Auburn, AL, 2018. Nematicide rates were applied as follows: Reklemel 2.2 L/ha at weeks 0, 4, 8, 12; 4.5 L/ha at weeks 0, 8; 9 L/ha at week 0; fluopyram 0.63 L/ha at weeks 0, 8. *Different from the untreated according to the pairwise comparison of each treatment to the untreated control (Tukey-Kramer; $P \leq 0.05$).

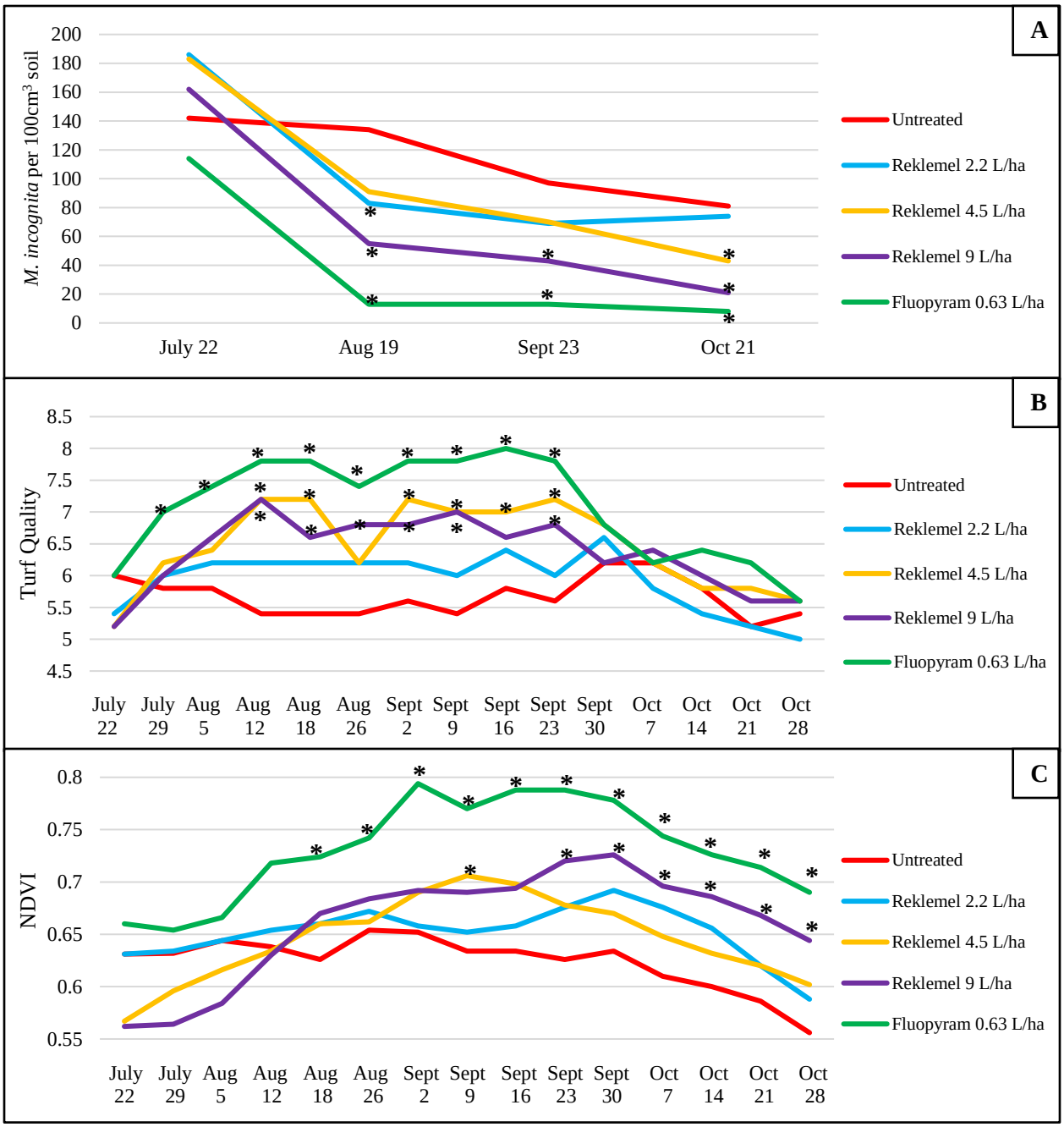

Figure 3.5. *Meloidogyne incognita* population density (A), visual turfgrass quality (B), and NDVI values (C) as affected by nematicide treatments in 2019 bermudagrass microplot evaluations, Auburn, AL. Nematicide rates were applied as follows: Reklemel 2.2 L/ha at weeks 0, 4, 8, 12; 4.5 L/ha at weeks 0, 8; 9 L/ha at week 0; fluopyram 0.63 L/ha at weeks 0, 8. *Different from the untreated according to the pairwise comparison of each treatment to the untreated control (Tukey-Kramer; $P \leq 0.05$).

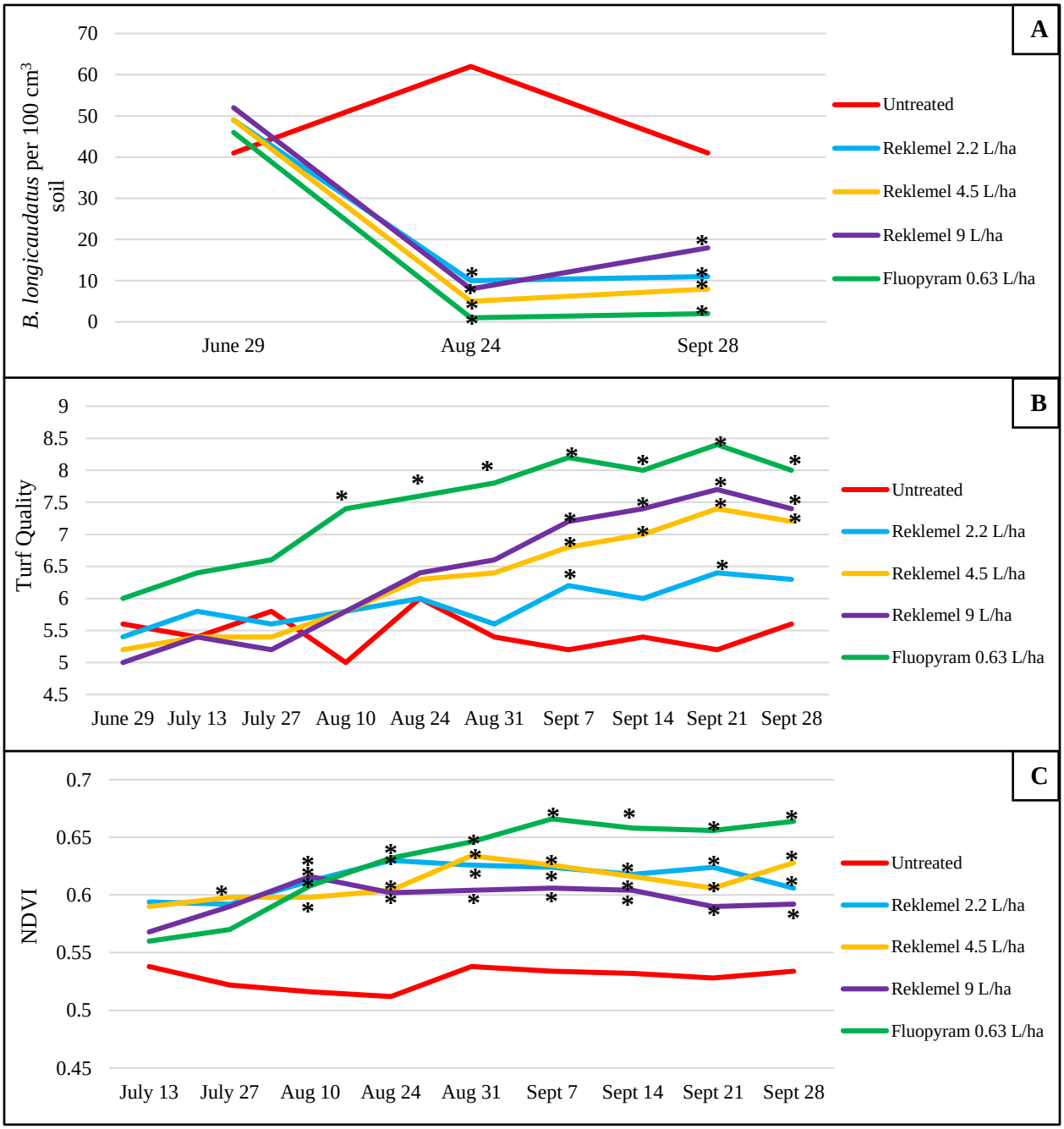

Figure 3.6. *Belonolaimus longicaudatus* population density (A), visual turfgrass quality (B), and NDVI values (C) as affected by nematicide treatments in 2018 bermudagrass microplot evaluations, Auburn, AL. Nematicide rates were applied as follows: Reklemel 2.2 L/ha at weeks 0, 4, 8, 12; 4.5 L/ha at weeks 0, 8; 9 L/ha at week 0; fluopyram 0.63 L/ha at weeks 0, 8. *Different from the untreated according to the pairwise comparison of each treatment to the untreated control (Tukey-Kramer; $P \leq 0.05$).

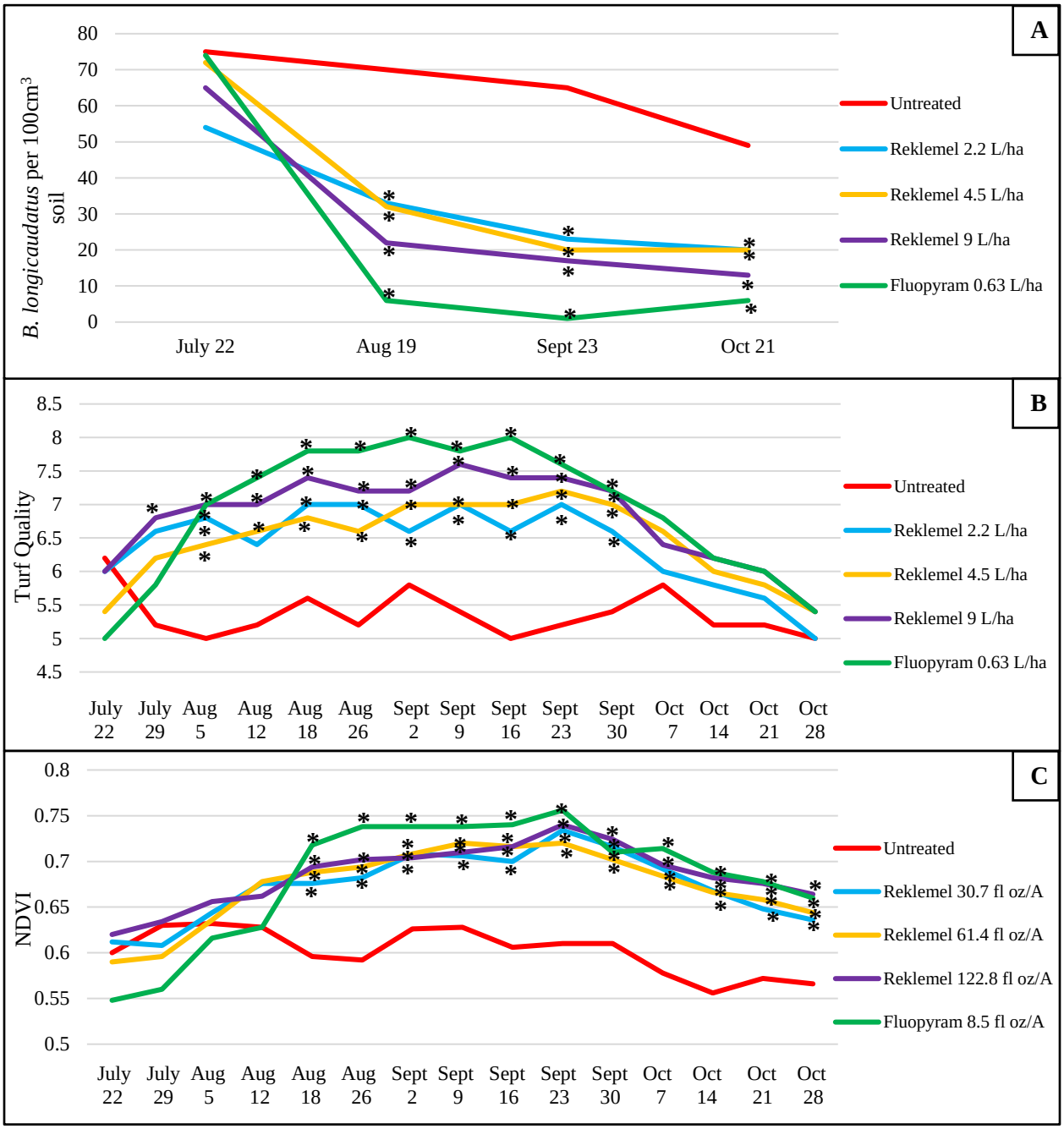

Figure 3.7. *Belonolaimus longicaudatus* population density (A), visual turfgrass quality (B), and NDVI values (C) as affected by nematicide treatments in 2019 bermudagrass microplot evaluations, Auburn, AL. Nematicide rates were applied as follows: Reklemel 2.2 L/ha at weeks 0, 4, 8, 12; 4.5 L/ha at weeks 0, 8; 9 L/ha at week 0; fluopyram 0.63 L/ha at weeks 0, 8. *Different from the untreated according to the pairwise comparison of each treatment to the untreated control (Tukey-Kramer; $P \leq 0.05$).

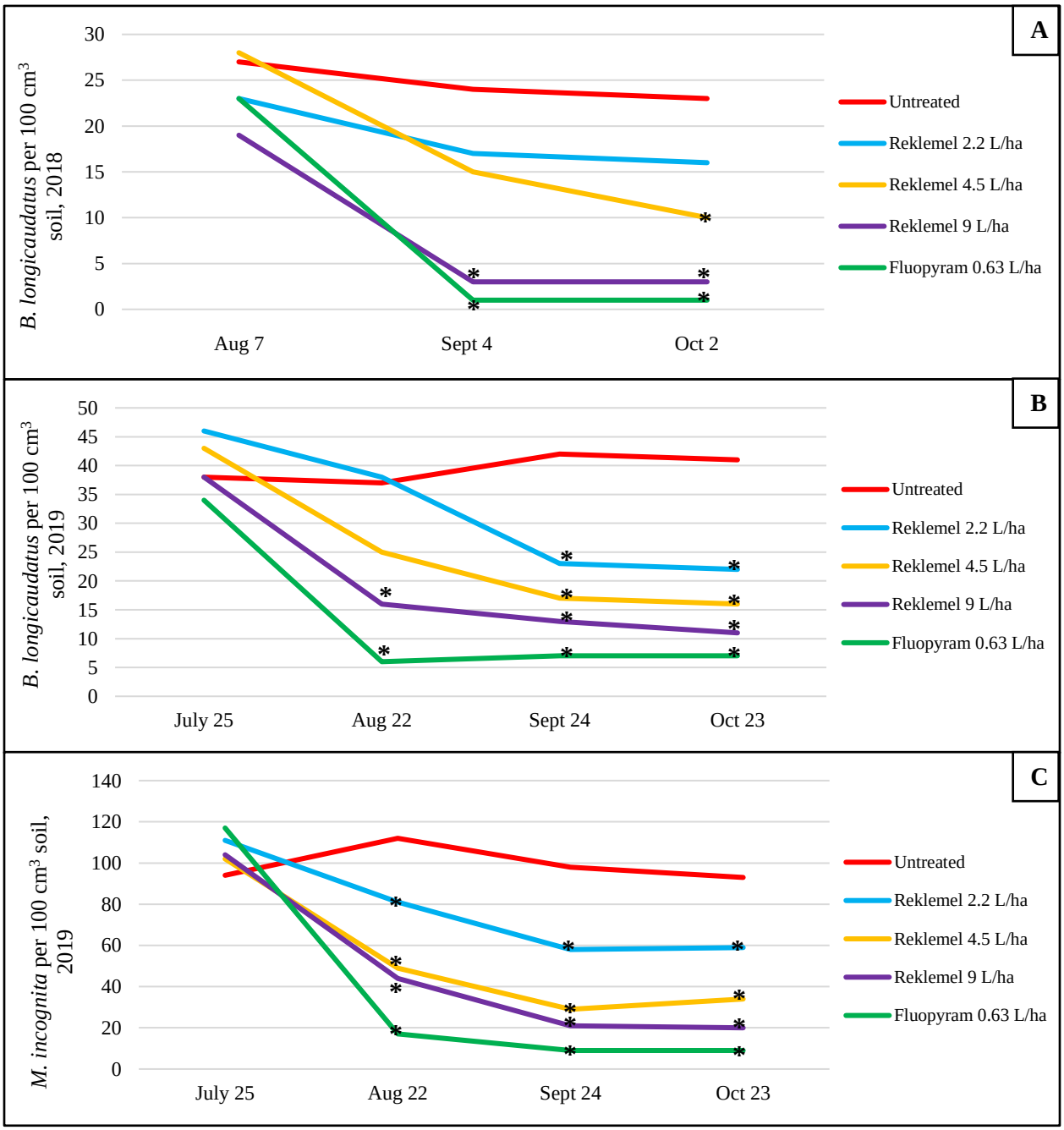

Figure 3.8. *Belonolaimus longicaudatus* population density in 2018 (A) and 2019 (B), and *Meloidogyne incognita* population density in 2019 (C) as affected by nematicide treatments in nematicide trials at Fairhope, AL. Nematicide rates were applied as follows: Reklemel 2.2 L/ha at weeks 0, 4, 8, 12; 4.5 L/ha at weeks 0, 8; 9 L/ha at week 0; fluopyram 0.63 L/ha at weeks 0, 8. *Different from the untreated according to the pairwise comparison of each treatment to the untreated control (Tukey-Kramer; $P \leq 0.05$).

Chapter IV: Plant growth-promoting rhizobacteria: a novel management strategy for *Meloidogyne incognita* on turfgrass

Abstract

Meloidogyne spp., root-knot nematodes, are among the most economically important plant-parasitic nematodes in turfgrass in the United States. Only a few nematicides are available or efficacious for plant-parasitic nematodes in turfgrass in the United States, and recent work has demonstrated the potential for microbial control of root-knot nematodes in field crops. The objectives of this study were to evaluate the efficacy of 104 plant growth-promoting rhizobacteria (PGPR) strains isolated from grasses in Alabama against *M. incognita in vitro,* and their ability to manage plant-parasitic nematodes in the greenhouse and microplot settings. *In vitro* mortality ranged from 0.9 to 94.6% mortality by PGPR strains screened. Ten individual PGPR strains and one three-strain blend (13 total PGPR strains) were advanced to greenhouse and microplot screening. In the greenhouse, six of the 11 PGPR treatments significantly reduced *M. incognita* population density, with a couple strains also promoting root growth. In the microplots, five of the 11 PGPR treatments significantly reduced *M. incognita* population density. Of these strains, 11 were identified as *Bacillus* spp., one as *Stenotrophomonas rhizophila*, and one as *Paenibacillus sonchi*. Eight of these strains were also found to have nitrogenase activity, and seven have the ability to produce siderophores, showing a potential mechanism for growth promotion. Overall, results indicate that multiple strains of *Bacillus* spp. and one strain of *S. rhizophila* have potential to reduce *M. incognita* population density and enhance turfgrass root growth.

Introduction

Plant-parasitic nematodes are a major pest of bermudagrass (*Cynodon* spp. L), a commonly grown warm-season turf and pasture grass in the southeastern United States (Luc et al. 2007). One of the most important genera of plant-parasitic nematodes on turfgrass in the southeast is *Meloidogyne* spp. (Root-knot nematode, RKN). *Meloidogyne* spp. reproduce at high levels in light, sandy soils, and population density declines in heavier soil types that have high percentages of silt and clay (Robinson et al. 1987; Starr et al. 1993). Since a majority of highly maintained turfgrass is built on a significant sand-based soil profile, RKN is commonly reported as a problem on turfgrass. RKN symptoms are often expressed as poorer turf quality, including overall plant decline and stunting, chlorosis, potential necrosis, and can predispose the turf to damage from other stressors such as extreme heat, drought, and nutrient deficiencies (Aryal et al. 2016). Current RKN management relies heavily on a limited number of chemical nematicides, and there is a great need for additional management tools for nematode suppression.

For decades, the organophosphate pesticide, fenamiphos, (Nemacur; Bayer CropScience, St. Louis, MO) was the standard for nematode management on turfgrass, but production halted in 2007 (Keigwin, 2014). Since fenamiphos's removal from the market, several other nematicides have shown efficacy for plant-parasitic nematodes. Abamectin (Divanem; Syngenta Crop Protection, Greensboro, NC), a nematicide made available on turf in the past decade, has shown effectiveness against plant-parasitic nematodes if applied at 2-4 week intervals (Crow, 2014b). Fluopyram (Indemnify; Bayer CropScience, Raleigh, NC), another chemical nematicide, entered the turfgrass market in 2016. Fluopyram has activity against a wide range of plant-parasitic nematodes. It also has a long soil half-life, ranging from six months to as long as two years, making it effective for long periods of time compared to most other chemical nematicides

available (Crow et al. 2017). Fluensulfone (Nimitz Pro G, Adama, Pasadena, Texas) became available for turf in 2017, and initial studies have shown effectiveness against plant-parasitic nematodes by applying 3-4 applications on a monthly basis (Crow et al. 2017). While each of these nematicides are strong products for plant-parasitic nematode management in turfgrass, relying on a limited number of chemical options for any pest management strategy is not ideal.

The need for additional options for integrated management of nematodes has led to the investigation of alternative strategies beyond chemical products. Biological control agents, specifically, plant growth-promoting rhizobacteria (PGPR), are a potential alternative option for use in addition to chemical nematicides. PGPR have the ability to promote plant growth, and are proven in some situations to elicit significant reductions in both severity and incidence of disease across a broad range of hosts (Kloepper et al. 2004). Multiple studies have shown the antagonistic ability of PGPR towards root-knot nematodes. A majority of these PGPR strains have been attributed to the genus *Bacillus*. Xiang et al. (2017) investigated the potential of 662 PGPR strains for antagonistic activity towards nematodes, and observed *Bacillus* spp. as the major genus capable of reducing *M. incognita* (Chitwood) population density on cotton (*Gossypium hirsutum* L.). Previous studies have also shown that specific strains of *B. subtilis* (Ehrenberg) Cohn can reduce *M. javanica* (Treub) infection on eggplant (*Solanum melongena* L.) (Abbasi et al. 2014). Other rhizobacteria such as *Streptomyces*, *Pseudomonas,* and *Pasteuria* also have antagonistic activity towards plant-parasitic nematodes (Xiang et al. 2018).

In turfgrass, plant and microbe interactions primarily refer to the relationship of fungi and cool-season grasses. There are over 80 examples of cool-season turfgrass cultivars that have these fungal endophytes incorporated into their growth and development (Meyer et al. 2013). Fungal endophytes can convey heat stress tolerance on tall fescue (*Festuca arundinacea* Schreb)

and perennial ryegrass (*Lolium perenne* L.) (Kane, 2011), in addition to drought stress tolerance for some cool-season grasses (Malinowski and Belesky, 2006). Bacterial endophytes such as *Pseudomonas* spp. can also provide suppression of gray leaf spot on perennial ryegrass and brown patch on creeping bentgrass (*Agrostis stolonifera* L.) (Viji et al. 2003; He et al. 2004). Historically, warm-season grasses are not known for their fungal endophyte relationships; however, there are multiple reported examples of bacterial endophyte relationships. Bacterial endophytes have been isolated from kallar grass (*Leptochola fusca* L.), saltmarsh grass (*Spartina alterniflora* Loisel), and switchgrass (*Panicum virgatum* L.) (McClung et al. 1983; Gagne-Bourgue et al. 2013; Reinhold-Hurek and Hurek, 1998).

More recently, Coy et al. (2019a) inoculated bermudagrass with multiple strains of *Bacillus* spp., and were able to reisolate these same strains up to 12 weeks after inoculation. This study also reported enhanced root growth by nitrogenase activity, phosphate solubilization, and siderophore production following inoculation with multiple *Bacillus* spp. *Bacillus firmus* (Bredemann and Werder) has also been studied for its antagonism towards plant-parasitic nematodes on warm season turfgrass, and a commercial formulation of *B. firmus* strain I-1582 (Nortica 5 WG; Bayer CropScience, St. Louis, MO) was developed. While this product has shown effectiveness for *Belonolaimus longicaudatus* (sting nematode, Rau) management on turfgrass, it is the only registered biological product currently used for nematode management (Crow, 2014a).

The purpose of this study was to evaluate PGPR strains for their biological control potential of root-knot nematode on bermudagrass. Specifically, the objectives were to assess PGPR potential for mortality of *M. incognita* J2 *in vitro* and to evaluate PGPR ability to reduce *M. incognita* population density and promote root growth on bermudagrass in greenhouse and

microplot settings. For the best performing strains, an additional objective was to determine their nitrogenase activity and ability to produce siderophores as mechanisms of growth promotion.

Materials and methods

PGPR strains

A total of 101 individual PGPR strains and 1 PGPR blend containing three *Bacillus* spp. were evaluated *in vitro*. The 101 strains were selected from a 600 strain library (DH library, Auburn University, Auburn, AL) isolated from various lawn, ornamental, and weedy grasses. These strains were selected based upon morphological characteristics that closely matched growth patterns on media to *Bacillus* spp. or a similar bacterial strain. Xiang et al. (2017; 2018) found that *Bacillus* spp. was the most successful bacteria for nematode suppression in their research, so focus was to find strains with similar efficacy. The PGPR blend evaluated consists of equal parts of three *Bacillus* strains – two strains (AP7 and AP18) of *B. pumilus* (Meyer and Gottheil), and one strain (AP282) of *B. sphaericus* (Meyer and Neide). These three strains were originally isolated and identified at Auburn University, Auburn, AL. This blend, known as 'Blend 20', was previously identified as a high performance blend with the ability to promote growth in bermudagrass (Coy, 2014). It was aptly included in this study as a control. All strains were stored in 30% glycerol at - 80°C. When ready for use, each strain was transferred to tryptic soy agar (TSA) plates and incubated at 28°C for 24-72 hours. Vegetative cells of each strain were then suspended in 5 mL of sterile distilled water in 15 mL plastic tubes, and the concentration was adjusted to 1 x 10^7 CFU/mL.

Nematode inoculum

Meloidogyne incognita race 3, originally isolated in 2016 from an infested field at the Plant Breeding Unit (PBU) at E.V. Smith Research Center of Auburn University was used as inoculum for the experiment (Groover et al. 2019). The *M. incognita* inoculum was increased on corn plants (Mycogen 2R042; Corteva Agriscience, Indianapolis, IN) in 500-cm^3 polystyrene pots in the greenhouse. To obtain the nematode inoculum, the eggs were extracted from the corn roots following a modified version of the methodology of Hussey and Barker (1973). The root mass was placed in a 0.625% sodium hypochlorite (NaOCl) solution and shaken for 4 minutes at 1 g-force on a Barnstead Lab Line Max Q 5000 E Class shaker (Conquer Scientific, San Diego, CA). Roots were then scrubbed by hand, and the eggs were collected on a 25-µm pore sieve and washed into a 50 mL centrifuge tube. The contents were centrifuged at 427 g-forces for 1 minute in a 1.14 specific gravity sucrose solution based on a modified Jenkins (1964) methodology. Eggs, now located in the supernatant of the sucrose solution, were recollected on a 25-µm pore sieve, rinsed with water to remove sucrose from eggs, and their presence confirmed via a Nikon TSX 100 inverted microscope at 40-x magnification. For *in vitro* experiments, *M. incognita* eggs were placed in a modified Baermann funnel (Castillo et al. 2013) on a slide warmer (Model 77; Marshall Scientific, Brentwood, NH) and incubated at 31°C for 5 to 7 days to obtain second-stage juveniles (J2). The J2 were collected on a 25-µm pore sieve, transferred to 1.5 mL micro-centrifuge tubes, centrifuged at 5,000 g-forces for 1 minute, rinsed with sterile distilled water, and centrifuged again at 5,000 g-forces for 1 minute. The J2 solution was adjusted to 30-40 J2 per 10 µL of water for *in vitro* assays (Xiang and Lawrence, 2016). For greenhouse and microplot experiments, eggs were diluted to inoculation levels of 2,000 eggs per 150cm^3 Cone-tainer and 50,000 eggs per microplot, respectively.

In vitro studies

In vitro evaluations were conducted to assess mortality of *M. incognita* J2 by PGPR strains. PGPR vegetative cell suspensions and *M. incognita* J2 inoculum were prepared as previously described. A 10 µL nematode suspension containing 30 to 40 *M. incognita* J2 was added to each well of a 100-µL, 96 well plate. Ninety µL of PGPR vegetative cell suspension was added into each test well of the plate. *Bacillus firmus* I-1582 (90 µL of cell suspension at 10 x 10^7 CFU/mL) (Nortica; Bayer CropScience) and fluopyram (90 µL of 500 PPM/mL) (Indemnify; Bayer CropScience) were used as biological and chemical controls, respectively. Sterile distilled water was included as a non-treatment control. Each plate was sealed with Parafilm (Bemis Company, Inc.; Neenah, WI) and incubated at room temperature (22 to 26°C) for 48 hours. Total live and dead *M. incognita* J2 were counted at experiment initiation and 48 hours after treatment exposure. Nematode viability was determined using the sodium hydroxide technique (Chen and Dickson, 2000). Mortality percentage of *M. incognita* J2 was calculated using the following equation as previously done by Xiang et al. (2017): [(live J2 prior to exposure – live J2 at 48 hour)/live J2 prior to exposure] x 100. PGPR strains and control treatments were replicated four times and the *in vitro* screening experiment was repeated once.

Candidate strain identification via 16S rDNA

Ten PGPR strains and one PGPR blend (Blend 20) were advanced from the *in vitro* screening for evaluation in the greenhouse and microplot for their ability to reduce *M. incognita* population density and promote turfgrass root growth. Each of these strains were taxonomically classified based upon the partial sequence of the 16S rDNA. Each strain was taken from storage at -80°C, streaked onto a TSA plate, and incubated at 28°C for 24-72 hours. Using an inoculating

loop, an individual bacterial colony was taken from the TSA plate culture and mixed with 20 µL of sterile distilled water in a 0.2 mL PCR tube. Each PCR tube with the bacterial strain sample was then placed in a MultiGene DNA thermal cycler (Labnet International, Edison, NJ) and incubated at 94°C until needed for PCR (~15 minutes). PCR amplification occurred in 50 mL reactions, with each reaction containing 17.8 µL of ddH_2O, 25 µL EconoTaq Plus Green 2x master mix (Lucigen Corp., Middleton, WI), 0.5 µL of each 100 µM forward and reverse primer, 0.2 RNase A, and 6 µL bacterial DNA template. Universal bacterial primers 8F (5'-AGAGTTTGATCCTGGCTCAG -3') and 1492R (5'-ACGGCTACCTTGTTACGACTT - 3') were used for amplification, and obtained from Invitrogen (ThermoFisher Scientific; Waltham, MA). PCR amplification consisted of initial denaturation at 95° C for 10 minutes, followed by 31 cycles of denaturation at 94° C for 1 minute, annealing at 57°C for 45 seconds, and extension at 70°C for 2 minutes, and a final extension at 70°C for 10 minutes. After amplification, samples were run on a 1.5% agarose gel stained with GelRed Nucleic Acid Stain (Biotium; Fremont, CA) and visualized on a midrange UV box. Four µL of PCR product was used for visualizing amplified gel patterns. Amplified products were then sent to Eurofins Genomics (Huntsville, AL) for sequencing. Sequence results were aligned using BioEdit Sequence Alignment Editor, and compared to previously published sequences in the Ribosomal Database Project (Michigan State University, East Lansing, MI) for species analysis. Isolation and amplification of each PGPR strain was replicated once to confirm accurate species identification via 16S rDNA.

Qualitative determination of nitrogenase activity

The nitrogenase activity of the ten PGPR strains and three strains in 'Blend 20' were determined using a nitrogen-free semisolid media (JNFb) to determine each strain's ability to exhibit nitrogen fixation as described by Döbereiner (1995). Each strain was grown in JNFb

medium, which contains, per liter, 5 g of malic acid, 0.6 ml of K_2HPO_4, 1.8 ml of KH_2PO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of NaCl, 0.2 g of $CaCl_2 \cdot H_2O$, 0.066 g of FeEDTA, 2.0 ml of bromothymol blue, 2.0 ml of micronutrients, 1.0 ml vitamin solution, 0.02 g of yeast extract, and 4.5 g of KOH (pH 5.8). The bromothymol blue solution contained 0.5 g bromothymol blue and 1.122 g KOH per 100 ml dH_2O. The micronutrient solution contained 0.04 g $CuSO_4 \cdot 5H_2O$, 0.012 g $ZnSO_4 \cdot 7H_2O$, 0.14 g H_2BO_3, 0.1 g $Na_2MoO_4 \cdot 2H_2O$, and 0.15 g $MnSO_4 \cdot H_2O$. The vitamin solution contained 0.01 g Biotin, and 0.02 g Pyridoxol-HCl in 100 ml dH_2O. After autoclaving, 7.0 ml of the JNFb media was dispensed into 10 mL sterile glass tubes. After allowing the media to reach room temperature, a single colony of bacteria was transferred into each tube. The tubes were capped and placed in an incubator at 28° C for 96 hours. The formation of a pellicle in the growth media indicated nitrogen fixation for each bacterium. Each bacterial inoculation on the media was replicated four times, and the test was repeated once.

Qualitative siderophore production

Siderophore production by each of the bacterial strains was also evaluated via Chrome azurol S (CAS) agar (Schwyn and Neilands, 1987; Lynne et al. 2011). Bacteria were grown on TSA at 28°C for 24-72 hours, followed by a transfer of a single bacterial colony to the CAS medium divided into four quadrants with a sterile inoculating loop. Each quadrant received a bacterial colony. The production of a yellow-orange halo around the growing bacterial colony confirmed siderophore production after a 72-hour incubation period at 28°C. The CAS agar was a mixture of four solutions prepared separately and sterilized before mixing – Fe-CAS indicator solution, buffer solution, sugar solution, and casamino acid solution. The Fe-CAS solution contained 10 mL of 1 mM $FeCl_3 \cdot 6H_2O$ (in 10 mM HCl), 50 ml of aqueous CAS solution (1.21 mg/ml); and 40 ml of aqueous hexadecyl-trimetylammonium bromide (HDTMA, 1.82 g/ml).

The buffer solution consisted of 750 ml of a salt solution, with 0.3 g KH_2PO_4, 0.5 g NaCl 1 g NH_4Cl, 30.24 g PIPES (peperazine-N, N'-bis [2-ethanesulfonic acid]), and 15 g agar. The sugar solution consisted of 2 g of glucose, 2 g of mannitol per 70 ml dH_2O. The casamino acid solution consisted of 30 ml filtered-sterilized 10% (W:V) casamino acid. The sugar solution, buffer solution, and casamino acid solution were all autoclaved, and then the sugar solution and acid solution were added to the buffer solution. The Fe-CAS solution was then added and stirred to ensure a thorough mixing of ingredients. This mixture yielded a blue media.

Greenhouse studies

The ten PGPR strains and Blend 20 were evaluated for their ability to reduce *M. incognita* population density and promote turfgrass root growth in the greenhouse. This experiment was conducted at the Plant Science Research Center (PSRC) located at Auburn University, Auburn, AL. Experiments were conducted in 150-cm^3 plastic Cone-tainer (Stuewe & Sons Inc., Tangent, OR) filled with 100% medium-coarse sand (0.25-1.0 mm). Cotton balls were placed in the bottom of the Cone-tainer to prevent sand from escaping the drainage holes. Each Cone-tainer was seeded with 2 grams of bermudagrass seed ('Princess 77', Pennington Seed, Inc., Madison, GA). Six weeks after germination and grass establishment, 1 mL of bacterial cell suspension (1 x 10^7 CFU/mL) was added to each Cone-tainer (6.7 x 10^4 CFU per cm^3 of sand). One mL of *Bacillus firmus* I-1582 (1 x 10^7 CFU/mL) and fluopyram (1µL of product mixed in 1 mL of water) were used as biological and chemical controls per Cone-tainer, respectively. One mL of tap water was included as a non-treated control. The experiment was arranged as a split-plot randomized complete block design (RCBD) with five replications and the entire test was repeated once. Plots consisted of two adjacent Cone-tainers, one inoculated with 1 mL of water containing 2,000 *M. incognita* eggs, and the other with no *M. incognita* inoculation.

Inoculation vs. no-inoculation with *M. incognita* in each Cone-tainer represented the whole plot of the split-plot design, and the subplot was the inoculation of individual PGPR treatments. Plants were watered as needed, and supplemental light of 1,000-watt halide bulbs producing 110,000 lumens was supplied to maintain a day length of 14 hours. Greenhouse temperature ranged from 21 to 35°C. Turfgrass shoot growth was clipped once a week to maintain a height of 2.5 cm.

Experiments were harvested at 60 days after inoculation of nematodes. Root and nematode samples were collected from each Cone-tainer. Roots were collected by removing the shoots, washed free of soil on an 853-µM pore sieve, and placed in a 50-mL plastic cup. Roots growth dynamics were evaluated using WinRhizo^{TM} root scanning equipment and software (Regent Instruments Inc., Ottawa, Canada). Root surface area, root volume, root length, and projected root area were recorded from the scanned images, and both root fresh weight and dry weight were recorded. Nematodes were extracted from the total soil in the Cone-tainer using the modified centrifugal flotation technique as previously described. The final nematode population density was determined under a Nikon TSX 100 inverted microscope at 40-x magnification.

Microplot studies

The same ten PGPR strains and Blend 20 evaluated in the greenhouse trials were also evaluated for their ability to reduce *M. incognita* population density and promote turfgrass root growth in microplots under natural environmental conditions. The experiments were conducted from May 22 to July 25, 2019 at the PSRC at Auburn University. For these trials, 26.5-liter plastic tree pots were used as microplots. Pots were nested one on top of the other with a brick in between to limit root growth by air pruning. The nested pot design was buried in the ground with

one inch of the pot above the soil surface. Microplots were then filled with 100% medium-coarse sand (0.25-1.0 mm). Experiments were arranged in a split-plot RCBD with five replications for each treatment and the entire test was repeated once. 'Tifway' hybrid bermudagrass sod was laid on top of the sand for each microplot and given 10 weeks for establishment. At the end of the 10-week period, *M. incognita* eggs were inoculated at a rate of 50,000 eggs per microplot on a weekly basis for 4 weeks to increase nematode population density in half of the microplots. After the 4-week inoculation period, a 100-cm^3 soil sample was taken from each plot to confirm *M. incognita* presence. Similar to the greenhouse trial, the whole plot was inoculation vs. no inoculation of *M. incognita*, and the subplot was the individual PGPR treatments. Five mL of bacterial suspension (1 x 10^7 CFU/mL) was applied to each microplot at the start of the trial via a small handheld spray bottle (1.9 x 10^6 CFU per L of sand). Five mL of *Bacillus firmus* I-1582 (1 x 10^7 CFU/mL) and fluopyram (11.5 mL/0.1 m^2) were again used as treatment controls. The non-treated control plots received a 5 mL application of tap water. Each microplot received water at 30 mL/min by an automated drip irrigation system adjusted throughout the season to run for 15 to 45 minutes twice a day every other day. Grass was trimmed on a weekly basis to a height of 2.5 cm. At 64 days after treatment, a 100-cm^3 soil sample was collected from each plot. For the *M. incognita* infested plots, nematodes were extracted from this sample as previously described and enumerated. Roots images and weights were collected as previously described using this sample for all microplots.

Statistical analysis

Data collected from *in vitro*, greenhouse, and microplot trials were analyzed using the PROC GLIMMIX procedure (SAS 9.4, SAS Institute, Cary, NC). Dependent variables included J2 mortality, root fresh weight (RFW), root dry weight (RDW), root length (RL), projected root

area (PRA), root surface area (RSA), root volume (RV), and total RKN. Fixed effects were RKN presence, PGPR strains or nematicide treatments, and random effects included replication, test repeat, and location. Student panels were generated to determine the normality of the residuals. For *in vitro* analysis, LS-means were compared between treatments, the water control, the chemical standard fluopyram, and the biological standard *B. firmus* I-1582 by Dunnett's ($P \leq 0.05$). In the greenhouse and microplot trials, LS-means were compared by Fisher's Protected Least Significance Difference ($P \leq 0.05$).

Results

Tests in vitro

Mortality of *M. incognita* J2 ranged from 0.9 (water) to 98.9% (fluopyram) among all treatments screened (Table 4.1). Of the screened PGPR strains, 18% had a significantly greater level of *M. incognita* J2 mortality than the non-treated control. Four strains (DH14, 40, 444, and 527) had a significantly similar level of mortality of *M. incognita* J2 as the chemical control, fluopyram. Compared to the biological control, *B. firmus* I-1582, 2.8% of the strains had a significantly higher level of *M. incognita* J2 mortality, and 65.7% had a significantly similar level of *M. incognita* J2 mortality (Table 4.1). A total of ten strains and Blend 20 (all with above 40% RKN mortality) were advanced to greenhouse and microplot screening (Figure 4.1). These strain isolates were taxonomically identified via 16S rDNA and assessed for nitrogenase activity and siderophore production.

Strain identification, nitrogenase activity, and siderophore production

Each of the PGPR strains advanced to greenhouse and microplot screening were identified to species level using 16S rDNA. Eight of the strains identified as *Bacillus* spp., one

identified as *Paenibacillus sonchi*, and one identified as *Stenotrophomonas rhizophila* (Table 4.2). Six of the PGPR strains inoculated in the liquid JNFb media grew after 72 hours, indicating nitrogen fixation capabilities. Strains DH30, 40, 44, 57, 267, and 527 grew in the JNFb media, and strains DH14, 140, 444, and 580 did not (Table 4.2). Strains AP7 and AP18 also had nitrification activity while AP 282 did not, as previously reported by Coy et al. (2019a). Five of the PGPR strains (DH14, 30, 267, 444, and 580) inoculated on the CAS-agar produced a yellow halo surrounding the bacterial colony after 72 hours, indicating siderophore production. Strains AP7 and AP18 also produced siderophores while AP 282 did not, as previously reported by Coy et al. (2019a). Siderophore production by bacteria can lead to binding of iron in the plant and enhance plant growth (Verma et al. 2011).

Tests in the greenhouse

The inoculation of *M. incognita* compared to no inoculation in the greenhouse was significant on all root parameters evaluated except for root dry weight (Table 4.3). Root dry weight in the non-inoculated plots averaged 0.222 ± 0.01 grams, and the root dry weight in the RKN inoculated plots averaged 0.245 ± 0.01 grams**.**

In the absence of nematodes, significant differences were observed for root fresh weight, root dry weight, projected root area, and root surface area (Table 4.4). Root length ranged on average from 207.10 ± 61.59 to 346.28 ± 54.29 cm. Average root volume ranged from 1.59 ± 0.15 to 2.23 ± 0.25 cm^3. There was no significant difference between PGPR treatments for root length or root volume. Blend 20, DH527, and fluopyram had significantly greater root fresh weight and dry weight compared to the non-treated control. The projected root area of bermudagrass treated with either DH40 or DH527 was significantly greater than the non-treated

control. Both DH30 and DH527 had significantly more surface area compared to the non-treated control.

Among bermudagrass inoculated with *M. incognita* (Table 4.5), root fresh weight and root length were similar among all treatments. Root fresh weight (grams) ranged from an average of 1.82 ± 0.44 to 2.81 ± 0.63, and root length (cm) ranged from 83.50 ± 14.85 to 128.19 ± 30.38. Bermudagrass treated with either DH44 or DH267 significantly increased root dry weight compared to the non-treated control. Both projected root area and root surface area of bermudagrass were significantly higher with DH140 or *B. firmus* I-1582 compared to the non-treated control. For root volume, DH267 had the largest total root volume, with all strains significantly larger than the non-treatment control. Six of PGPR treatments (DH14, 30, 40, 57, 140, and 444) evaluated in the trial significantly reduced total *M. incognita* population density compared to the non-treated water control. Both fluopyram and *B. firmus* I-1582 significantly lowered *M. incognita* population density compared to the non-treated control.

Tests in the microplots

Inoculation of *M. incognita* in the microplots had a significant impact on all root parameters except for root length (Table 4.3). Root length (cm) in the non-inoculated plots averaged 309.91 ± 15.17, and the inoculated plots averaged 331.37 ± 13.88**.**

Significant differences were observed in the microplots among PGPR treatments in the absence of *M. incognita* (Table 4.6). However, there were no significant differences among root fresh weights, which ranged from an average of 12.44 ± 3.95 to 23.36 ± 8.67. While all PGPR strains numerically increased root dry weight, only strain DH44 significantly increased root dry weight relative to the non-treated control. DH267 supported the greatest root length in the study,

and while it was similar to the water control, it was significantly higher than fluopyram, DH30, 57, 527, and 580. Bermudagrass treated with DH267, again, had the largest projected root area, and significantly increased projected root area compared to DH580 and fluopyram. *Bacillus firmus* I-1582 and DH267 had the highest root surface area, significantly increasing surface area compared to DH580 and fluopyram. *Bacillus firmus* I-1582 also had the largest root volume, and was significantly larger than DH140, 580, fluopyram and the water control.

In the *M. incognita* inoculated microplots, significant differences among PGPR strains were documented for all data parameters (Table 4.7). Fluopyram had the highest root fresh weight and root dry weight, with both parameters significantly higher than six evaluated PGPR strains. Both DH267 and the water control had the greatest observed root length, and were significantly greater than Blend 20. Fluopyram also had the largest projected root area, and was significantly higher than six PGPR strains. Fluopyram also had the largest root volume, but was only significantly greater than one other strain, DH527.

All PGPR strains had numerically lower *M. incognita* population density compared to the water control, with five (DH30, 444, 527, 580, and Blend 20) of the PGPR treatments, fluopyram, and *B. firmus* I-1582 significantly lowering population density. Fluopyram had the lowest numerical *M. incognita* population density observed, and seven (DH30, 44, 140, 444, 527, 580, and Blend 20) of the evaluated PGPR treatments were significantly similar to fluopyram.

Discussion

Among all PGPR strains evaluated, 18% had a significantly higher level of mortality of *M. incognita* J2 than the non-treated control. While only 4% of evaluated PGPR strains had a statistically similar level of mortality compared to the chemical control fluopyram, 66% of these

strains caused a statistically similar mortality when compared to the biological control, *B. firmus* I-1582. *Bacillus* was the primary genus causing mortality, with 11 of the total 13 PGPR strains used for greenhouse and microplot testing belonging to the genus. The additional testing beyond *in vitro* confirmed that some of these PGPR strains not only suppressed population density of *M. incognita* in both greenhouse and microplot systems, but have nitrogenase activity and siderophore production as well. Bacteria that possess the ability to fix nitrogen or produce siderophores have the potential to influence the plant by promoting root growth or assisting with nutrient uptake (Coy et al. 2019a; Verma et al. 2011; Day et al. 1975). Specific benefits may also be linked to an increased chlorophyll content, bioremediation, and disease suppression (Coy et al. 2019a; Calvo et al. 2014). Over half of the strains evaluated either had nitrogenase activity and/or siderophore production, and demonstrates a potential for further evaluation of these PGPR strains for biofertilization of bermudagrass and possibly other grasses.

The *in vitro* screening indicates that *Bacillus* spp. can cause high levels of mortality to *M. incognita* J2. This confirms previous reports of *Bacillus* spp. antagonism on various plant-parasitic nematodes across multiple host plants. Xiang et al. (2017; 2018) evaluated 662 PGPR strains for mortality of both *M. incognita* and *Heterodera glycines in vitro,* and found a wide range of nematode mortality, with the highest mortality rates also caused by *Bacillus* spp. These studies also found the ability of top performing *in vitro* PGPR strains to reduce *M. incognita* and *H. glycines* nematode population density in cotton and soybean greenhouse, microplot, and field trials, respectively. Similar to this study, they found multiple strains of *B. subtilis, B. aryabhattai, B. simplex,* and *B. pumilus* with strong antagonistic activity towards *M. incognita*. Kloepper et al. (1992) also previously reported that strains of *B. megaterium* and *B. pumilus*

significantly reduced *M. incognita* galling on soybean, and Siddiqui et al. (2001) reported a strain of *B. subtilis* demonstrated antagonistic activity on *M. javanica* in mungbean.

Beyond *Bacillus* spp., this study also found two additional strains, identified as *Paenibacillus sonchi* and *Stenotrophomonas rhizophila,* with *M. incognita* antagonism potential. Khan et al. (2012) reported antagonistic activity of *Paenibacillus polymyxa* against *M. incognita* in tomato, and Son et al. (2009) reported suppression of the root-knot nematode and Fusarium wilt disease complex by *P. polymyxa* and *P. lentimorbus*. However, to our knowledge, this is the first report of antagonistic activity to *M. incognita* by a strain of *P. sonchi*. Li et al. (2014) found multiple strains of *S. rhizophila* with similar mortality *in vitro* studies, ranging from 56 to 83% mortality against *M. incognita*. Multiple species of *Stenotrophomonas,* including *S. rhizophila*, have also been reported to inhibit *Rhizoctonia solani* (Kai et al. 2009).

Results from both greenhouse and microplot studies confirm the antagonism towards *M. incognita* observed by multiple PGPR strains *in vitro*. Strains DH14, 30, 40, 57, 140, and 444 all significantly lowered *M. incognita* population density in the greenhouse compared to the non-treated control. Strains DH30, 444, 527, 580, and Blend 20 significantly lowered *M. incognita* population density in the microplots compared to the non-treated control. Between these two trials, DH30 and 444 were significant in both, showing a consistent antagonism of *M. incognita.* Also of note in both trials, all but one treatment, DH267, were statistically similar to the biological control, Nortica (*B. subtilis* I-1582). Crow (2014a) found that *B. firmus* I-1582 could be an effective tool for *Belonolaimus longicaudatus* management on golf course bermudagrass. Primarily, the results of that study showed early season preventative applications led to the most success for nematode management, making it a good tool for nematode IPM programs in

turfgrass. These results indicate that the strains evaluated in this research may have similar potential for root-knot nematode management in turfgrass.

In both greenhouse and microplot evaluations, PGPR effects on growth promotion were significantly impacted by the infection of *M. incognita.* In the greenhouse, RKN infection led to an increase in both root weight and volume, but a reduction in parameters related to root architecture (root length, projected root area, and root surface area). In the microplot experiment, the inoculation of *M. incognita* led to an increase in all root parameters measured except for root length. Coy et al. (2019b) noted that similar applications of PGPR treatments led to a significant increase in turfgrass root weight as a response to white grub feeding. One striking example of this potential microbe effect occurring can be seen with DH267. This strain had average root growth promotion in the non-inoculated greenhouse trial compared to other treatments, but had significantly higher root dry weight and root volume compared to other treatments when inoculated with *M. incognita*. We do not have a proposed mechanism for this result, but Pineda et al. (2013) suggests the effects of inoculated microbes may be strengthened under either abiotic or biotic stresses.

In the growth promotion experiments, Blend 20 had the greatest root weight in the greenhouse, and DH44 had the greatest root weight in the microplot. This confirms previous reports of growth promotion with the addition of Blend 20 by Coy et al. (2014). Hong et al. (2009) also reported the ability of *P. sonchi* to provide root growth promotion. However, there were differences observed in the *M. incognita* infested trials. Growth promotion by PGPR strains was not as evident where *M. incognita* was present. In the greenhouse trial, the only PGPR strain with a significant growth promotion benefit that also significantly lowered *M. incognita* population density was DH140. Projected root area and root surface area were significantly

higher with the addition of DH140 compared to the water control. None of the PGPR treatments that significantly lowered *M. incognita* population density in the microplots showed significant root growth promotion compared to the water control. Despite these results, the reduction in *M. incognita* population density is a strong indicator that these PGPR strains are promising for a turfgrass nematode IPM program.

In summary, DH14 (*B. megaterium*), DH30 (*S. rhizophila*), DH40 (*Bacillus pumilus*), DH57 (*B. subtilis*), DH140 (*B. subtilis*), DH444 (*B. aryabhattai*), DH527 (*B. simplex*), DH580 (*B. aryabhattai*), and Blend 20 (*B. pumilus*, *B. pumilus*, *B. sphaericus*) are promising biological control agents for plant-parasitic nematode management. Further evaluations - primarily field trials – are needed to investigate the potential for these PGPR strains as a tool in IPM for plant-parasitic nematode management in turfgrass.

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Table 4.1: Effect of select plant growth-promoting rhizobacteria strains on *Meloidogyne incognita* J2 mortality percentage compared to a water, chemical, and biological control in laboratory trials[†].

		Dunnett's *P* versus[§] (*P* ≤ 0.05)		
Code	***Meloidogyne incognita* J2 mortality (%)[‡]**	**Water[#]**	**Fluopyram**	***B. firmus***
DH3	6.5	1.0000	<0.0001	0.0067
DH4	16.8	0.9150	<0.0001	0.1877
DH5	8.1	1.0000	<0.0001	0.0124
DH6	14.6	0.9901	<0.0001	0.1048
DH7	4.4	1.0000	<0.0001	0.0028
DH8	12.9	0.9994	<0.0001	0.0648
DH9	8.0	1.0000	<0.0001	0.0119
DH10	5.6	1.0000	<0.0001	0.0046
DH11	3.9	1.0000	<0.0001	0.0023
DH12	6.8	1.0000	<0.0001	0.0074
DH13	27.4	0.1393	<0.0001	0.9666
DH14	75.4	<0.0001	0.2904	0.0171
DH15	11.1	1.0000	<0.0001	0.0358
DH16	30.0	0.0659	<0.0001	0.9993
DH17	3.8	1.0000	<0.0001	0.0022
DH18	18.0	0.8301	<0.0001	0.2503
DH19	15.2	0.9786	<0.0001	0.1247
DH20	17.1	0.8955	<0.0001	0.2028
DH23	11.6	1.0000	<0.0001	0.0424
DH25	4.7	1.0000	<0.0001	0.0032
DH27	5.5	1.0000	<0.0001	0.0044
DH28	2.4	1.0000	<0.0001	0.0012
DH29	8.5	1.0000	<0.0001	0.0142
DH30	65.3	<0.0001	0.0151	0.3132
DH31	8.6	1.0000	<0.0001	0.0149
DH32	18.1	0.8223	<0.0001	0.2559
DH33	2.9	1.0000	<0.0001	0.0015
DH34	21.8	0.4790	<0.0001	0.5407
DH35	5.6	1.0000	<0.0001	0.0046
DH36	29.1	0.0863	<0.0001	0.9962
DH37	26.0	0.1978	<0.0001	0.9011
DH38	37.1	0.0068	<0.0001	1.0000
DH39	19.9	0.6602	<0.0001	0.3755
DH40	78.2	<0.0001	0.5131	0.0056
DH41	16.9	0.9087	<0.0001	0.1926
DH42	20.3	0.6187	<0.0001	0.4095
DH43	11.0	1.0000	<0.0001	0.0353

DH44	49.6	<0.0001	<0.0001	1.0000
DH45	18.2	0.8131	<0.0001	0.2624
DH46	15.5	0.9724	<0.0001	0.1330
DH47	5.9	1.0000	<0.0001	0.0053
DH48	4.9	1.0000	<0.0001	0.0034
DH49	12.3	0.9999	<0.0001	0.0525
DH50	12.1	0.9999	<0.0001	0.0498
DH57	62.7	<0.0001	0.0054	0.5246
DH79	22.3	0.4410	<0.0001	0.5820
DH86	26.0	0.3020	<0.0001	0.9619
DH89	20.1	0.6363	<0.0001	0.3949
DH98	21.6	0.5029	<0.0001	0.5161
DH113	17.5	0.8689	<0.0001	0.2225
DH132	31.3	0.0429	<0.0001	1.0000
DH134	39.0	0.0023	<0.0001	1.0000
DH135	20.7	0.5810	<0.0001	0.4421
DH140	51.8	<0.0001	<0.0001	1.0000
DH146	22.2	0.4517	<0.0001	0.5701
DH176	9.9	1.0000	<0.0001	0.0240
DH178	14.2	0.9942	<0.0001	0.0940
DH192	16.6	0.9251	<0.0001	0.1794
DH213	17.7	0.8556	<0.0001	0.2321
DH251	27.1	0.1472	<0.0001	0.9593
DH265	17.2	0.8917	<0.0001	0.2057
DH267	67.4	<0.0001	0.0317	0.1931
DH295	38.5	0.0028	<0.0001	1.0000
DH296	36.3	0.0070	<0.0001	1.0000
DH301	26.1	0.1933	<0.0001	0.9069
DH310	25.4	0.2280	<0.0001	0.8603
DH313	10.0	1.0000	<0.0001	0.0244
DH317	19.6	0.6840	<0.0001	0.3569
DH386	5.6	1.0000	<0.0001	0.0045
DH400	9.8	1.0000	<0.0001	0.0227
DH404	17.0	0.8988	<0.0001	0.2003
DH419	16.0	0.9555	<0.0001	0.1519
DH420	16.5	0.9326	<0.0001	0.1731
DH426	36.0	0.0082	<0.0001	1.0000
DH434	9.1	1.0000	<0.0001	0.0181
DH439	37.7	0.0040	<0.0001	1.0000
DH444	77.8	<0.0001	0.4790	0.0066
DH447	10.1	1.0000	<0.0001	0.0253
DH455	22.4	0.4298	<0.0001	0.5947
DH462	33.2	0.0229	<0.0001	1.0000

DH466	14.8	0.9867	<0.0001	0.1115
DH490	23.0	0.3840	<0.0001	0.6492
DH500	29.5	0.0760	<0.0001	0.9982
DH503	13.9	0.9965	<0.0001	0.0857
DH507	18.8	0.7617	<0.0001	0.2990
DH511	14.5	0.9914	<0.0001	0.1016
DH518	20.2	0.6254	<0.0001	0.4039
DH522	22.3	0.4423	<0.0001	0.5805
DH527	94.6	<0.0001	1.0000	<0.0001
DH542	9.5	1.0000	<0.0001	0.0209
DH545	17.3	0.8801	<0.0001	0.2143
DH552	15.7	0.9642	<0.0001	0.1427
DH570	23.4	0.3539	<0.0001	0.6873
DH573	11.1	1.0000	<0.0001	0.0359
DH574	20.7	0.5849	<0.0001	0.4386
DH580	61.5	<0.0001	0.0034	0.6292
DH582	17.7	0.8499	<0.0001	0.2362
DH588	25.5	0.2226	<0.0001	0.8677
DH591	20.3	0.6232	<0.0001	0.4057
DH593	13.1	0.9992	<0.0001	0.0679
DH594	12.5	0.9998	<0.0001	0.0562
DH598	24.8	0.2611	<0.0001	0.8139
Blend 20	56.3	<0.0001	0.0003	0.9813
Control				
Fluopyram	98.9	<0.0001	-	<0.0001
Water	0.9	-	<0.0001	0.0006
Bacillus firmus I-1582	41.6	0.0006	<0.0001	-

[†]*In vitro* tests were performed in 96-well plates. Data of 101 PGPR strains and 1 blend (Blend 20) mortality ranging from 0 to 95% are presented in the table. All PGPR strains and controls had 4 replications. Data were analyzed in SAS 9.4 using PROC Glimmix procedure at significant level of $\alpha \leq 0.05$. *P* values less than 0.05 indicate a significant effect. LS-means and adjusted *P values* are presented in the table.

[‡]Mortality percentage was determined using the following equation: [(live J2 prior to exposure – live J2 at 48 hours) / live J2 prior to exposure] x 100. As defined by Xiang *et al*. 2017.

[§]Dunnett's option was used in the LS-means statement to assess the differences between bacterial strains and the Indemnify, Nortica, and water control.

Table 4.2. List of PGPR strains advanced to greenhouse and microplot evaluation for turfgrass root-knot nematode management.

Code	Scientific Name[†]	Nitrogenase Activity	Siderophore Production
DH14	*Bacillus megaterium*	No	Yes
DH30	*Stenotrophomonas rhizophila*	Yes	Yes
DH40	*Bacillus pumilus*	Yes	No
DH44	*Paenibacillus sonchi*	Yes	No
DH57	*Bacillus subtilis*	Yes	No
DH140	*Bacillus subtilis*	No	No
DH267	*Bacillus subtilis*	Yes	Yes
DH444	*Bacillus aryabhattai*	No	Yes
DH527	*Bacillus simplex*	Yes	No
DH580	*Bacillus aryabhattai*	No	Yes
Blend 20			
AP7	*Bacillus pumilus*	Yes	Yes
AP18	*Bacillus pumilus*	Yes	Yes
AP282	*Bacillus sphaericus*	No	No

[†]Taxonomic identification was based upon a partial sequence of the 16S rDNA of each PGPR strain. Each partial sequence was blasted against the type strain in the Ribosomal Database Project for identification.

Table 4.3. Split-plot analysis (Mean ± SEM) of PGPR treated greenhouse and microplot bermudagrass inoculated with *Meloidogyne incognita* compared to non-inoculated bermudagrass[†].

Greenhouse	**RFW**[‡]	**RDW**[§]	**RL**[¶]	**RA**[††]	**SA**[‡‡]	**RV**[§§]
Non-inoculated	0.907 ± 0.03b	0.222 ± 0.01	278.02 ± 19.11a	24.38 ± 0.86a	74.05 ± 2.38a	1.94 ± 0.09b
Inoculated	2.357 ± 0.17a	0.245 ± 0.01	104.24 ± 6.72b	19.56 ± 0.48b	61.13 ± 1.55b	3.65 ± 0.30a
P-value	< 0.0001	0.0548	<0.0001	<0.0001	<0.0001	<0.0001
Microplot	**RFW**	**RDW**	**RL**	**RA**	**SA**	**RV**
Non-inoculated	17.81 ± 1.23b	7.64 ± 0.62b	309.91 ± 15.17a	59.09 ± 2.64b	186.11 ± 8.23b	10.02 ± 0.81b
Inoculated	27.53 ± 1.42a	9.65 ± 0.71a	331.37 ± 13.88a	86.18 ± 2.95a	270.62 ± 9.29a	22.27 ± 2.48a
P-value	<0.0001	0.0335	0.2990	<0.0001	<0.0001	<0.0001

[†]Greenhouse and microplot trials 60 and 64 days after treatment (DAT), respectively. Means within a column followed by the same letter are not significantly different (Fisher's LSD, $P \leq 0.05$).
[‡]RFW = Root fresh weight (grams)
[§]RDW = Root dry weight (grams)
[¶]RL = Root length (centimeters)
[††]RA = Projected root area (centimeters^2)
[‡‡]SA = Root surface area (centimeters^2)
[§§]RV = Root volume (centimeters^3)

Table 4.4: Evaluation of 10 PGPR strains and 1 PGPR blend (Mean ± SEM) on bermudagrass root architecture under greenhouse conditions at 60 DAT.[†]

Code	Scientific Name	RFW[‡]	RDW[§]	RA[¶]	SA[††]
DH14	*Bacillus megaterium*	0.89 ± 0.20ab	0.24 ± 0.05abc	23.38 ± 2.49bc	65.14 ± 12.78abc
DH30	*Stenotrophomonas rhizophila*	0.88 ± 0.13ab	0.22 ± 0.04abc	25.66 ± 1.89abc	80.60 ± 5.97ab
DH40	*Bacillus pumilus*	0.92 ± 0.09ab	0.20 ± 0.04abc	30.54 ± 3.45a	71.61 ± 13.62abc
DH44	*Paenibacillus sonchi*	0.85 ± 0.14ab	0.20 ± 0.03abc	22.96 ± 3.12bc	69.14 ± 10.95abc
DH57	*Bacillus subtilis*	0.94 ± 0.25ab	0.23 ± 0.06abc	22.56 ± 3.74bc	70.88 ± 11.76abc
DH140	*Bacillus subtilis*	0.83 ± 0.12ab	0.23 ± 0.05abc	22.97 ± 2.81bc	72.16 ± 8.83abc
DH267	*Bacillus subtilis*	0.97 ± 0.11ab	0.23 ± 0.02abc	24.91 ± 1.98abc	78.24 ± 6.22abc
DH444	*Bacillus aryabhattai*	0.80 ± 0.12ab	0.19 ± 0.03abc	24.19 ± 2.38abc	76.00 ± 7.45abc
DH527	*Bacillus simplex*	0.99 ± 0.12a	0.25 ± 0.03ab	27.17 ± 2.20ab	85.36 ± 6.89a
DH580	*Bacillus aryabhattai*	0.90 ± 0.08ab	0.21 ± 0.02abc	22.56 ± 1.88bc	70.88 ± 5.91abc
Blend 20[‡‡]		1.04 ± 0.15a	0.27 ± 0.03a	25.06 ± 2.21abc	78.71 ± 6.91abc
Control	**Active Ingredient**				
Non-treated	Water	0.73 ± 0.07b	0.18 ± 0.02c	20.00 ± 1.58c	62.82 ± 4.98c
Nortica	*Bacillus firmus* I-1582	0.94 ± 0.14ab	0.19 ± 0.03bc	25.47 ± 3.48abc	79.99 ± 10.95abc
Indemnify	Fluopyram	1.00 ± 0.12a	0.25 ± 0.04ab	23.89 ± 2.29bc	75.00 ± 7.19abc

[†]Greenhouse trials 60 days after treatment (DAT). Means within a column followed by the same letter are not significantly different (Fisher's LSD, $P \leq 0.05$).
[‡]RFW = Root fresh weight (grams)
[§]RDW = Root dry weight (grams)
[¶]RA = Projected root area (centimeters^2)
[††]SA = Root surface area (centimeters^2)
[‡‡]Blend 20 contains equal parts strains AP7 (*B. pumilus*), AP18 (*B. pumilus*) and AP282 (*B. sphaericus*).

Table 4.5: Evaluation of 10 PGPR strains and 1 PGPR blend (Mean ± SEM) on bermudagrass root architecture when infested with *Meloidogyne incognita* under greenhouse conditions at 60 DAT. †

Code	Scientific Name	RDW‡	RA§	SA¶	RV††	RKN‡‡
DH14	*Bacillus megaterium*	0.21 ± 0.03ab	18.61 ± 1.55abc	58.52 ± 4.88abc	3.64 ± 0.58ab	234 ± 123c
DH30	*Stenotrophomonas rhizophila*	0.25 ± 0.05ab	19.34 ± 1.24abc	56.32 ± 6.77bc	4.28 ± 1.54ab	188 ± 69c
DH40	*Bacillus pumilus*	0.23 ± 0.07ab	19.73 ± 1.79abc	61.98 ± 5.62abc	3.28 ± 1.18ab	129 ± 29c
DH44	*Paenibacillus sonchi*	0.31 ± 0.07a	20.11 ± 1.87abc	63.17 ± 5.86abc	3.91 ± 1.09ab	341 ± 188abc
DH57	*Bacillus subtilis*	0.27 ± 0.07ab	19.09 ± 2.32abc	59.99 ± 7.29abc	3.15 ± 0.54ab	229 ± 44c
DH140	*Bacillus subtilis*	0.26 ± 0.04ab	21.28 ± 1.71ab	66.87 ± 5.36ab	3.73 ± 0.67ab	193 ± 70c
DH267	*Bacillus subtilis*	0.29 ± 0.07a	20.33 ± 2.28abc	63.88 ± 7.14abc	5.33 ± 2.93a	639 ± 495a
DH444	*Bacillus aryabhattai*	0.22 ± 0.03ab	18.45 ± 1.35bc	57.98 ± 4.23abc	3.11 ± 0.39ab	179 ± 62c
DH527	*Bacillus simplex*	0.22 ± 0.03ab	20.47 ± 1.79abc	64.30 ± 5.61abc	3.14 ± 0.62ab	355 ± 228abc
DH580	*Bacillus aryabhattai*	0.26 ± 0.06ab	19.18 ± 2.12abc	60.27 ± 6.66abc	3.39 ± 0.73ab	251 ± 93bc
Blend 20§§		0.24 ± 0.04ab	18.93 ± 1.93abc	59.48 ± 6.08abc	3.96 ± 0.97ab	332 ± 99abc
Control	**Active Ingredient**					
Non-treated	Water	0.18 ± 0.03b	17.40 ± 1.82c	54.65 ± 5.73c	2.86 ± 0.5b	567 ± 171ab
Nortica	*Bacillus firmus* I-1582	0.26 ± 0.05ab	21.96 ± 1.55a	69.00 ± 4.88a	3.66 ± 0.58ab	197 ± 56c
Indemnify	Fluopyram	0.22 ± 0.04ab	18.88 ± 1.47abc	59.37 ± 4.63abc	3.70 ± 0.99ab	80 ± 11c

†Greenhouse trials 60 days after treatment (DAT). Means within a column followed by the same letter are not significantly different (Fisher's LSD, $P \leq 0.05$).
‡RDW = Root dry weight (grams).
§RA = Projected root area (centimeters^2).
¶SA = Root surface area (centimeters^2).
††RV = Root volume (centimeters^3).
‡‡RKN = Root-knot nematodes per 100cm^3 of soil at 60 DAT.
§§Blend 20 contains equal parts strains AP7 (*B. pumilus*), AP18 (*B. pumilus*) and AP282 (*B. sphaericus*).

Table 4.6: Evaluation of 10 PGPR strains and 1 PGPR blend (Mean ± SEM) on bermudagrass root architecture in microplots at 60 DAT. [†]

Code	Scientific Name	RDW[‡]	RL[§]	RA[¶]	SA[††]	RV[‡‡]
DH14	*Bacillus megaterium*	7.05 ± 3.26ab	363.38 ± 43.47ab	62.66 ± 11.52ab	196.86 ± 36.18ab	9.02 ± 2.76ab
DH30	*Stenotrophomonas rhizophila*	7.60 ± 2.63ab	239.71 ± 38.70bc	56.35 ± 7.88ab	177.02 ± 24.74ab	10.67 ± 1.53ab
DH40	*Bacillus pumilus*	7.75 ± 2.15ab	309.24 ± 8.27abc	60.22 ± 7.55ab	189.20 ± 23.79ab	9.78 ± 1.86ab
DH44	*Paenibacillus sonchi*	11.85 ± 3.08a	291.27 ± 74.58abc	61.25 ± 10.65ab	192.44 ± 33.46ab	11.36 ± 3.05ab
DH57	*Bacillus subtilis*	10.13 ± 3.08ab	232.32 ± 33.72bc	55.02 ± 9.87ab	172.86 ± 30.99ab	10.35 ± 2.17ab
DH140	*Bacillus subtilis*	6.03 ± 2.08ab	347.27 ± 48.97abc	53.82 ± 10.28ab	169.07 ± 32.29ab	7.97 ± 2.65b
DH267	*Bacillus subtilis*	7.88 ± 1.52ab	436.81 ± 72.13a	74.29 ± 7.05a	233.38 ± 22.14a	10.51 ± 1.51ab
DH444	*Bacillus aryabhattai*	7.08 ± 1.26ab	382.59 ± 39.12ab	64.72 ± 5.26ab	203.31 ± 16.51ab	9.15 ± 1.81ab
DH527	*Bacillus simplex*	8.81 ± 2.22ab	259.02 ± 40.82bc	58.73 ± 10.83ab	184.50 ± 34.04ab	11.38 ± 3.23ab
DH580	*Bacillus aryabhattai*	5.71 ± 0.57b	266.69 ± 23.82bc	44.63 ± 5.56b	146.93 ± 15.45b	6.20 ± 1.06b
Blend 20[§§]		9.22 ± 3.45ab	314.87 ± 22.71abc	67.31 ± 12.31ab	211.45 ± 38.67ab	12.39 ± 3.59ab
Control	**Active Ingredient**					
Non-treated	Non-treated	5.02 ± 0.95b	370.49 ± 106.10ab	50.28 ± 4.14ab	157.97 ± 13.02ab	6.76 ± 1.31b
Nortica	*Bacillus firmus* I-1582	7.80 ± 1.19ab	315.68 ± 58.97abc	73.99 ± 17.61a	232.47 ± 55.31a	16.97 ± 7.89a
Indemnify	Fluopyram	5.05 ± 1.37b	209.34 ± 55.23c	43.95 ± 11.37b	138.06 ± 35.73b	7.76 ± 2.33b

[†]Microplot trials 64 days after treatment (DAT). Means within a column followed by the same letter are not significantly different (Fisher's LSD, $P \leq 0.05$).
[‡]RDW = Root dry weight (grams).
[§]RL = Root length (centimeters).
[¶]RA = Projected root area (centimeters^2).
[††]SA = Root surface area (centimeters^2).
[‡‡]RV = Root volume (centimeters^3).
[§§]Blend 20 contains strains equal parts AP7 (*B. pumilus*), AP18 (*B. pumilus*) and AP282 (*B. sphaericus*).

Table 4.7: Effect of 10 PGPR strains and 1 PGPR blend (Mean ± SEM) on bermudagrass root architecture when infested with *Meloidogyne incognita* in microplots at 60 DAT. [†]

Code	Scientific Name	RFW[‡]	RDW[§]	RL[¶]	RA[††]	SA[‡‡]	RV[§§]	RKN[¶¶]
DH14	*Bacillus megaterium*	28.45 ± 7.53abc	7.93 ± 1.30bc	372.42 ± 64.73ab	94.86 ± 6.06abc	298.01 ± 19.04abc	21.08 ± 3.22ab	134 ± 46ab
DH30	*Stenotrophomonas rhizophila*	29.70 ± 4.22abc	9.95 ± 1.88abc	368.37 ± 95.98ab	100.93 ± 10.02ab	317.07 ± 31.48ab	28.19 ± 9.57ab	75 ± 19bc
DH40	*Bacillus pumilus*	24.16 ± 3.90bc	7.12 ± 1.01bc	372.47 ± 21.58ab	89.83 ± 8.99abcd	282.22 ± 28.25abcd	17.88 ± 3.54ab	111 ± 35ab
DH44	*Paenibacillus sonchi*	21.97 ± 3.65bc	6.92 ± 2.17bc	305.66 ± 27.44ab	73.87 ± 11.79bcd	232.07 ± 37.06bcd	16.87 ± 7.09ab	82 ± 17abc
DH57	*Bacillus subtilis*	24.59 ± 7.89bc	10.01 ± 4.64abc	330.44 ± 40.66ab	79.51 ± 12.24bcd	249.80 ± 38.46bcd	16.89 ± 5.19ab	111 ± 20ab
DH140	*Bacillus subtilis*	19.20 ± 1.38c	5.66 ± 0.81c	280.56 ± 21.05ab	67.59 ± 4.12cd	212.35 ± 12.95cd	13.39 ± 2.02ab	93 ± 32abc
DH267	*Bacillus subtilis*	27.23 ± 3.28abc	10.10 ± 0.87abc	380.60 ± 76.79a	90.08 ± 5.31abcd	282.99 ± 16.69abcd	19.24 ± 3.49ab	152 ± 23a
DH444	*Bacillus aryabhattai*	34.49 ± 4.82ab	13.24 ± 3.33ab	304.69 ± 78.78ab	98.02 ± 12.54ab	307.93 ± 39.39ab	34.31 ± 13.75ab	70 ± 17bc
DH527	*Bacillus simplex*	18.31 ± 3.27c	6.91 ± 1.96bc	336.75 ± 32.38ab	65.06 ± 3.23d	204.39 ± 10.15d	10.56 ± 1.79b	62 ± 19bc
DH580	*Bacillus aryabhattai*	34.34 ± 1.13ab	15.21 ± 1.38a	317.80 ± 32.63ab	82.56 ± 2.39bcd	257.84 ± 8.20bcd	20.53 ± 2.92ab	55 ± 13bc
Blend 20[†††]		23.31 ± 7.53bc	5.72 ± 1.87c	232.13 ± 17.38b	74.34 ± 13.98bcd	233.55 ± 43.94bcd	20.04 ± 6.78ab	78 ± 18bc
Control	**Active Ingredient**							
Non-treated	Water	31.38 ± 8.07abc	11.79 ± 4.50abc	379.51 ± 37.77a	93.92 ± 17.19abcd	295.06 ± 54.02abcd	19.55 ± 0.79ab	154 ± 18a
Nortica	*Bacillus firmus* I-1582	28.19 ± 6.23abc	9.19 ± 2.18abc	303.57 ± 73.54ab	84.16 ± 8.54abcd	264.41 ± 26.83abcd	35.94 ± 22.06ab	72 ± 18bc
Indemnify	Fluopyram	40.05 ± 6.33a	15.29 ± 3.01a	354.18 ± 49.11ab	111.74 ± 15.84a	351.04 ± 49.78a	37.26 ± 17.05a	23 ± 5c

[†]Microplot trials 64 days after treatment (DAT). Means within a column followed by the same letter are not significantly different (Fisher's LSD, $P \leq 0.05$).
[‡]RFW = Root fresh weight (grams).
[§]RDW = Root dry weight (grams).
[¶]RL = Root length (centimeters).
[††]RA = Projected root area (centimeters^2).
[‡‡]SA = Root surface area (centimeters^2).
[§§]RV = Root volume (centimeters^3).
[¶¶]RKN = Root-knot nematodes per 100 cm^3 of soil per microplot at 60 DAT.
[†††]Blend 20 contains equal parts strains AP7 (*B. pumilus*), AP18 (*B. pumilus*) and AP282 (*B. sphaericus*).

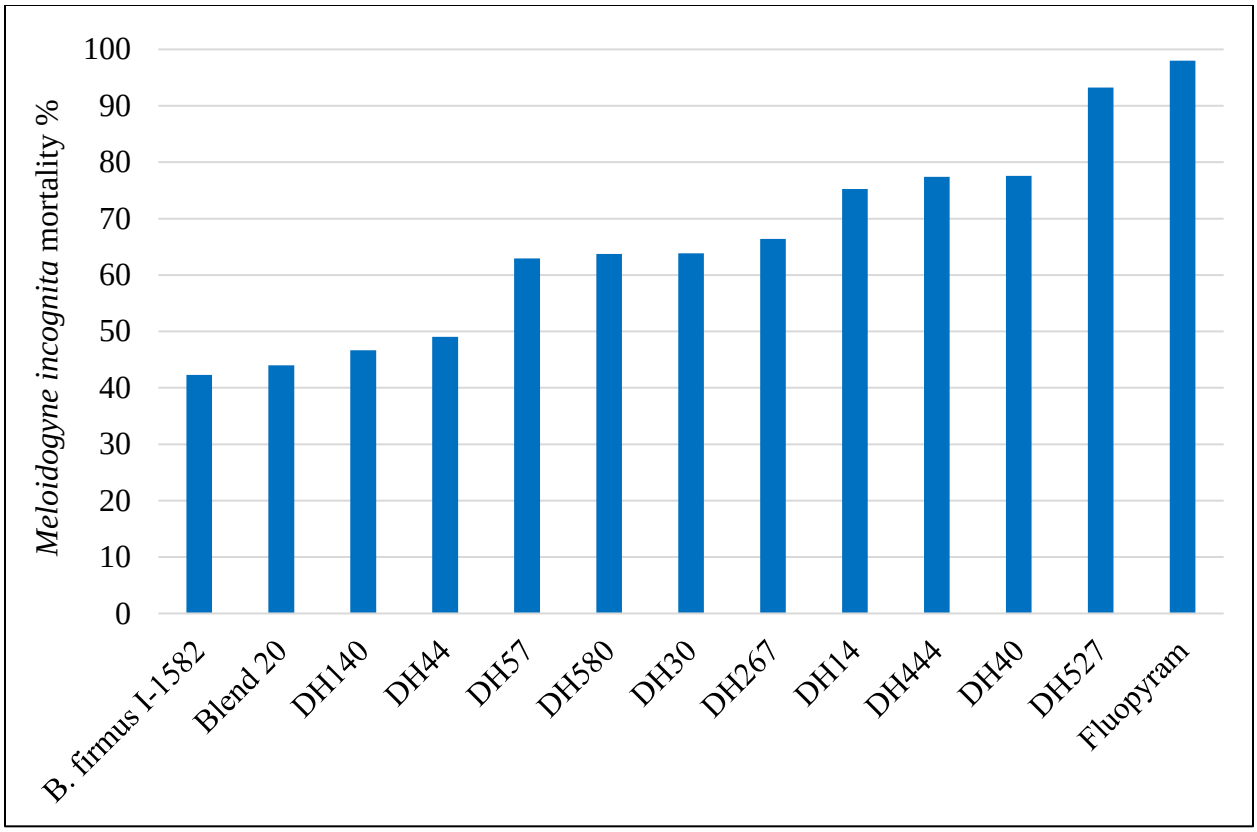

Figure 4.1. *In vitro* evaluation of ten PGPR strains, one PGPR blend (Blend 20), *B. firmus* I-1582, and fluopyram for their antagonistic ability against *M. incognita* at 48 hours after inoculation. Each of these treatments were advanced for greenhouse and microplot evaluations. See Table 4.1 for means comparisons.

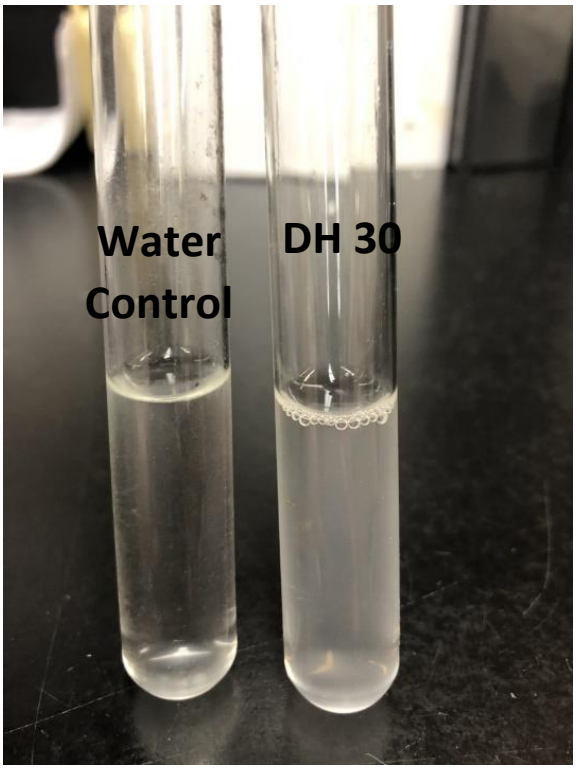

Figure 4.2. Determination of a qualitative nitrogenase activity using liquid JNfB media. Formation of a cloudy liquid after inoculation of a single bacterial colony into the media indicates nitrogenase activity.

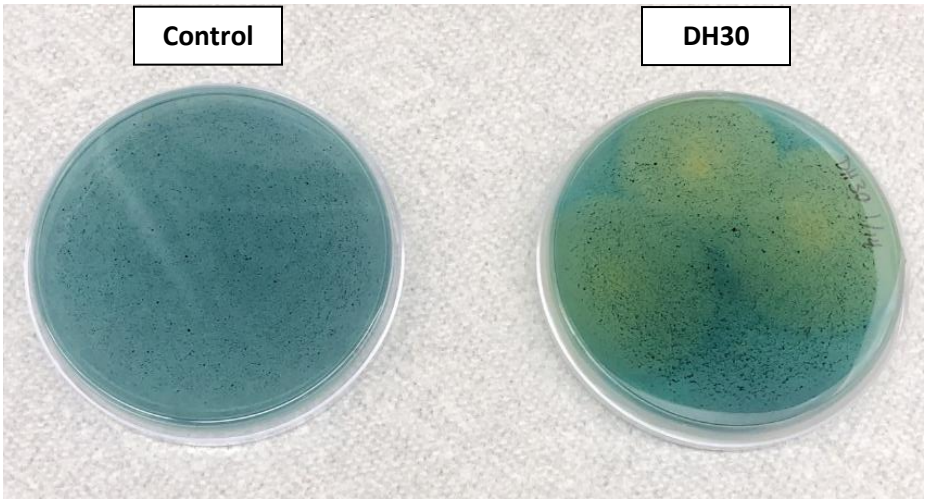

Figure 4.3. Qualitative determination of siderophore production using CAS media. Yellow halos surrounding bacterial colonies indicates siderophore production by the strain.

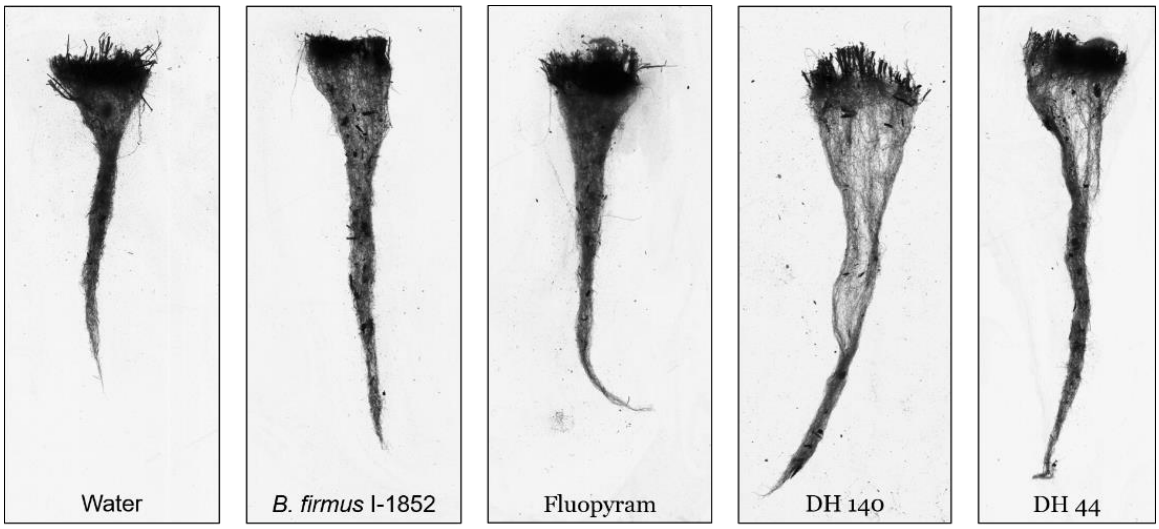

Figure 4.4. WinRhizo images of water, *Bacillus firmus* I-1582, fluopyram, DH140, and DH44 inoculated bermudagrass in a greenhouse setting.

Chapter V: Remote sensing assisted evaluation of chemical nematicides in plant-parasitic nematode infested bermudagrass

Abstract

The objective of this study was to evaluate the ability of an unmanned aerial system (UAS) equipped with a multispectral sensor to track plant health in the presence of plant-parasitic nematodes in conjunction with nematicide applications. Four nematicides were evaluated for their ability to suppress *Belonolaimus longicaudatus* and *Meloidogyne incognita* in microplots, and three nematicides were evaluated on a golf course for their ability to suppress multiple plant-parasitic nematode genera. Visual ratings, NDVI, and NDRE were reported throughout the trial to assess plant health. *Belonolaimus longicaudatus* and *Meloidogyne incognita* population density was significantly lowered by nematicide treatments in microplots and correlated with visual ratings, NDVI, and NDRE plant health ratings. On the golf course, all nematicides reduced total plant-parasitic nematode population density at 28, 56, and 84 DAT. Visual turf quality ratings, NDVI, and NDRE were positively correlated with lower nematode population density in the majority of evaluation dates. In the microplot and golf course settings, the parameters evaluated for plant health were correlated with plant-parasitic nematode population density: as nematode population density declined, visual ratings, NDVI, and NDRE increased. These results show that remote sensing has the potential to be a beneficial tool for assessing plant-parasitic nematode infested bermudagrass.

Introduction

Bermudagrass (*Cynodon* spp. L.), one of the most commonly grown turfgrass species in the southern United States, is very susceptible to a wide range plant-parasitic nematodes (Crow, 2005). Examples of genera known to parasitize turfgrass in the United States include *Belonolaimus longicaudatus* (sting nematode, Rau), *Helicotylenchus* spp. (spiral nematode), *Hoplolaimus galeatus* (lance nematode, Cobb), and *Meloidogyne* spp. (root-knot nematode), and *Mesocriconema* spp. (ring nematode) (*sensu lato*) (Crow, 2005; Sikora et al. 2001; Zeng et al. 2012). Feeding on the turfgrass root system by these nematodes results in inhibition of root growth, and can lead to a decrease in water and nutrient uptake (Lucas et al. 1982; Trenholm et al. 2005; White and Dickens, 1984). This impact on root development, in turn, can lead to visible foliar symptoms including chlorosis and necrosis, wilting, and a thinning of the turf canopy (Aryal et al. 2017; Crow and Han, 2005; Johnson, 1970). With a wide range of potential plant-parasitic nematodes that can inhibit turfgrass growth, early and accurate detection of symptoms caused by nematode feeding could be important for more timely and effective management.

Over the past decade, there has been a significant rise in interest of unmanned aerial system (UAS) and remote multispectral sensing technology for turfgrass management. Spectral reflectance of the turfgrass canopy has been shown to provide valuable information on turfgrass species quality (Bremer et al. 2011; Fitz-Rodriguez and Choi, 2002), drought stress (Jiang and Carrow, 2007), nutrient levels (Caturegli et al. 2016; Volterrani et al. 2005), and fungal diseases (Sykes et al. 2017). A majority of this research has focused on using the normalized difference vegetation index [NDVI = (NIR – Red)/(NIR + Red), NIR = reflectance in the near-infrared region and Red = reflectance in the red light region], and is a commonly used plant stress indicator (Barton, 2012; Labus et al. 2002). Multiple research groups have shown that NDVI

strongly correlates with a range of parameters related to turfgrass, including visual ratings, nitrogen applications, and shoot density (Bell et al. 2009; Caturegli et al. 2016; Trenholm et al. 1999). Trenholm et al. (2005) also reported significant improvement in NDVI by nematicides on turfgrass infested with *B. longicaudatus* in greenhouse evaluations. Thus, this is a proven vegetative index for rating bermudagrass in conjunction with visual ratings

Another vegetation index used in crop stress management is the normalized difference red edge index [NDRE = (NIR – Red Edge)/(NIR + Red Edge), NIR = reflectance in the near-infrared region and Red Edge = reflectance in the change from red light to near-infrared light, approximately 680 – 750 nanometers] (Fitz-Rodriguez and Choi, 2002). NDRE has been shown to correlate with wheat and corn growth, and is a valuable tool for vegetative chlorophyll status (Horler et al. 1983). Krienke et al. (2017) found that NDRE values strongly correlate with nitrogen variability in maize production, and Tilling et al. (2007) found that NDRE was able to account for nitrogen level variability in wheat. In turfgrass, Hong et al. (2019) found that NDRE can be useful for drought stress analysis on creeping bentgrass (*Agrostis stolonifera* L.). Previous studies have also shown that NDRE has a positive correlation with clipping weight from hybrid bermudagrass (Fitz-Rodriguez and Choi, 2002).

While multiple studies have demonstrated the benefits of using these indices in turfgrass research, no one has evaluated these indices for their ability to track changes in plant health as a result of plant-parasitic nematodes. Research on *Heterodera glycines* (soybean cyst nematode, Ichinode) has shown differences in spectral reflectance correlate with pathogen severity (Bajwa et al. 2017; Nutter et al. 2002). Joalland et al. (2018) were also able to correlate multiple spectral indices with yields of tolerant and susceptible sugar beets to *Heterodera schachtii* (beet cyst nematode, Schmidt). A research group in Brazil recently reported that red, red edge, and near-

infrared spectral ranges were significant for discrimination of healthy coffee plants and coffee plants infected with *Meloidogyne* spp. at or above damage thresholds with an accuracy rate of 78% (Martins et al. 2017).

Visual ratings are the predominant means of assessing the impact of plant-parasitic nematode damage on turfgrass, yet visual evaluations and rating scales can lead to inconsistences between evaluators, and assessing detailed turfgrass features can be subjective (Horst et al. 1984; Trenholm et al. 1999). Thus, the addition of NDVI and NDRE values in conjunction with visual turfgrass ratings may provide some stability in plant-parasitic nematode damage assessment. With this information in mind, the goal of this study was to evaluate UAS equipped with multispectral sensors for their ability to track plant health in the presence of plant-parasitic nematodes in conjunction with nematicide applications. Specifically, the objectives were to (1) assess turfgrass chemical nematicides for their ability to impact visual quality ratings, NDVI, and NDRE values on *Meloidogyne incognita* and *Belonolaimus longicaudatus* infested bermudagrass in microplots, and (2) to assess the ability of these chemical nematicides for their ability to impact these same vigor ratings on a golf course infested with multiple genera of plant-parasitic nematodes.

Materials and methods

Microplot evaluations

Microplot trials were conducted at the Plant Science Research Center (PSRC) at Auburn University, AL during the summers of 2018 and 2019. Two trials were conducted: one to evaluate four nematicides for their ability to reduce *Belonolaimus longicaudatus* population density, and the other to reduce *Meloidogyne incognita* population density. Trials were

established in 26.5-liter plastic tree pots nested one on top of the other with a brick in between to limit root growth by air pruning and filled with 100% sand. Experiments were arranged in a randomized complete block design (RCBD) with five replications for each treatment. In 2018, 'Tifway' hybrid bermudagrass was laid as sod in each plot and given ten weeks for establishment. Each microplot received water at 30 mL/min by an automated drip irrigation system adjusted throughout the season to run for 30 minutes twice a day every other day. Grass was trimmed twice a week to a height of 2.5 cm. At the end of the 10-week period, *M. incognita* eggs were inoculated on half of the microplots at a rate of 50,000 eggs per pot on a weekly basis for 4 weeks to build up the nematode population density. The remaining microplots received an inoculation rate of 100 *B. longicaudatus* nematodes per pot on a weekly basis for 4 weeks. After the 4-week inoculation period, a 100-cm^3 sample was taken from each plot to confirm *M. incognita* or *B. longicaudatus* population density.

Microplot nematode inocula

Half of the microplots were inoculated with *Meloidogyne incognita* race 3, originally isolated from an infested field at Plant Breeding Unit (PBU) at E.V. Smith Research Center of Auburn University in 2017 (Groover et al. 2019). The *M. incognita* inoculum was increased on corn plants (Mycogen 2R042; Corteva Agriscience, Indianapolis, IN) in 500 cm^3 polystyrene pots in the greenhouse. Nematode eggs were extracted from the corn roots following a modified version of the methodology of Hussey and Barker (1973). The root mass was placed in a 0.625% sodium hypochlorite (NaOCl) solution and shaken for 4 minutes at 1 g-force on a Barnstead Lab Line Max Q 5000 E Class shaker (Conquer Scientific, San Diego, CA). Roots were then scrubbed by hand, and the eggs were collected on a 25-µm pore sieve and washed into a 50 mL centrifuge tube. The contents were centrifuged at 427 g-forces for 1 minute in a 1.14 specific

gravity sucrose solution based on Jenkins (1964) methodology. Eggs, now located in the supernatant of the sucrose solution, were recollected on a 25-µm pore sieve, rinsed with water to remove sucrose from eggs, and their presence confirmed via a Nikon TSX 100 inverted microscope at 40-x magnification. For the microplot experiment, eggs were diluted to inoculation levels of 50,000 eggs per microplot.

For the other microplot experiment, *B. longicaudatus*, originally isolated from a golf course in east Alabama (specific location redacted by request of the golf course), was used as inoculum. The *B. longicaudatus* was maintained on 'Princess 77' bermudagrass in 500 cm^3 polystyrene pots in the greenhouse. The nematode population was obtained from total soil. Soil from each pot was collected on a 25-µm pore sieve and the modified sucrose centrifugal flotation technique was used to extract nematodes as previously described. The final *B. longicaudatus* population was collected on a 25-µm pore sieve, rinsed with water to remove sucrose from nematodes, and their presence confirmed via a Nikon TSX 100 inverted microscope at 40-x magnification. For the microplot experiment, *B. longicaudatus* population density was diluted to inoculation levels of 100 nematodes per microplot.

Microplot nematicide treatments

Four nematicides were evaluated in the microplot setting during the study. Nematicides used were as follows: 1) abamectin (Divanem; Syngenta, Greensboro, NC) at 0.89 L/Ha; 2) fluopyram (Indemnify; Bayer CropScience, St. Louis, MO) at 1.25 L/Ha; 3) fluensulfone (Nimitz Pro G; Adama, Pasadena, TX) at 134 kg/Ha; 4) furfural (Multiguard Protect; Agriguard Company, LLC, Cranford, NJ) at 75 L/Ha; and 5) an untreated control. Abamectin, fluopyram, and furfural were applied once at the start of the trial via a handheld spray bottle, and each treatment was diluted so that two sprays of the bottle was the calibrated rate. Fluensulfone was

broadcast with a Scotts Easy Hand-Held Broadcast Spreader. All nematicides were watered in with 0.64 cm of water after application.

Microplot data collection

Data collection occurred on July 5 [0 days after treatment (DAT)], Aug. 4 (30 DAT), and Sept. 3 (60 DAT) in 2018, and July 19 (0 DAT), Aug. 18 (30 DAT), and Sept. 17 (60 DAT) in 2019. Data collected included nematode population density, visual turf quality assessment, NDVI, and NDRE. At each collection date, a 100-cm^3 soil sample was taken from each plot. For *M. incognita,* population density, the soil sample was placed in a modified Baermann funnel (Castillo et al. 2013), and after 48 hours, juveniles were collected on a 25-µm pore sieve. For *B. longicaudatus,* the 100-cm^3 sample was collected on a 25-µm pore sieve and nematodes were extracted using the modified centrifugal flotation technique as previously described. Once extracted, both *M. incognita* and *B. longicaudatus* presence were confirmed and quantified via a Nikon TSX 100 inverted microscope at 40-x magnification.

Turfgrass vigor was calculated using the National Turfgrass Evaluation Program (NTEP) guidelines (Parsons et al. 2015). Visual ratings consisted of a 1-9 rating scale, where 1 was very poor quality turf, 6 was minimal acceptable turf quality, and 9 was exceptional turf quality (Morris, 2004). Multispectral data was collected the same day that visual ratings were made. Drone flights occurred within two hours of solar noon on clear sunny days or light overcast days when ambient light was not changing. A DJI Phantom 4 Professional (SZ DJI Technology Co.; Shenzhen, China) equipped with a MicaSense RedEdge-M (MicaSense, Inc.; Seattle, WA) was used for flight and image acquisition. Flight speed was 10 meters per second at a height of 35 meters, and imagery was spaced with 80% front overlap and 88% side overlap. The sensor measured wavelengths of blue (475 nm), green (560 nm), red (668 nm), red edge (RE, 717 nm),

and near infrared (NIR, 840 nm). Automated image processing was performed with Pix4Dmapper (Pix 4D; Prilly, Switerzland), and wavelength values were calculated using ArcMap (Esri; Redlands, California). Wavelengths red, red edge, and near infrared were used to calculate NDVI and NDRE values for each plot in ArcMap. NDVI and NDRE were calculated as previously described.

Field evaluations

A field trial was conducted in the summer of 2019 at Montevallo Golf Club in Montevallo, AL to assess the ability of multispectral imagery to track nematicidal responses on a plant-parasitic nematode infested golf course. Four putting greens were selected for the experiment: three with a history of high plant-parasitic nematode population density, and one with low population density. Each green was divided into four quadrants, with three quadrants receiving a nematicide application, and one left as an untreated control. Nematicide applications were as follows: 1) untreated control; 2) abamectin at 0.89 L/Ha; 3) fluopyram at 1.25 L/Ha; and 4) fluensulfone at 134 kg/Ha. Each treatment was mixed with water to a total volume of two gallons and sprayed on the plots with a CO_2-powered backpack sprayer (R&D Sprayers, Bellspray, Inc.; Opelousas, LA).

Field data collection

The trial was initiated on July 22. Nematode samples were collected at 0 (July 22), 28 (Aug. 19), 56 (Sept. 16), and 84 (Oct. 14) DAT. At each sample data, six soil cores (2.22 cm x 10 cm) were taken at roughly equal intervals in a zigzag pattern across each quadrant of a green. Collected soil samples were mixed and a 100-cm^3 subsample was processed to determine the plant-parasitic nematode population density from each quadrant. Nematodes were extracted by

gravity sieving followed by sucrose centrifugation following the methodology of Jenkins (1964) as previously described. Plant-parasitic nematodes were confirmed and enumerated by a Nikon TSX 100 inverted microscope at 40-x magnification, and morphologically identified to genus or species if possible (Mai and Lyon, 1975; Eisenback, 2002).

Drone flights occurred during the trial on approximately 14-day intervals. Specific dates were July 22 (0 DAT), Aug. 5 (14 DAT), Aug. 19 (28 DAT), Sept. 3 (56 DAT), Sept. 16 (60 DAT), Sept. 30 (74 DAT), and Oct. 14 (84 DAT). Flights were performed as previously described during microplot evaluations, capturing NDVI and NDRE values for each treatment quadrant. Visual turfgrass quality ratings were also conducted on the 1-9 scale as previously described using the NTEP guidelines.

Statistical analysis

Data collected from microplot and field evaluations were statistically analyzed using the PROC GLIMMIX procedure (SAS version 9.4; SAS Institute, Cary, NC). Dependent variables included plant-parasitic nematodes per 100 cm^3 soil, turf visual quality, NDVI, and NDRE. The fixed effect was nematicide treatment, and random effects included replication, test repeat, and location. Student panels were generated to determine the normality of the residuals. LS-means were compared by Tukey's multiple range test for each evaluation date ($P \leq 0.05$). Rating methods (NTEP, NDVI, and NDRE) were also compared statistically by the Pearson correlation coefficient at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$ to determine the strength of the linear relationship between nematode populations and foliar ratings.

Results

Microplot evaluations

Meloidogyne incognita population density was significantly impacted by nematicide treatments in both 2018 and 2019. All nematicides significantly lowered *M. incognita* population density at 30 and 60 days after treatment (DAT) for both years ($P \leq 0.05$) (Figure 5.1A,B). In 2018, only fluopyram significantly increased turf quality compared to the untreated control at both 30 and 60 DAT ($P \leq 0.05$) (Figure 5.1C). In 2019, both fluopyram and fluensulfone significantly increased turf quality compared to the untreated control at 30 DAT, and fluopyram and abamectin significantly increased turf quality compared to the untreated control at 60 DAT ($P \leq 0.05$) (Figure 5.1D). NDVI was significantly higher in both years for bermudagrass treated with fluopyram and abamectin at 30 DAT, and all nematicides at 60 DAT ($P \leq 0.05$) (Figure 5.1E,F). No significant differences were observed for NDRE in 2018 at 30 DAT, and fluopyram, abamectin, and furfural had a significantly higher NDRE value compared to the untreated plots at 60 DAT ($P \leq 0.05$) (Figure 5.1G). In 2019, all nematicides significantly increased NDRE at 60 DAT compared to the untreated control ($P \leq 0.05$) (Figure 5.1H).

In 2018, fluopyram and fluensulfone significantly reduced *B. longicaudatus* population density compared to the untreated control at 30 and 60 DAT, and in 2019 fluopyram, fluensulfone, and furfural significantly reduced population density at 30 and 60 DAT compared to the untreated control ($P \leq 0.05$) (Figure 5.2A,B). Turf quality was significantly improved by fluopyram and fluensulfone compared to the untreated control at 30 DAT and abamectin, fluopyram, and fluensulfone at 60 DAT in 2018 ($P \leq 0.05$) (Figure 5.2C). In 2019, fluopyram significantly increased turf quality compared to the untreated control at 30 DAT, and abamectin, fluopyram, and fluensulfone increased turf quality compared to the untreated control at 60 DAT ($P \leq 0.05$) (Figure 5.2D). In 2018, abamectin and fluopyram significantly increased NDVI compared to the untreated control at 30 DAT, and fluopyram significantly increased NDVI

compared to the untreated control at 60 DAT ($P \leq 0.05$) (Figure 5.2E). For 2019, NDVI was significantly improved by abamectin, fluensulfone, and fluopyram at 30 DAT, and abamectin and fluopyram at 60 DAT ($P \leq 0.05$) (Figure 5.2F). Abamectin and fluopyram both significantly improved NDRE at 30 DAT in 2018 compared to the untreated control, and fluopyram significantly improved NDRE at 60 DAT compared to the untreated control ($P \leq 0.05$) (Figure 5.2G). In 2019 only fluopyram significantly improved NDRE at 30 DAT compared to the untreated, and fluopyram and abamectin significantly improved NDRE compared to the untreated control at 60 DAT ($P \leq 0.05$) (Figure 5.2H).

Turfgrass visual ratings, NDVI, and NDRE were correlated with *M. incognita* and *B. longicaudatus* population density in both 2018 and 2019. In 2018, turf visual ratings significantly correlated with *M. incognita* population density at 30 DAT ($P \leq 0.05$), and all three sample dates in 2019 ($P \leq 0.05$) (Table 5.1). NDVI was significantly correlated with *M. incognita* population density at 30 DAT ($P \leq 0.05$) and 60 DAT ($P \leq 0.001$) in 2018 and 2019. NDRE was significantly correlated with *M. incognita* population density 60 DAT in 2018 ($P \leq 0.01$) and 2019 ($P \leq 0.001$). *Belonolaimus longicaudatus* population density was significantly correlated with turf visual ratings at 0 DAT ($P \leq 0.001$) and 60 DAT ($P \leq 0.01$) in 2018, and 30 and 60 DAT ($P \leq 0.01$) in 2019 (Table 5.2). Both NDVI and NDRE were significantly correlated with *B. longicaudatus* population density at 60 DAT in 2018 ($P \leq 0.001$), and all sample dates in 2019 ($P \leq 0.01$) (Table 5.2).

Plant health ratings were also highly correlated with each other throughout both the *M. incognita* and *B. longicadatus* microplot evaluations. In the *M. incognita* trial, turf quality was positively correlated with NDVI at 0 and 30 DAT ($P \leq 0.01$) in 2018, and 60 DAT in 2019 ($P \leq 0.001$) (Table 5.1). NDRE was positively correlated with turf quality at 30 DAT in 2018 ($P \leq$

0.05), and 60 DAT in 2019 ($P \leq 0.001$). NDVI and NDRE were positively correlated at every evaluation date in 2018 and 2019 ($P \leq 0.001$), except for 0 DAT in 2018. For the *B. longicaudatus* trial, turf quality was positively correlated with NDVI and NDRE at 60 DAT in 2018 ($P \leq 0.001$), and both 30 and 60 DAT in 2019 ($P \leq 0.05$) (Table 5.2). NDVI and NDRE were positively correlated at every flight date in 2018 and 2019 ($P \leq 0.001$).

Field evaluations

Five plant-parasitic nematode genera were identified throughout the duration of the 2019 field trial at Montevallo Golf Club. These include *Hoplolaimus galeatus* (Lance nematode, Figure 5.3A), *Helicotylenchus* spp. (Spiral nematode, Figure 5.3B), *Meloidogyne* spp. (Figure 5.3C), *Belonolaimus longicaudatus* (Figure 5.3D), and *Mesocriconema* spp. (Ring nematode, Figure 5.3E) (*sensu lato*). All nematicides reduced total plant-parasitic nematode population density at 28, 56, and 84 DAT compared to the untreated control ($P \leq 0.05$) (Figure 5.4A). Fluopyram significantly improved visual turf quality and NDVI at 28, 43, 56, 70, and 84 DAT ($P \leq 0.05$) (Figure 5.4B,C), and NDRE at 14, 28, 43, 56, 70, and 84 DAT ($P \leq 0.05$) (Figure 5.4D) compared to the untreated control. Abamectin and fluopyram also significantly improved visual turf quality at two evaluation dates apiece ($P \leq 0.05$) (Figure 5.4B). Abamectin significantly improved NDVI at 70 and 84 DAT compared to the untreated control, and significantly improved NDRE at 43, 70, and 84 DAT ($P \leq 0.05$) (Figure 5.4C,D). Fluensulfone significantly improved NDRE at 70 DAT ($P \leq 0.05$) (Figure 5.4D).

Total plant-parasitic nematode population density was highly correlated with turfgrass visual ratings, NDVI and NDRE. Correlation occurred with visual ratings at 0 DAT ($P \leq 0.01$), 28 DAT, 56 DAT, and 84 DAT ($P \leq 0.001$) (Table 5.3). Nematode population density and NDVI were correlated at 0 DAT ($P \leq 0.05$), 28 DAT, 56 DAT, and 84 DAT ($P \leq 0.001$) (Table 5.3).

NDRE and nematode population density were correlated at 28 DAT ($P \leq 0.05$), 56 DAT ($P \leq 0.01$), and 84 DAT ($P \leq 0.001$) (Table 5.3). NDVI and turf quality ratings were positively correlated at five of the seven evaluation dates, and NDRE and turf quality were positively correlated at four of the seven evaluation dates ($P \leq 0.01$) (Table 5.3). NDVI and NDRE were positively correlated at five of the seven evaluation dates ($P \leq 0.05$) (Table 5.3).

Discussion

Evaluating new technology for its ability to improve turfgrass management is very important, and the potential uses for UAS in the turfgrass industry are widespread. This study confirmed previous reports of the correlation between both NDVI and NDRE and turfgrass quality (Bell et al. 2009; Caturegli et al. 2016; Hong et al. 2019). These results also indicate that NDVI and NDRE captured via remote sensing are reliable metrics for incorporating into evaluating turfgrass damage caused by plant-parasitic nematodes. In all nematicide trials, a significant positive correlation was observed for multiple evaluation dates between visual turfgrass quality and both NDVI and NDRE, meaning that as turfgrass visually improved, so did NDVI and NDRE. Conversely, multiple evaluation dates for all trials displayed a significant negative correlation between plant-parasitic nematode genera and both NDVI and NDRE. As plant-parasitic nematode population density increased, both NDVI and NDRE declined.

Meloidogyne incognita and *B. longicaudatus* population density were high in microplot trials for both 2018 and 2019, with initial population density at or near levels that have previously been reported to recommend a nematicide application in Alabama (Sikora et al. 2001). For the *M. incognita* infested microplots, the strongest correlations between nematode population density and NDVI and NDRE occurred at 60 DAT for both years. In fact, nematode population density had a significantly stronger correlation with both NDVI and NDRE in 2018

and 2019 at 60 DAT compared to visual turf quality ratings. Similar results were observed in the *B. longicaudatus* microplots, with strongest correlations of visual turfgrass quality, NDVI, and NDRE compared to population density occurring at 60 DAT. While both NDVI and NDRE had a higher correlation with population density at 60 DAT numerically compared to the visual ratings, only 2018 correlations were significantly higher. This is similar to greenhouse evaluations by Trenholm et al. (2005), who found significant improvement of NDVI in *B. longicaudatus* infested turfgrass by nematicide treatment. Previous research has also shown that each of the nematicides evaluated in this study can be effective tools for lowering plant-parasitic nematode population density (Aryal et al. 2016; Baird et al. 2017; Blackburn et al. 1996; Crow et al. 2017). Feeding by plant-parasitic nematodes inhibit root growth and function, so as root growth and development improve after a nematicide treatment, turfgrass vigor should improve as well (Crow, 2005; Luc et al. 2007).

Another interesting finding from the microplot evaluations was that NDVI and NDRE ratings significantly indicated nematicide reductions of nematode population density more frequently than the visual turfgrass ratings. For example, in the 2018 *M. incognita* microplot trial, only fluopyram significantly improved visual turfgrass quality compared to the untreated control at both 30 and 60 DAT. However, two of the four nematicides had a significant improvement in NDVI at 30 DAT compared to the untreated control, and all nematicides significantly improved NDVI at 60 DAT compared to the untreated control. Three of the four nematicide treatments also improved NDRE compared to the untreated control at 60 DAT. A similar trend was observed in 2019 *M. incognita* inoculated microplots, as more nematicide treatments reduced nematode numbers and significantly increased NDVI and NDRE compared to the untreated control than with visual turfgrass ratings. Bremer et al. (2010; 2011) reported

similar findings, noting that significant differences were frequently observed in NDVI among turfgrasses even when all were visually rated at the same quality level. While the mechanism for this occurrence is not fully understood, factors such as chlorophyll content, plant water status, and leaf cell constituents all play a role in influencing red and NIR reflectance.

At the field site, multiple genera of plant-parasitic nematodes were identified. While average population density for each individual genus of nematode was not, on its own, above traditional treatment thresholds, it is clear that the combined presence of the total nematode population density did have a detrimental impact on the turfgrass. Field results from this study were consistent with microplot data. Visual turf quality and NDVI were both significantly correlated with total plant-parasitic nematode population density at all evaluation dates, and NDRE was significantly correlated with nematode population density at all but the first evaluation date.

Visual evaluations can be inconsistent among evaluators, and studies have shown that even the same turfgrass evaluator may not be consistent on a day-to-day basis (Bell et al. 2009; Horst et al. 1984). However, digital imagery captured and processed quickly for immediate use, can help eliminate potential inconsistencies in turfgrass evaluation. While it is still vital to use visual assessments in conjunction with proper soil sampling to diagnose plant-parasitic nematode damage on turfgrass, the results of this research show that UAS-assisted multispectral imagery analysis may provide an additional tool to help assess and track the impact of plant-parasitic nematodes on intensively maintained turfgrass.

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Table 5.1. Pearson correlation coefficients[†] resulting from linear correlation of data parameters from 2018 and 2019 *Meloidogyne incognita* infested bermudagrass microplots in Auburn, AL.

	July		August		September	
	2018	2019	2018	2019	2018	2019
	Visual Quality[‡]					
NDVI[§]	0.59**	NS	0.54**	NS	NS	0.63***
NDRE[¶]	NS	NS	0.47*	NS	NS	0.66***
	NDRE					
NDVI	NS	0.60**	0.96***	0.72***	0.93***	0.95***
	Meloidogyne incognita					
Visual Quality	NS	-0.64***	-0.38*	-0.39*	NS	-0.59**
NDVI	NS	NS	-0.48*	-0.37*	-0.76***	-0.82***
NDRE	NS	NS	NS	NS	-0.61**	-0.76***

[†]NS, *, **, *** tests of linear correlation between variables were not significant (NS) or were significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively.

[‡]Visual quality ratings were assigned on a 1 to 9 scale, where 1 = poorest turf quality, 6 = minimally acceptable turf quality, and 9 = exceptional turf quality.

[§]NDVI = (840 nm reflectance – 668 nm reflectance) ÷ (840 nm reflectance – 668 nm reflectance) collected using a MicaSense RedEdge-M (MicaSense, Inc.; Seattle, WA) sensor.

[¶]NDRE = (840 nm reflectance – 717 nm reflectance) ÷ (840 nm reflectance – 717 nm reflectance) collected using a MicaSense RedEdge-M (MicaSense, Inc.; Seattle, WA) sensor.

Table 5.2. Pearson correlation coefficients[†] resulting from linear correlation of data parameters from 2018 and 2019 *Belanolaimus lonigcaudatus* infested bermudagrass microplots in Auburn, AL.

	July		August		September	
	2018	2019	2018	2019	2018	2019
	Visual Quality[‡]					
NDVI[§]	NS	NS	NS	0.43*	0.63***	0.41*
NDRE[¶]	NS	NS	NS	0.45*	0.66***	0.56**
	NDRE					
NDVI	0.60**	0.91***	0.72***	0.96***	0.95***	0.84***
	Belonolaimus longicaudatus					
Visual Quality	-0.64***	NS	NS	-0.56**	-0.59**	-0.54**
NDVI	NS	-0.51**	NS	-0.57**	-0.82***	-0.56**
NDRE	NS	-0.53**	NS	-0.61**	-0.76***	-0.63**

[†]NS, *, **, *** tests of linear correlation between variables were not significant (NS) or were significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively.

[‡]Visual quality ratings were assigned on a 1 to 9 scale, where 1 = poorest turf quality, 6 = minimally acceptable turf quality, and 9 = exceptional turf quality.

[§]NDVI = (840 nm reflectance – 668 nm reflectance) ÷ (840 nm reflectance – 668 nm reflectance) collected using a MicaSense RedEdge-M (MicaSense, Inc.; Seattle, WA) sensor.

[¶]NDRE = (840 nm reflectance – 717 nm reflectance) ÷ (840 nm reflectance – 717 nm reflectance) collected using a MicaSense RedEdge-M (MicaSense, Inc.; Seattle, WA) sensor.

Table 5.3. Pearson correlation coefficients[†] resulting from linear correlation of vigor ratings and plant-parasitic nematode density from four bermudagrass putting greens treated with three different nematicides in 2019 in Montevallo, AL.

	July 22	August 5	August 19	September 3	September 16	September 30	October 14
				Visual Quality[‡]			
NDVI[§]	NS	NS	0.63**	0.70**	0.62**	0.78***	0.80***
NDRE[¶]	NS	NS	NS	0.59*	0.51*	0.67**	0.60**
				NDVI			
NDRE	NS	NS	0.49*	0.76***	0.93***	0.79***	0.68**
				Total plant-parasitic nematodes			
Visual Quality	-0.64**		-0.81***		-0.85***		-0.84***
NDVI	-0.47*		-0.84***		-0.80***		-0.82***
NDRE	NS		-0.59*		-0.70**		-0.70**

[†]NS, *, **, *** tests of linear correlation between variables were not significant (NS) or were significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively.

[‡]Visual quality ratings were assigned on a 1 to 9 scale, where 1 = poorest turf quality, 6 = minimally acceptable turf quality, and 9 = exceptional turf quality.

[§]NDVI = (840 nm reflectance – 668 nm reflectance) ÷ (840 nm reflectance – 668 nm reflectance) collected using a MicaSense RedEdge-M (MicaSense, Inc.; Seattle, WA) sensor.

[¶]NDRE = (840 nm reflectance – 717 nm reflectance) ÷ (840 nm reflectance – 717 nm reflectance) collected using a MicaSense RedEdge-M (MicaSense, Inc.; Seattle, WA) sensor.

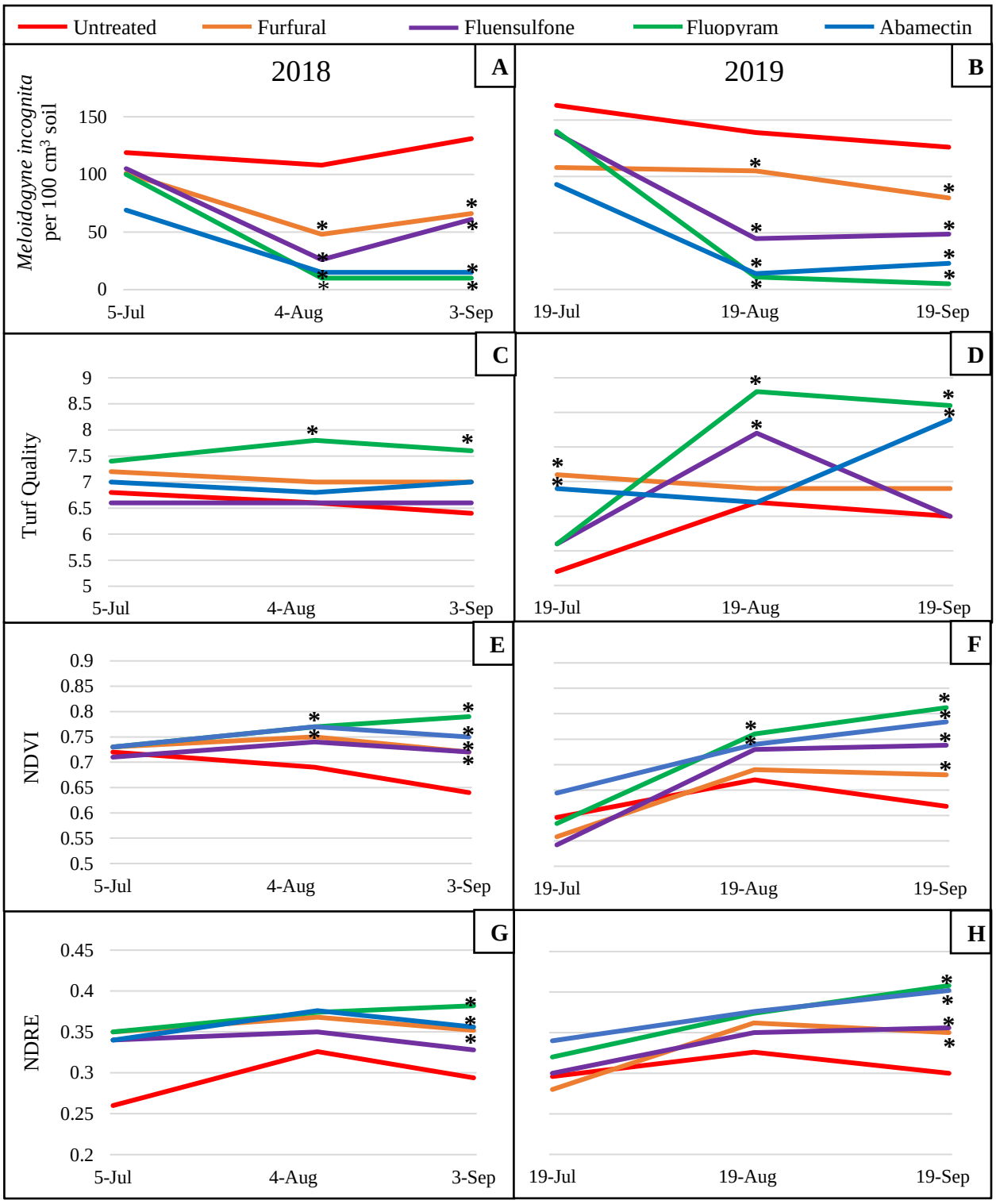

Figure 5.1: Auburn University *Meloidogyne incognita* nematicide microplot trials for 2018 and 2019 showing *M. incognita* population density for 2018 (A) and 2019 (B), visual turf quality ratings for 2018 (C) and 2019 (D), NDVI ratings for 2018 (E) and 2019 (F), and NDRE values for 2018 (G) and 2019 (H). *Different from the untreated control according to the pairwise comparison of each treatment to the untreated (Tukey-Kramer, $P \leq 0.05$).

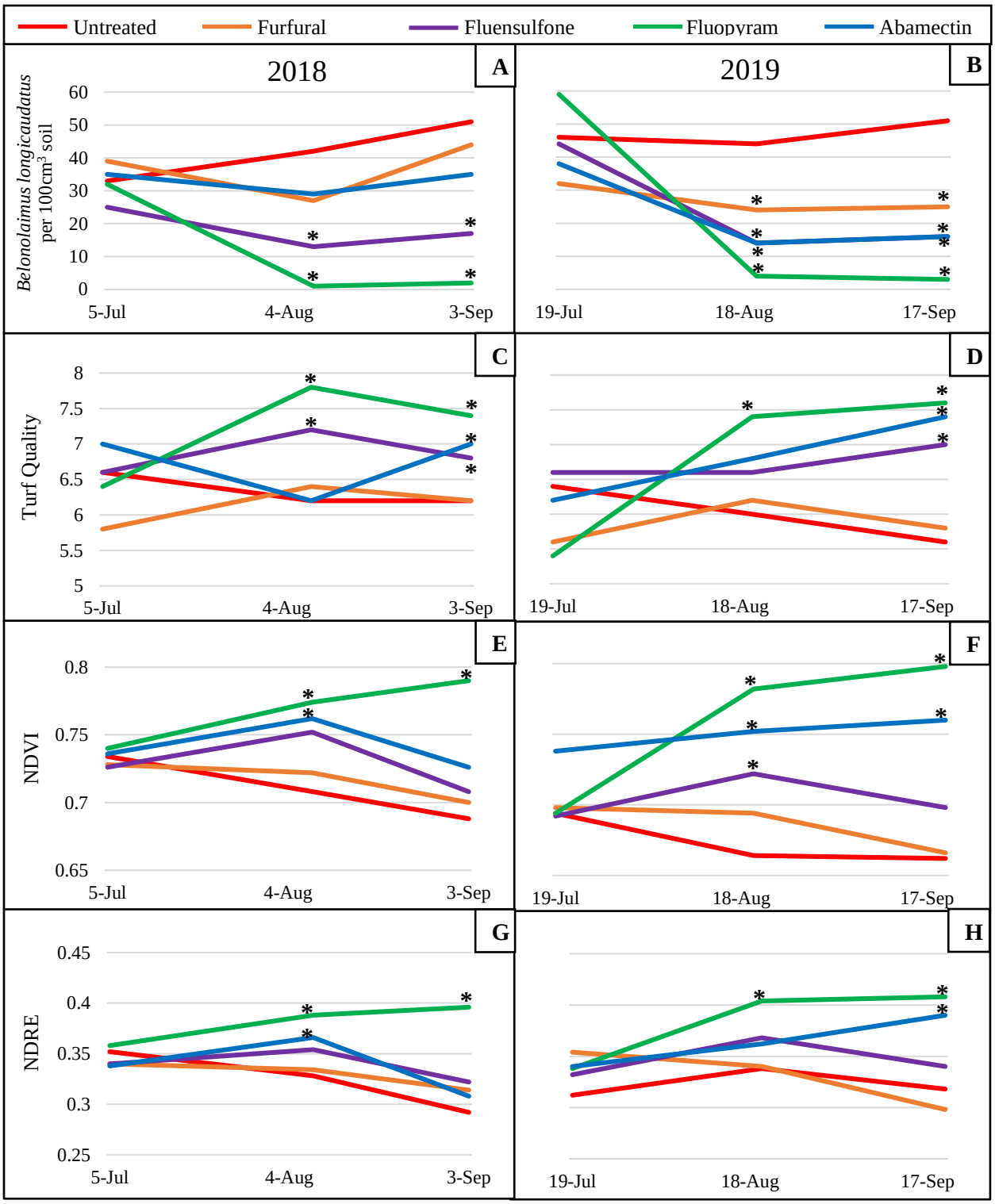

Figure 5.2: Auburn University *Belonolaimus longicaudatus* nematicide microplot trials for 2018 and 2019 showing *B. longicaudatus* population density for 2018 (A) and 2019 (B), visual turf quality ratings for 2018 (C) and 2019 (D), NDVI ratings for 2018 (E) and 2019 (F), and NDRE values for 2018 (G) and 2019 (H). *Different from the untreated control according to the pairwise comparison of each treatment to the untreated (Tukey-Kramer, $P \leq 0.05$).

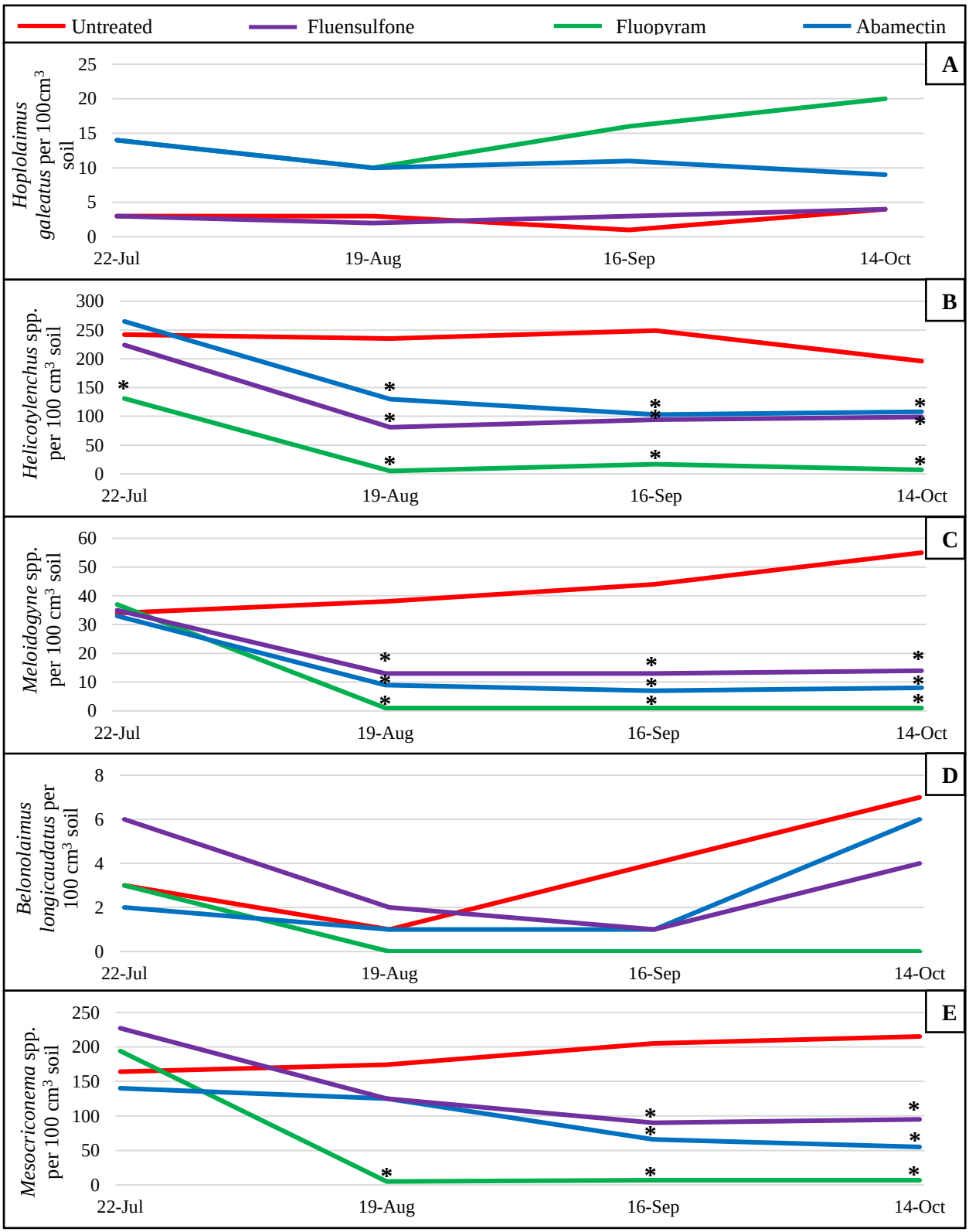

Figure 5.3: Plant-parasitic nematode genera identified at Montevallo Golf Course, Montevallo, AL in 2019. Genera include *Hoplolaimus galeatus* (A), *Helicotylenchulus* spp. (B), *Meloidogyne* spp. (C), *Belonolaimus longicaudatus* (D), and *Mesocriconema* spp. (E). *Different from the untreated control according to the pairwise comparison of each treatment to the untreated (Tukey-Kramer, $P \leq 0.05$).

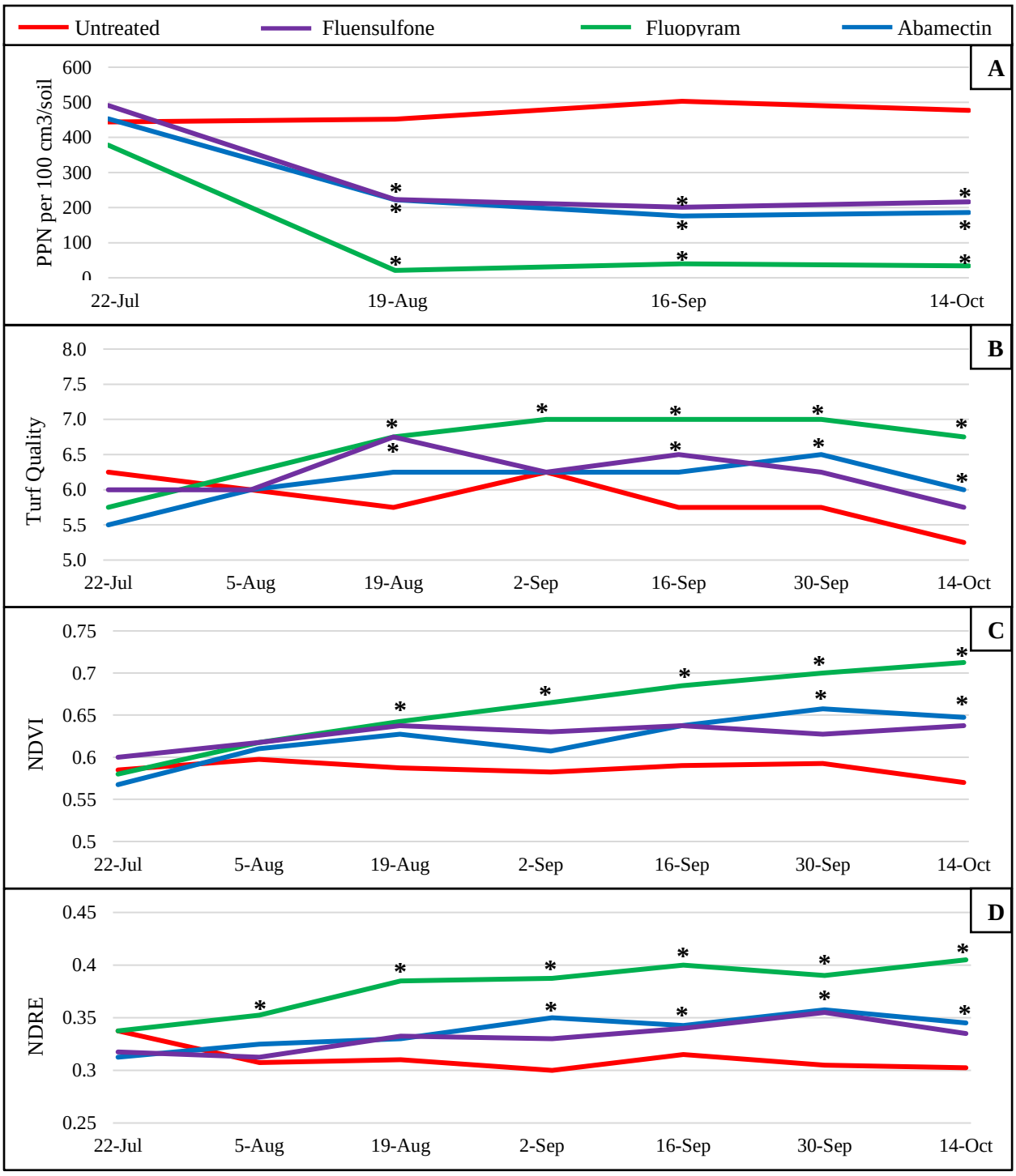

Figure 5.4: Nematicide effects on total plant-parasitic nematode population density (A), visual turf quality (B), NDVI (C), and NDRE (D) at Montevallo Golf Course, Montevallo AL, 2019. *Different from the untreated control according to the pairwise comparison of each treatment to the untreated (Tukey-Kramer, $P \leq 0.05$).

Summary

This research's primary focus was on gaining a deeper understanding of plant-parasitic nematodes on turfgrass, and working towards improving current management strategies. These results clearly demonstrate the impact that plant-parasitic nematodes have on turfgrass. Monthly sampling on six turfgrass locations revealed that most turfgrass managers should sample for nematodes three times per year: early spring, mid-summer, and early fall. However, more frequent sampling may be warranted on turfgrass with a history of plant-parasitic nematodes at high population density levels. While this study identified seven genera of plant-parasitic nematodes on turfgrass in Alabama, and only two genera at high population density, previous studies have shown that more genera are likely present in the state (Sikora et al. 2001). It is also important to recognize that each of these genera have the ability to affect turfgrass at varying population density levels, so understanding for each genus what population density may lead to turfgrass damage is important.

A large collection of PGPR strains were also evaluated throughout this research for root-knot nematode management on turfgrass, with multiple individual strains showing promise for nematode management. Of the 104 PGPR strains screened in this study, ten individual PGPR strains and Blend 20 all had above 40% mortality of *M. incognita in vitro.* Seven of these PGPR strains significantly lowered population density of *M. incognita* in greenhouse evaluations and five of these strains lowered *M. incognita* population density in microplot trials. Three PGPR strains, one identified as *Stenotrophomonas rhizophila* and two identified as *Bacillus aryabhatti*, had a significant reduction of population density in both trials. This research is novel because there are currently minimal published research articles evaluating the potential of PGPR strains for nematode management on turfgrass. While these findings are very encouraging for the future

of biological control of nematodes on turfgrass, more studies on this PGPR collection are needed. This should primarily focus on field trials. It would also be beneficial to evaluate some of the top performing PGPR strains from this research as blends, and determine if that not only improves the potential population density reduction, but also may lead to a stronger increase in root growth.

Results from this study also indicate the value that NDVI and NDRE may provide for evaluating nematicide efficacy on plant-parasitic nematode infested turfgrass. This research showed a strong negative correlation between both NDVI and NDRE values with population density of multiple genera of plant-parasitic nematodes. As the population density of these nematodes declined, NDVI and NDRE values significantly increased. Strong correlations were also observed between NDVI, NDRE, and visual turfgrass ratings. As visual turfgrass ratings improved, a significant increase in both NDVI and NDRE was observed. This is beneficial, because having NDVI and NDRE ratings to go alongside visual turfgrass ratings can help limit any inconsistencies that occur between visual ratings. Moving forward, there are additional studies that could build upon these findings. Primarily, full season and multi-year field trials need to be conducted evaluating nematicide programs that turfgrass managers have available for plant-parasitic nematode suppression. While this research showed that there is a correlation between NDVI and NDRE for a single nematicide application, it is important to understand how that trend may go over multiple years as the nematode population changes.