DEVELOPING INTEGRATED PEST MANAGEMENT FOR RHODESGRASS MEALYBUG (HEMIPTERA: PSEUDOCOCCIDAE) ON GOLF COURSE PUTTING GREENS

by

ROBERT MICHAEL WOLVERTON

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ABSTRACT

Rhodesgrass mealybug, *Antonina graminis* Maskell (Hemiptera: Pseudococcidae) is an invasive pest of turfgrass. Their feeding on putting greens causes yellowing and eventual turfgrass mortality which affect the aesthetics and playability of golf. Because little is known on the phenology and integrated pest management for *A. graminis*, a series of studies were conducted to develop integrated pest management (IPM) tactics for *A. graminis* on the putting greens. The phenology data from 2019 to 2022 suggest that the *A. graminis* population remained at low densities in the spring until June or July, when high densities of all stages of *A. graminis* were observed. *Antonina graminis* population declined by October to nondetectable levels. In 2021 and 2022, among six trap types evaluated to determine a potential sampling tool for crawlers on the putting greens, the paper-folded sticky card method consistently collected greater numbers of crawlers after placing sticky cards on the putting green surface than those sampled from grass plugs or other remaining methods. Acephate, flupyradifurone, imidacloprid and thiamethoxam achieved greater and more consistent reduction in *A. graminis* abundance than other insecticides in multiple experiments. A single application of flupyradifurone during the early summer (June or July only treatments) did not reduce the *A. graminis* densities or improve turfgrass quality, whereas a single application in late summer (August only treatment) reduced *A. graminis* densities and improved turfgrass quality for at least 30 d post-application. The results show that applying a high dose of N fertilizer improved turfgrass quality without increasing *A. graminis* densities on the golf course green. Although flupyradifurone application reduced *A. graminis* densities regardless of N fertilizer treatments, suppression of *A. graminis* densities improved at the high fertilizer dose with flupyradifurone. The numbers of *A. graminis* were not significantly affected by rolling treatment in the 2021 and 2022 experiments. Similarly, the sand topdressing alone had no significant effect on the *A. graminis* densities on the putting greens. The numbers of *A. graminis* were significantly lower for the insecticide (thiamethoxam) and combination of sand topdressing + insecticide treatment than for the nontreated treatment.

INDEX WORDS: *Antonina graminis,* flupyradifurone, thiamethoxam, rolling, topdressing, application timing, insecticide efficacy, phenology, crawler emergence

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CHAPTER 1

LITERATURE REVIEW AND INTRODUCTION

Rhodesgrass mealybug, *Antonina graminis* Maskell (Hemiptera: Pseudococcidae) is a serious pest of grasses (Poaceae). Native to Asia, it was accidentally introduced to the USA and was first detected in Texas in the 1940s. Soon, it emerged as a serious pest of pastures. By the 1950s, *A. graminis* had emerged as an important pest on golf course putting greens in Florida. Golf course superintendents are charged with the maintenance and management of the turfgrass surfaces within their facilities. Because the aesthetic appearance of the golf course is the major responsibility of superintendents, they spend most of their time maintaining quality turfgrass. Pest problems develop in the golf course due to poor pest management decisions, lack of adequate resources for management, misdiagnosis of problems, and infestation by invasive species. *Antonina graminis* infestations on golf course putting greens could be easily misdiagnosed as initial feeding symptoms, such as yellowing and stunting are often attributed to abiotic or biotic stress to turfgrass from inadequate irrigation, insufficient nutrient availability, or disease. These feeding symptoms become severe as stunting of turfgrass and yellowing turn into browning and mortality when *A. graminis* densities increase typically during the fall. Management tactics, such as biological control and cultural control, are helpful but have not proved effective in suppressing *A. graminis* on putting greens. There was limited information on effective insecticides for *A. graminis* management on the golf course. Therefore, there is a critical need to develop integrated pest management (IPM) for *A. graminis* on golf course putting greens.

Classification

In 1897, Maskell first described this insect as *Sphaerococcus graminis*, sp. nov., after a collection trip to Hong Kong, China (Maskell 1897). In 1898, Maskell changed the name to *Chaetococcus graminis*, sp. nov. (Maskell 1898). In 1899, T. D. A. Cockerell attempted to systematically position *Chaetococcus* as a subgenus of *Kermicus* based on the number of hairs around the anal ring (Cockerell 1899). In 1899, Popenoe and Parrott described a new genus of coccid *Antonina* that associated with grasses, and they named a new species, *Antonina graminis*, a common mealybug on grasses (Popenoe and Parrott 1900). In 1903, Mrs. Maria E. Fernald completed the Catalogue of the Coccidae of the world, and the nomenclature of *Antonia graminis* Maskell was first formalized (Fernald 1903). Collection efforts by E. E. Green during the same period thoroughly described a 'scale' referred to as *Antonina indica* (Green 1908). Another junior synonym associated with *A. graminis* included *Antonina littoralis* (Cockerell and Bueker 1930), and *Antonia graminis* was misspelled by Beardsley in 1960 (Aaron 2013) .

Host association

Antonina graminis is primarily a pest on grasses. It was introduced in the USA in 1942 when Rhodesgrass (*Chloris gayana* Kunth) samples from the King Ranch in Kingsville, Texas were examined (Riherd 1950, Chada and Wood 1960). Later, it was reported as a pest of grasses on rangeland, lawn, and golf courses (Wene and Riherd 1950, Chada and Wood 1960). In 1936, Schmidt reported children being repeatedly stung by honey bees on the turfgrass near the University of Hawaii. These bees were foraging on honeydew on the turfgrass infested with *Antonina indica* (Schmidt 1937).

In 1950, Dr. D. W. Clancy reported the presence of *A. graminis* in Hawaii since 1910 and an encyrtid wasp, *Anagyrus antoninae* Timberlake parasitizing on this pest. Later, colonies of the

A. antoninae wasps were shipped to the Weslaco research facility in Texas from Hawaii. The biological control efforts were led by Dr. Paul Riherd to manage *A. graminis* in pasture grasses (Clancy 1950).

By the early 1950s, *A. graminis* became a nuisance pest on golf courses, especially on the putting greens. *Antonina graminis* was reported attacking bermudagrass [*Cynodon dactylon* (L.) Pers], St. Augustinegrass [*Stenotaphrum secundatum* (Walt) Kuntze] and zoysiagrass (*Zoysia japonica* Steud). This pest was a serious problem, especially on bermudagrass putting greens in Rio Grande Valley, Texas (Henry 1950), four courses in greater Miami, Florida, and one in Palm Beach, Florida (Lawrence 1952). The United States Golf Association also reported problems on golf courses related to a scale from South Texas to Dallas (Ferguson 1953). The superintendent of Houston country club used a vertical mower to remove *A. graminis* from the turfgrass (Ferguson 1954).

Outside the USA, *A. graminis* was first reported as the felted coccid attacking lawns in Australia (Brimblecombe 1966). It was also reported on the golf course putting greens in Caesarea, Israel (Berlinger and Barak 1981), Japan (Kawazoe et al. 1987), and on zoysiagrass in Korea (Gyu-Yul and Kim 1994).

Beginning the mid-1950s, university research focused on developing turfgrass varieties adapted for golf course use (Burton 1964, Burton and Powell 1971, Burton 1991, Reasor et al. 2016, Baxter and Schwartz 2018). Based on many trials through many years, ultradwarf bermudagrass [*C. dactylon* (L.) Pers × *C. transvaalensis* Burtt-Davy] varieties, such as 'Tifgreen' (Burton 1964), 'Tifdwarf' (Burton 1966) and 'TifEagle' (Hanna and Elsner 1999) with finer leaf texture suitable for use on golf course putting greens were developed. 'TifEagle' was the popular variety currently planted on many putting greens. It was developed after genetic modification forcedly controlled by cobalt-60 gamma-radiation on hybrid bermudagrass, 'TifWay II' (also referred to as 'TW-72') (Hanna and Elsner 1999).

Lifecycle, ecology, and distribution

In 1960, the biology of *A. graminis* was studied in detail by Drs. H. L. Chada and E. A. Wood at Texas Agricultural Experiment Station and USDA in Weslaco, Texas. *Antonina graminis* is parthenogenetic. The eggs develop into females without mating with males rarely observed. The adult female produces 150-200 eggs, depending on the season. First instar larvae, referred to as crawlers, are reproduced ovoviviparously (hatch within the oviduct and give live birth) and are the only fully mobile stages of *A. graminis*. The crawlers can be dispersed by walking, wind, water, or phoresy. After 10 d post-emergence, the crawlers settle and begin feeding. The crawlers are thigmotropic, seeking refuge within the crown area or beneath a leaf sheath at a node of the grass stem. Once settled, the crawlers feed on photosynthates from the plant phloem after inserting its thread-like mouthparts into the plant. They molt into the second nymphal stage, lose their legs, and become sessile. The second and third nymphal stages, and adults produce a white, waxy coating on their purple-colored bodies. Excess water and honeydew are excreted from a characteristic white filamentous anal tube. The only easily distinguishable character between the nymphs and adult females is size. Adults average 3 mm × 1.5 mm (length ×width) and are dark maroon colored. A single generation is completed in 60-70 d, dependent on temperatures. The time to complete a generation in the spring, summer, and fall is generally half the time it takes in the winter. In southern coastal regions of the USA, it has five generations each year (Riherd 1954, Chada and Wood 1960, Brimblecombe 1968).

Antonina graminis is a subtropical pest that prefers the latitudes within the 32nd parallel and prefers 29-32 °C. However, between 38-42 °C, the activity of *A. graminis* declines and the insect dies if exposed to 42 °C for 24 h. Similarly, its development arrests at 0 °C, and it does not survive if exposed to -2 °C for 24 h (Chada and Wood 1960). Currently, *A. graminis* is distributed worldwide in 98 countries (Morales et al. 2016). In the USA, *A. graminis* is reported from Alabama, Arizona, California, Florida, Georgia, Louisiana, Mississippi, New Mexico, South Carolina, and Texas (McKenzie 1967, Chada and Wood 1960, BenDov 1994, Hendricks and Kosztarab 1999).

Damage

The feeding damage to turfgrass appears as the yellowing of the older leaves. The yellowing expands to irregular patches on the putting greens. The damage is often misdiagnosed as abiotic stress, such as localized dry spots or lack of fertility. These abiotic stressors are most prevalent during periods of extreme drought (Ferguson 1953, Watschke et al. 1995, Vittum 2020). When damage to turfgrass is suspected, it is often misdiagnosed as fungal infections, other insects, or nematode activity (Lawrence 1952, Wolverton personal observation.). Signs of *A. graminis* infestations include the foraging activity of honey bees, predatory wasps, and ants clustered on the turf surfaces, especially on the infested patches of turfgrass with *A. graminis* (Schmidt 1937, Watschke et al. 1995, Helms and Vinson 2002, 2008). Eventually, as the *A. graminis* densities build up, the turfgrass on the putting green turns brown. Dead turfgrass is often recovered from the brown areas of the putting greens. The impact of damage can be observed in the following seasons.

Management

Host range and host plant resistance

Antonina graminis can attack grasses (Poaceae) in ~60 genera and 120 species. It was also reported on members of Cyperaceae, Euphorbiaceae, and Orchidaceae (Morales et al. 2016). On golf courses, the major grass species affected are buffalograss [*Buchloe dactyloides* (Nutt) Engelm], bermudagrass, centipedegrass [*Eremochloa ophiuroides* (Munro) Hack], tall fescue (*Festuca arundinacea* Schreb), seashore paspalum (*Paspalum vaginatum* Swartz), bahiagrass (*Paspalum notatum* Flugge), kikuyugrass (*Pennisetum clandestinum* Hochst), St. Augustinegrass and zoysiagrass. These grasses were identified as important for the golf course industry (Reinert et al. 2009, Reinert and Vinson 2010). From varietal experimentation, bermudagrass is more susceptible to *A. graminis* infestation and damage than kikuyugrass, is more susceptible than St. Augustinegrass followed by buffalograss, zoysiagrass, centipedegrass, seashore paspalum, bahiagrass and tall fescue (Reinert and Vinson 2010). When the cultivars of bermudagrasses were compared, the *A. graminis* infestations were greater on 'TifEagle' than on 'Patriot' and 'Tifgreen' (Reinert et al. 2009).

Biological control

Classical biological control is used as an important tactic to manage *A. graminis* in the grass, especially in pastures in the USA. Many biological control agents from six families and 10 genera were introduced from native ranges of *A. graminis* worldwide. Most of them were parasitic wasps (Hymenopterans in Chalcididae and Encyrtidae), beetles (Coccinellidae and Cybocephalidae), and one midge (Cecidomyiidae) (García Morales et al. 2016). Two widely released and established species of biological control agents on *A. graminis* were *A. antoninae*, and *Neodusmetia sangwani* (Rao) (Riherd 1950, Dean and Schuster 1957, Narayanan et al. 1957, Questel and Genung 1957, Rao 1957, Dean et al. 1961, Schuster 1965, Schuster and Boling 1971, Mescheloff and Dubitzki 1975, Chantos et al. 2009, Filho et al. 2017).

Following the first report of *A. graminis* at the King Ranch in 1942, the economic impact of *A. graminis* on native rangelands was serious with widespread colonization (Schuster and

Boling 1971). In Hawaii, this pest was successfully suppressed on the lawn by *A. antoninae*. Following that discovery, live specimens of *A. antoninae* were shipped to Texas from Hawaii to establish it in rangeland grass infested with *A. graminis* (Clancy 1950). Unfortunately, *A. antoninae* performed poorly in Texas due to the hot, dry climate. On the rangeland, this parasitoid only thrived in the irrigation ditches and along stream banks (Riherd 1950, Riherd 1951, Dean and Schuster 1957). Other parasitoids introduced from France failed to establish due to the mismatch in climatic conditions between Texas and France (Dean and Schuster 1957). *Anagyrus antoninae* was shipped to Florida for *A graminis* suppression, and it was established and effectively suppressed *A. graminis*. This success was related to the hot and humid climate in Florida (Questel and Genung 1957).

In 1957, an encyrtid wasp, *Dusmetia sangwani* was observed attacking *A. graminis* in Bangalore, India (Dean et al. 1961). In 1959, samples of this newly described parasitoid, *Neodusmetia sangawani* (Rao) were shipped to the USDA Parasite Receiving Station in Moorestown, NJ (Dean et al. 1961, Rao 1965, Schuster and Boling 1971, Dean et al. 1979). *Neodusmetia sangwani* was an effective agent for controlling *A. graminis* (Schuster 1965). The apterous females of *N. sangwani* systematically colonized *A. graminis* on the rangelands. They were dispersed by phoresy and wind (Schuster and Boling 1971) or released by airplane (Schuster et al. 1971). Within three years, *N. sangwani* reduced the population of *A. graminis* below economic threshold levels in pastures (Schuster and Boling 1971, Dean et al. 1979). The effectiveness of this biological control was lauded as a prominent success story for biological control strategy and was described as ‘complete’ biological control and “The Classic for Texas” (Dean et al. 1979). *Neodusmetia sangwani* was also introduced into other countries that struggled with *A. graminis* infestations, such as Brazil in 1967 (Fihlo et al. 2017) and Israel in 1971

(Mescheloff and Dubitzki 1975). The follow-up evaluations of parasitoids in the continental USA confirmed the abundance and establishment of *N. sangwani* throughout the southern coastal areas of the USA (Chantos et al. 2009).

In contrast, a mutualistic relationship was reported with red imported fire ant, *Solenopsis invicta* Buren (Hymenopteran: Formicidae), where the foraging ants harvested honeydew secretions from *A. graminis* and in return, *A. graminis* received protection from parasitoids (Helms and Vinson 2002, Helms and Vinson 2008).

Chemical control

In 1947, oil emulsion insecticides were first used to manage *A. graminis*. However, the residual activity of oil emulsions did not last for more than 3 d (Wene and Riherd 1950, Richardson 1953). By 1953, chlordane, DDT, parathion, and systox were determined as effective insecticides for *A. graminis*. Chlordane, parathion, and systox effectively reduced crawlers. Additionally, parathion was effective on nymphal stages but was used with extreme caution on golf courses because of its toxicity (Henry 1950, Richardson 1953, Watson Jr. 1953, Ferguson 1954). However, in 1962, parathion was prohibited due to toxicity issues (Bernard 1962). In 1955, Chada and Wood (1960) tested systemic organophosphates, demeton (systox), schradan, and Geary E-20/86, and demeton and schradan were effective on *A. graminis* adults with residual activity. Because these insecticides demanded large amounts of irrigation post application (22,450 L per ha) for effective translocation within the grass, they were deemed impractical for large pasture production. Therefore, their uses were limited to lawns and golf course putting greens (Chada and Wood 1960). By 1971, more applicator-friendly formulations of organophosphates became available to managers, which included Akton, diazinon, chlorpyrifos, and leptophos. The insecticides were applied at 2, 4, and 6 weeks apart for one year (22

September 1971 to 23 September 1972). Most of the tested chemistries, at any interval, were successful in controlling *A. graminis* on Sunturf bermudagrass (*Cynodon magennissii* Hurcombe) (Murdoch and Mitchell 1976).

Research objectives

Objective 1: Phenology of Rhodesgrass mealybug (Hemiptera: Pseudococcidae) and evaluation of trap types for sampling crawlers in Georgia golf course putting greens.

The phenology of *A. graminis* has not been documented from Georgia golf course putting greens. Because feeding damage of *A. graminis* severely affects the aesthetics of putting greens, understanding the phenology of *A. graminis* on putting greens is necessary to develop management strategies. To develop management strategies, the temporal emergence of crawlers is determined; however, a sampling tool for *A. graminis* crawlers on putting greens has not been developed. Thus, this study aimed to determine (1) the phenology of *A. graminis* and (2) the best trap types for sampling crawlers on putting greens in Georgia. The results of this study will help us determine (1) the best period for management sprays and (2) the trap type that can be used to monitor the crawlers of *A. graminis* on putting greens.

Objective 2: Efficacy of selected insecticides in reducing Rhodesgrass mealybug density on golf course putting greens.

In 2017, little was known about effective insecticides that can be used to manage Rhodesgrass mealybug on the putting greens of the golf course. Previously, organophosphates were determined to be effective on *A. graminis,* but those insecticides are no longer available for *A. graminis* management on golf courses. Therefore, we conducted a series of experiments using currently available and novel insecticides on putting greens in golf courses to determine effective insecticides to manage *A. graminis*.

Objective 3: Effects of application timing of systemic insecticides on the Rhodesgrass mealybug (Hemiptera: Pseudococcidae) densities and turfgrass quality on golf course putting greens.

This research was designed to explore the best time to apply insecticide for effective suppression of *A. graminis* on golf course putting greens. Although systemic insecticides, such as flupyradifurone, have proven effective against *A. graminis*, their application timing during the growing season has not been studied. Thus, the study aimed to determine the effects of single and repeated applications of flupyradifurone on the densities of *A. graminis* and turfgrass quality. Ultimately, the aim was to determine limited early and late application of insecticide to develop an integrated pest management (IPM) strategy for *A. graminis* on the putting greens.

Objective 4: Effects of fertilizer and insecticide on *Antonina graminis* on golf course putting greens.

The putting greens are intensively managed golf course areas where fertilizers are routinely applied to maintain and enhance turfgrass quality, playability and aesthetics. It is unclear if nitrogen fertilizer use can be optimized to reduce *A. graminis* densities which can be effectively managed using systemic insecticides. The hypothesis is that *A. graminis* densities can be optimized by reducing nitrogen (N) fertilizer, which can be effectively managed using systemic insecticides. Thus, this study aimed to determine the effects of various levels of N fertilizer and flupyradifurone on the *A. graminis* population and turfgrass quality on the golf course putting green.

Objective 5: Determining the effects that light weight rolling and frequent sand topdressing have on Rhodesgrass mealybug viability on golf course putting greens.

Aside from the daily mowing, several other practices are employed to improve the surfaces of golf course putting greens, including sand topdressing and lightweight rolling. Lightweight rolling and sand topdressing are standard cultural practices on the golf course. Sand topdressing is the addition of a thin layer of sand and lightweight rolling is simply the use of a roller that compresses the turf surface. However, whether they can reduce *A. graminis* densities and provide additional suppression when combined with insecticide is unclear. Thus, this study aimed to determine the effects of (1) lightweight rolling and sand topdressing and (2) when combined with a systemic insecticide, thiamethoxam, on *A. graminis* densities on golf course putting greens. The assumption is that the semi-angular edges of sand and pressure can cause *A. graminis* mortality and can be effectively used for *A. graminis* management.

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CHAPTER 2

PHENOLOGY OF RHODESGRASS MEALYBUG (HEMIPTERA: PSEUDOCOCCIDAE) AND EVALUATION OF TRAP TYPES FOR SAMPLING CRAWLERS IN GEORGIA GOLF COURSE PUTTING GREENS

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Abstract Rhodesgrass mealybug, *Antonina graminis* Maskell (Hemiptera: Pseudococcidae) is an emerging pest of turfgrass in Georgia golf course putting greens. Because the feeding damage of *A. graminis* severely affects the aesthetics of the putting surface, it is necessary to understand the phenology of *A. graminis* on putting greens. To develop management strategies, the temporal emergence of crawlers is determined; however, a sampling tool for *A. graminis* crawlers on putting greens has not been developed. Thus, the objectives were to determine (1) the phenology of *A. graminis* and (2) the best trap types for sampling crawlers on the putting greens in Georgia. From 2019 to 2022, 10-20 turfgrass plugs were sampled from the putting greens at biweekly intervals from the spring to fall. The numbers of crawlers, nymphs, and adults of *A. graminis* were quantified from these plug samples. In the spring, the *A. graminis* densities remained low until June or July, then all stages of *A. graminis* increased. In the late fall and winter, *A. graminis* densities declined and remained low. The turfgrass quality improved temporally from April to June but progressively declined from the mid-to-late summer to fall. In 2021 and 2022, six trap types were evaluated for sampling crawlers on the putting greens. Significantly greater numbers of crawlers were sampled in the paper-folded sticky card method than in the turfgrass plug method. This information will be utilized to develop management strategies for *A. graminis* on the putting greens.

Key words *Antonina graminis*, sticky cards, bermudagrass

Rhodesgrass mealybug, *Antonina graminis* Maskell (Hemiptera: Pseudococcidae) is an important insect pest of warm-season turfgrasses in the southern USA (Riherd 1950, Riherd and Chada 1952, Chada and Wood 1960, Reinert et al. 2009). Native to Asia, *A. graminis* was accidentally introduced to the continental USA in 1942 (Riherd 1950) and is now distributed from the Carolina coast in the east to California in the west (Gracía Morales et al. 2016, Vittum 2020). The freezing winter temperatures restrict the northward range of *A. graminis* (Chada and Wood 1960). In Georgia, *A. graminis* populations are prevalent in southern and coastal regions (Joseph and Hudson 2019). *Antonina graminis* infest more than 120 species of grasses (Poacaea) (Chada and Wood 1960, Helms and Vinson 2000), including ultradwarf 'TifEagle' bermudagrass [*Cynodon dactylon* CL. (Pers) × *C. transvaalensis* (Burtt-Davy)] (Chada and Wood 1960, Berlinger and Barak 1981, Reinert et al. 2009, Reinert and Vinson 2010). *Antonina graminis* infestation can be misdiagnosed as stress symptoms, such as nutrient deficiency and inadequate irrigation (Watschke et al. 1995, Vittum 2020). The initial feeding symptoms appear as yellowing and stunted turfgrass, but gradually, the entire turfgrass turns from yellow to brown and quickly dies.

Antonina graminis reproduces parthenogenetically, where all individuals are females (Chada and Wood 1960). A female can produce 150-300 eggs (Chada and Wood 1960) during her lifetime. The eggs hatch ovoviparously to crawlers, the only mobile stage of *A. graminis* with appendages. The crawlers are thigmotactic and settle on nodes covered with leaf sheath. The settled crawlers insert their thread-like mouthparts into the phloem vessels, molt into second instars, and lose their legs (Joseph and Hudson 2019). These sessile instars produce waxy coatings on their bodies and secrete honeydew through a filamentous anal tube. They can reach reproductive maturity as adults within 60-70 d during optimal temperatures of 27 ºC and about three to four months in the winter (Chada and Wood 1960). *Antonina graminis* stages do not undergo diapause and are susceptible to near-freezing temperatures. This causes considerable population decline during the winter, especially in transition zones, such as southern and coastal Georgia and South Carolina. Temperatures exceeding 37 ºC will also reduce *A. graminis* survival (Chada and Wood 1960). However, in the coastal and southern Florida and coastal regions of the Gulf coast states, *A. graminis* undergoes up to five generations per year (Chada and Wood 1960, Dale 2017).

Understanding the phenology of *A. graminis* is critical for developing management strategies on putting greens, as most of the research was focused on pastures or nonturfgrass systems (Riherd 1950). *Antonina graminis* has been reported to affect golf course putting greens since the early 1950s in Texas, Louisiana, and Florida in the USA (Lawrence 1952, Riherd and Chada 1952, Ferguson 1953, Sander 1953, Watson Jr. 1953, Bernard 1962), Queensland in Australia (Champ 1961) and Caesarea in Israel (Berlinger and Barak 1981). Because the *A. graminis* population can cause mortality of turfgrass on the putting greens, it can affect the aesthetic appeal. Thus, it is critical to determine the phenology of *A. graminis* on the putting greens.

To manage piercing and sucking insects, such as scales, applying contact insecticides, such as horticultural oils or pyrethroids, targets the crawler stage, especially when crawlers begin to emerge after the egg hatch (Kosztarab 1996, Vafaie et al. 2020). Typically, the crawlers are monitored using double-sided sticky tapes wrapped around the stem or twig, or trunk of plants or trees (Dreistadt et al. 1994, Grafton-Cardwell and Reagan 1995, Taylor et al. 2002, Hodges and Braman 2004, Sazo et al. 2008). However, this sampling method does not directly apply to *A. graminis* monitoring as they colonize the turfgrass. To date, no sampling tool has been developed to monitor crawlers of *A. graminis* or other similar insect pests in turfgrass, especially on the putting greens. Thus, the objectives of this study were to determine (1) the phenology of *A. graminis* on the putting greens in Georgia and (2) the effectiveness of various trap designs for sampling crawlers of *A. graminis* on the golf course putting greens.

Materials and Methods

Study site and insects. From 2019 to 2022, experiments were conducted at the Key Golf Studio at Columbus State University (32.4976332, -84.9320184), Columbus, GA, USA. The putting greens at this facility were constructed with 30.5 cm deep sand and peat moss (17: 3) on a gravel layer for drainage, following the United States Golf Association's construction guidelines (USGA 1989). The putting greens in the facility were planted with 'TifEagle' bermudagrass [*Cynodon dactylon* CL. (Pers) × *C. transvaalensis* (Burtt-Davy)]. These putting greens had steady traffic from golfers all year round. The putting greens were mowed five times weekly using a riding mower (TORO® Greensmaster 3150 triplex mower, Bloomington, MN) or walk-behind mower (TORO® Greensmaster 1000 mower, Bloomington, MN), and heights between 2.3-3 mm were maintained. The greens were aerified using a Toro 648 (TORO® Bloomington, MN),with four tine block holders and 1.2 cm tines at 5 cm × 5 cm spacing twice yearly for adequate root growth and development. To reduce turfgrass thickness and remove excessive thatch, the putting greens were vertically mowed with a Toro triplex 3150 with 2 mm blades set at 5 mm spacing, ~2 mm deep, three times yearly.

Along with vertical mowing, the putting greens were sand topdressed with 310 sand (Unimin Sand, Butler, GA) at ~7 kg per ha. In addition, the research plots were treated with monthly preventative fungicides and wetting agents and irrigated, when necessary, using an overhead irrigation system to prevent wilt. The plant growth regulator, trinexapac-ethyl (Primo

Maxx (11.3%), Syngenta, Basel, Switzerland), was applied at 146 mL per ha every 14 d intervals from May to September every year. Urea nitrogen (Harrell's, Lakeland, FL) was applied from April to October at ~1.5 g N per m^2 or ~20 g N per m^2 per year. The mean temperatures at the experimental site from 2019 to 2022 were ~21 ºC from May to October and 12 ºC from November to April. The average precipitation received in the experimental site was 124.5 cm per year. Located along the fall line of the coastal plains and piedmont section of GA, Columbus does not trend towards longer-term freezing events in the winter. Thus, the *A. graminis* populations actively developed at a slow rate and successfully overwintered at low densities.

Sampling. Two putting greens were selected for this study and they were entirely infested with *A. graminis*. The selected putting greens were 80 m apart. Sampling was conducted on one putting green in 2019, and the other putting green was used from 2020 to 2022. In 2020, the putting greens were switched because the putting green used in 2019 developed unacceptable *A. graminis* damage.

The turfgrass samples were collected using a 53.3 cm (length) × 1.91 cm (diameter) soil core probe (SiteOne® Landscape Supply, Roswell, GA). This probe is referred to as a "grass plugger" in the current study. The turfgrass plugger was randomly pushed on the putting green surface about 5 cm deep into the soil profile. Initially in 2019, 20 turfgrass plug samples were collected from January to mid-April. Following crawler emergence in mid-May, 10 turfgrass plug samples were collected biweekly from May to late October when bermudagrass was actively growing. In 2020, 10 plugs were taken monthly from March until May 4th. On May 20, collection of five turfgrass plug samples per week were extracted from the putting green until crawler emergence on July 20. Once crawler emergence was detected, 10 turfgrass plug samples were collected up to October at 14 d intervals. In 2021 and 2022, 10 plugs were consistently collected every other week from February to October/November. The turfgrass plug samples were examined under a dissecting microscope (AmScope, Irvine, CA) at 10× magnification. Under the dissecting microscope, the numbers of crawlers, sessile nymphs, and adults were quantified per plug from each sampling date. An individual turfgrass plug was deemed as an experimental unit. Adults were pierced using an entomological pin to determine if they were alive, as reddish fluid indicates alive individuals, whereas yellow or brown dark viscous fluid or none indicates dead individuals (Chada and Wood 1960). Adults were carefully examined as they could be covered with black sooty mold. The turfgrass plug samples were collected on 24 January, 6 March, 1 April, 1 and 22 June, 8, 15 and 26 July, 19 and 26 August, 1 and 18 September and 1, 11 and 24 October 2019; 1 and 23 March, 20 April, 4, 20 and 27 May, 1, 8, 11, 15, 22 and 29 June, 7, 15 and 22 July, 7, 15 and 30 August, 13 and 25 September, and 16 October 2020; 28 February, 15 and 27 March, 19 and 28 April, 12 and 31 May, 14 and 28 June, 7 and 21 July, 5 and 28 August, 7 and 17 September, 1 October and 11 and 26 November 2021; and 17 February, 3, 22 and 31 March, 15 and 22 April, 4, 18 and 30 May, 15 and 27 June, 16 and 30 July, 15 and 28 August and 10 and 23 September, 8 and 23 October 2022. Weather data were obtained from www.underground.com as they were updated from the Columbus Metropolitan Airport Weather Station 32.46 °N, 84.99 °W (which is < 1 km from the study site) from 2019 to 2022. Mean monthly high, average, and low temperatures, and mean monthly precipitation were recorded.

Turfgrass quality. From 2019 to 2022, the putting greens were randomly photographed at various intervals. Three photos were collected using an iPhone 12 pro, at 1.5m focal length at any given time, and those photos were visually rated using a rating system developed by the NTEP (National Turfgrass Evaluation Program, Beltsville, MD) ratings (1-9), where 1 = poorest quality and 9 = outstanding quality (Morris 2022). The rating system was developed based on color, turfgrass density, and percentage of living ground cover. The photos of putting greens were captured on 16 May, 25 June, 1, 17 and 25 July, 19 and 26 August, 10 and 25 September, 13 October and 8 November 2019; 14 January, 27 March, 17 and 27 May, 19 and 29 June, 16 and 27 July, 16 and 25 August, 21 September and 3, 11, 19 and 28 October 2020; 10 March, 10 and 30 May, 3 June, 30 August and 9 September 2021; and 4, 19, 6, 16 and 28 June, 28 July 4, 19, 24 and 31 August and 2 and 10 September 2022.

Crawler sampling. Various sampling methods were designed based on the easiness of obtaining materials, preparation, deployment on putting green surface, cost of materials, labor, and exposure time. The sampling methods considered were sticky roller, grass clipping, macro photo or non-macro photo, Berlese funnel, paper folded sticky card, no-folded sticky card, and grass plugger (Figure 2.1). The description and deployment and evaluation procedures of each method are outlined in the following sections.

2021 experiment. In 2021, preliminary trials were conducted to determine which sampling methods should be systematically evaluated. Thus, six sampling methods were selected after preliminary trials and evaluated in 2021. These sampling methods were treatments, and they were (1) sticky roller, (2) grass clipping, (3) macro photo, (4) Berlese funnel, (5) paper folded sticky card, and (6) grass plugger. Three replicates of these sampling treatments were arranged in a randomized complete block design (RCBD) on a putting green surface. Each plot was 2 m^2 and treatments were randomly assigned within the plot. This experiment was repeated four times on 8 and 11 August, 1 and 4 September 2021.

For the sticky roller treatment, an 8.75 cm wide and 23 cm long lint roller tape (3M®, St Paul, MN) was attached to a paint roller handle. The roller was rolled on the putting green surface with one full roll in a random 2.5 × 2.5 cm^2 area was randomly chosen on the putting green surface (Figure 2. 1A). The number of crawlers stuck on the tape was counted. Using the sticky roll method, the crawlers could be quickly sampled from the putting green surface, and there was no need to leave any material behind on the green surface that may disrupt the play.

For the grass clipping treatment, the turfgrass clippings were collected within a replicated plot using a Toro® Greensmaster 1000 mower. The grass clippings removed from the putting green surface plot were collected in a bucket attached to the mower. The grass clippings were mowed from a 1 m × 0.53 m plot. From each replicated plot, four ~1 g of fresh grass clippings was placed on a 2.5 cm^2 area of a yellow sticky card (Alpha Scents Inc., Canby, OR) during each sampling date. The sticky cards were placed on a bench. After 48 h, the number of crawlers around the turfgrass clippings was quantified under a dissecting microscope at 10× magnification (Figure 2. 1B).

For the macro photo treatment, a 15× macro lens (Go Micro®, Tonsley, Australia) was attached to an iPhone 12Pro camera to capture a 2.5 cm × 2.5 cm photo. The camera was positioned ~1 cm focal length from the putting green surface (Figure 2. 1C). The camera was positioned randomly within the block area on the putting green surface, and three random photos were captured in each replicated plot area. The photos were evaluated, and the number of crawlers was quantified from these three photos.

For the Berlese funnel treatment, 11.5 cm diameter, 19.5 cm tall small Berlese funnels (BioQuip Products, Inc., 2845, Rancho Dominguez, CA) were purchased. These funnels were modified by placing a 1 mm × 0.5 mm mesh screen (Phifer Inc., Tuscaloosa, AL) inside the receiving container (Figure 2. 1D). Freshly cut turfgrass clippings (1 g) from each replicated plot were collected and immediately deposited on the mesh screen of a Berlese funnel (Figure 2. 1D).

The turfgrass clippings were removed from the putting green using a mower (as previously described, 'grass clippings'). On the top of a Berlese funnel, a 60 W incandescent bulb (Osram Sylvania, Wilmington, MA) was positioned as a heat and light source for 48 h. The crawlers moved down against the heated gradient and were collected in the jar underneath the funnel (Figure 2. 1D). The collected crawlers were stored in 70% ethanol. The number of crawlers trapped in the sampling jar was quantified.

For the paper-folded sticky card treatment, 10 cm × 2.5 cm sticky card (Alpha Scents Inc., Canby, OR) strips were prepared (Figure 2. 1F). On a sticky card strip, a 2.5 cm × 2.5 cm area was selected. Then two diagonal incisions were made on the 2.5 cm × 2.5 cm area to peel the nonstick paper covered on the sticky card. The 2.5 cm × 2.5 cm area was then exposed after rolling back the nonstick paper from the center to all four margins or sides of the square area (Figure 2. 1F). The nonstick papers were not completely removed from the sticky card square area when exposed to crawlers. These strips were randomly placed on the surface of the experimental plot on the putting green. The strips were exposed to crawlers for 24 h. The crawlers are mostly found along the margin (crease area) between the nonstick fold of paper and the sticky card (Figure 2. 1G). For evaluation, the paper folds were peeled off, and the number of crawlers from each side of the square on the sticky card was counted.

For the grass plugger treatment, three 1.9 cm core turfgrass plugs, 5 cm deep from the soil surface, were sampled. The turfgrass plug samples were collected using the grass plugger randomly within the replicated plot area. The turfgrass plug samples were carefully dismantled, and the number of crawlers was carefully quantified using a dissecting microscope at 10× magnification.

2022 experiment. In 2022, the experiment did not include sticky roller, grass clipping, and Berlese funnel treatments after assessing the user-friendliness of various methods and effectiveness in capturing crawlers in 2021. The anticipated end users of the monitoring methods will be golf course superintendents, and they are likely to adopt a rapid method that provides reliable data.

Four treatments were included in the 2022 experiment were: (1) photo [no macro lens], (2) no-folded sticky card, (3) paper-folded sticky card, and (4) grass plugger. The treatments were replicated four times with RCBD. For the photo treatment, two photos were captured. The phone device, used in 2021, was used again in 2022 to capture photos. However, the macro lens was not attached to the iPhone in 2022.

For the no-folded sticky card treatment, the procedure used for the paper-folded sticky card method in 2021 was utilized with some modifications. Sticky card strips (10 cm × 2.5 cm) were prepared as described for the fold-paper sticky card method (Figure 2. 1F). On a sticky card strip, instead of partially retaining the nonstick paper folds, the entire nonstick paper was removed, exposing 2.5 cm × 2.5 cm area (Figure 2. 1E) before deployment in the putting green surface. These sticky strips were randomly deployed by placing them on the surface of the replicated plot areas. After 24 h of exposure, the numbers of crawlers were carefully quantified under a dissecting microscope at 10× magnification. For the folded sticky card treatment, the procedure described in the 2021 experiment was repeated in 2022.

For the grass plugger treatment, a single turfgrass plug sample was collected in the 2022 experiment instead of three plugs sampled in 2021. The experiment was repeated four times on 30 May, 6 and 18 June and 28 July 2022.

Statistical analyses. SAS software (SAS Institute 2016) was used when statistical analyses were conducted on any data. For the phenology data, the crawlers, nymphs, and adults from 2019 to 2022 were not statistically analyzed. They were presented descriptively in Figure 2. 2 because the incidence and abundance of damaging stages of *A. graminis* (adults and nymphs) were mostly overlapping and concurrent when detected in the turfgrass plug samples. To determine the temporal decline pattern of turfgrass quality, turfgrass quality score data were subjected to a one-way analysis of variance (ANOVA) using the general linear model procedure (PROC GLM), where the date of capture was the treatment with three replications. The turfgrass quality score data were not transformed before analysis after checking the normality of the residual using PROC UNIVARIATE in SAS. The means were separated using Tukey's HSD method (α = 0.05).

To determine the effects of sampling methods on crawler captures, the numbers of *A. graminis* crawlers observed or collected in the various methods were subjected to ANOVA using the general linear model procedure (PROC GLM) in 2021 and 2022. In 2021, six methods were evaluated, whereas only four were evaluated in 2022 as treatments. The treatments were trap-type methods and replicated three (2021) or four (2022) times. The experiments were repeated four times in both years and analyzed separately. The *A. graminis* crawler data were log-transformed (ln[x +1]) before analysis. The residuals were analyzed using the PROC UNIVARIATE in SAS to check the normal distribution. The means were separated using Tukey's HSD method (α= 0.05). The means and standard errors of various treatments for turfgrass quality data and crawler data by trap type were calculated using PROC MEAN procedure in SAS and presented in the figures.

Results

Phenology of *A. graminis*. In 2019, the *A. graminis* population was low from January to May (Figure 2. 2). The temperatures gradually increased with limited precipitation events (Figure 2. 3). The *A. graminis* activity began in June and steadily increased in densities in the summer and fall until October. The temperatures also increased in the early summer and remained high until the late fall. In October, the temperatures steadily decreased (Figure 2. 3A), and a reduction in *A. graminis* densities was noticed (Figure 2. 2).

In 2020, temperatures increased gradually in the spring as compared to the rise in 2019 spring. The first crawler emergence was delayed by almost a month compared to the previous year. Once crawlers were detected, *A. graminis* densities rapidly and steadily increased in the summer until late fall. The precipitation was high in the summer of 2020 compared to 2019 (Figure 2. 3). A distinct number of *A. graminis* generations was not evident from the data, possibly because of overlapping densities of various stages (Figure 2. 2).

In 2021, high densities of nymphs and adults were observed in the winter (Figure 2. 2). Following cool temperatures in January (Figure 2. 3A), temperatures increased through March, with many precipitation events. A steady increase in temperature was observed in the summer. Similarly, *A. graminis* densities increased during the period. During October, *A. graminis* densities declined and never rebounded in 2021. During this period, a couple of cold fronts and heavy precipitations (> 19 cm) were recorded in Columbus, GA (Figure 2. 3B). A frost event was also observed in November. During 2021-22, the winter temperatures fluctuated but were colder than in previous years.

In 2022, the densities of *A. graminis* were low in late winter (Figure 2. 2). From February through April, precipitation was consistent. Temperatures increased gradually but steadily from

February to June (Figure 2. 3A). The *A. graminis* population spiked in July (Figure 2. 2) but moderated with the high temperatures (Figure 2. 3A). From the beginning of September, the temperatures steadily decreased (Figure 2. 3A). The *A. graminis* densities also declined in October (Figure 2. 2).

Turfgrass quality. In 2019 and 2020, the quality of putting greens improved from the spring to mid-summer, then deteriorated from the late summer to fall (2019: $F = 59.1$; df = 10, 20; $P < 0.001$; 2020: $F = 44.9$; df = 14, 28; $P < 0.001$; Figure 2. 4A and 2. 4B). In 2021, the quality of putting green improved in May and did not change much thereafter, although the quality remained substandard for the entire year ($F = 26.0$; df = 5, 10; $P < 0.001$; Figure 2. 4C). In 2022, the quality of the putting green remained above acceptable standards from May to August ($F = 9.3$; df = 11, 22; $P < 0.001$; Figure 2. 4D).

Method for crawler sampling. In 2021, in trial 1, significantly more crawlers were collected in the paper-folded sticky card treatment followed by the Berlese funnel treatment (~3× less) than in the remaining treatments (~8-10× less) (Table 2. 1; Figure 2. 4A). There was no significant difference between paper folded sticky card and sticky roller treatments. No crawlers were collected from the grass clipping treatment. In trials 2 and 3, the paper-folded sticky card treatment collected significantly greater numbers of crawlers than in the remaining treatments (Table 2. 1; Figure 2. 4B and 2. 4C). In trial 4, significantly greater densities of crawlers were collected in the paper-folded sticky card treatment than in the sticky roller treatment, followed by the grass plugger treatment (Table 2. 1; Figure 2. 3D). No crawlers were collected in grass clipping or the Berlese funnel treatments.

In 2022, in trials 1 and 4, the paper-folded sticky card and no-fold sticky card treatments collected significantly more crawlers than in the photo or grass plugger treatments (Table 2. 1;

Figure 2. 5A and 2.5D). In trials 2 and 3, the paper-folded sticky card treatment collected significantly greater numbers of crawlers than the no-fold sticky card treatment, followed by the remaining treatments. In all the trials, photo and grass plugger treatments did not record any crawlers (Figure 2. 5).

Discussion

A. graminis densities were substantially lower in the winter and early spring. Their densities did not increase until the beginning of June or July on the golf course putting green. The increases and decreases in *A. graminis* populations are likely in response to changing temperatures during the seasons, although cause and effect studies were not validated. In 2019 and 2021, when the spring temperatures were relatively greater than in the springs of 2020 and 2022, an early *A. graminis* population increase was observed in June. In contrast, in 2020 and 2022, *A. graminis* population gradually increased and reached high densities with a delay in July. The cold temperatures in winter can naturally reduce the population size of *A. graminis* (Chada and Wood 1960, Watschke et al. 1995). This suggests that milder winter temperatures (< 21 °C) may not trigger a rapid increase in *A. graminis* population in late spring and that increases are determined by the temperatures during the spring itself (Figures 2. 2 and 2. 3). Thus, it is critical to monitor the persistence of warmer (> 21°C) or cooler (< 21°C) temperatures in late winter and spring as it can determine the *A. graminis* population dynamics with early or delayed peak crawler emergence and subsequent population growth and damage in the summer and fall.

The results showed that the highest densities, of all stages, of *A. graminis* were collected in the June and/or July samples. This suggests that crawlers were active before nymphal captures, possibly during May or June, depending on spring temperatures. The *A. graminis* populations were high in July, August, and September in all years. The monthly temperatures in the summer remained high between 21 and 32 °C, and these conditions favor a spike in *A. graminis* populations. In the fall, however, when temperatures decreased below 18 °C, *A. graminis* populations declined and reached nondetectable levels. The turfgrass quality briefly improved in the late spring and early summer during the normal growing period for bermudagrass. However, a gradual decline in quality became noticeable from the mid-to-late summer into fall, associated with growing densities of *A. graminis* populations. Consequently, reduced densities of *A. graminis* in late September could be associated with the poor quality of the putting green turfgrass resulting from intense feeding from *A. graminis* populations in the summer. Similarly, biological control agents introduced for *A. graminis* control, such as *Anagyrus antoninae* (Timberlake) (Clancy 1950, Riherd 1950) and *Neodusmetia sangwani* Rao, (Rao 1957, Dean et al. 1979), were also found parasitizing *A. graminis* on the putting greens (RW unpublished data). Additional signs of *A. graminis* activity on putting greens are other insects, such as beneficial bees and wasps, foraging for honeydew secretions (Schmidt 1937, Watschke et al. 1995, RW unpublished data) as well as ants tending to *A. graminis* adults (Helms and Vinson 2002, Chantos et al. 2009). Thus, this phenology information of *A. graminis,* specifically from the putting greens, will critically help in developing effective integrated pest management (IPM) strategies, such as determining the application timing of effective insecticides (as identified by Joseph et al. 2021), and adequate amount or application timing of nitrogen fertilizers and plant stimulants. Clearly, any reduction in *A. graminis* densities would help maintain turfgrass quality, improve playability, and conserve beneficial insects on the putting greens.

The crawlers can be effectively monitored using sticky cards by placing them on the surface of the putting greens. The sticky cards with and without paper folds trapped greater densities of crawlers than other methods investigated, including turfgrass plugs. The turfgrass plug method can only effectively sample crawlers when they are present within the plugs, and prolonged exposure does not guarantee high captures. The distribution, prevalence, and movement of crawlers might be indirectly related to the time of the day (daytime or nighttime) when samples were drawn and prevailing abiotic factors, such as temperature and precipitation. The folded or no-fold sticky card methods were exposed to crawlers for 24 h on putting green surface. Thus, an extended exposure period and retention of visiting crawlers must be incorporated into the sampling method for an effective monitoring tool.

More crawlers were collected on the sticky cards with paper folds than without folds. The paper-fold sticky card likely provided more surface area for the crawlers to crawl around and interact than the no-fold sticky card. Moreover, crawlers of *A. graminis* exhibited thigmotropic behavior (Chada and Wood 1960). It is possible that those crawlers seeking refuge along the margins of the folded crease were trapped. This suggests that the paper-folded sticky card method is the best method for sampling crawlers among all methods evaluated. The sticky cards could be deployed on the putting green surface for a period convenient to the clientele, and it appears to have minimal disruption to golfers. The sticky card method was also easy to deploy and store for future evaluations. However, this method may not be useful during windy days if not secured firmly to the ground or if deployed during heavy rain or placed along heavily irrigated areas of the putting greens.

Other sampling methods, such as sticky roller, grass clipping, Berlese funnel, photo, and grass plugger, did not effectively capture or sample crawlers of *A. graminis* on the putting greens. Perhaps they were conducted too quickly or needed multiple repeats to ascertain the presence of crawlers. The golf course superintendents likely prefer methods that are easy to deploy and less cumbersome to evaluate crawler activity on the putting greens. These methods may not yield consistent or reliable crawler density data with time for pest management decisions. Some methods, such as Berlese funnel and turfgrass plug methods, require further steps in processing, such as cleaning the debris from the samples or training to distinguish other soil-borne arthropods from *A. graminis* crawlers. The turfgrass clipping method was relatively easy, as putting greens are mowed almost every day. However, mowing operations are typically conducted in the morning before the golfers begin to play, and crawlers may not be at their peak activity. The grass plugger method was adopted in the current study to determine the phenology of *A. graminis,* as this method consistently sampled sessile stages of *A. graminis*. However, compared to the sticky card method, the grass plugger method may not provide a reliable indication of crawler activity on the greens unless the crawlers are exceptionally abundant in spots on a given putting green and samples were drawn from those spots. Moreover, the grass plugger method will leave holes on the putting green surface, which could affect ball roll and playability even after filling the holes with sand.

Before the current study, no other study or record indicated the problems with *A. graminis* on golf courses in inland areas of southern Georgia (RW unpublished data). Thus, turfgrass samples were not routinely examined for *A. graminis* infestation as a possible causal agent for the loss of turfgrass quality. Because of their small size, *A. graminis* can easily be overlooked (Aaron 2013) and misdiagnosed (Lawrence 1952, Bernard 1962). The phenology data from 2019 to 2022 suggest that the *A. graminis* population remained at low densities in the spring until June or July, when high densities of all stages of *A. graminis* were observed. *Antonina graminis* population declined by October to nondetectable levels. In general, the quality of turfgrass on putting greens improved temporarily from April to June but progressively declined from the mid-to-late summer to fall as *A. graminis* densities spiked. In 2021 and 2022, among six trap types evaluated to determine a potential sampling tool for crawlers on the putting greens, the paper-folded sticky card method consistently collected greater numbers of crawlers after placing sticky cards on the putting green surface than those sampled from grass plugs or other remaining methods. Thus, crawler captures on sticky card should be further evaluated to determine the relationship between their captures on cards and application timing of contact insecticide for effective management of *A. graminis*. Similarly, more research is warranted to develop IPM tactics, utilizing the information developed in the current study, and minimizing the impacts on beneficial arthropods, such as parasitoids of *A. graminis* and pollinators and predatory arthropods foraging on honeydew.

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Table 2.1. Effects of trap type treatments on capture crawlers of *A. graminis* on the putting green surface of golf course.

Trial[a]	Experiment date	*F*	df	*P*
2021				
1	8 Aug	6.5	5,18	0.001
2	11 Aug	23.9	5,18	< 0.001
3	1 Sept	407.7	5,18	< 0.001
4	4 Sept	87.2	5,18	< 0.001
2022				
1	30 May	9.6	3,12	0.002
2	6 Jun	29.1	3,12	< 0.001
3	18 Jun	100.7	3,12	< 0.001
4	28 Jul	76.2	3,12	< 0.001

Trials were completed or evaluated immediately or 24 to 48 h post-deployment.

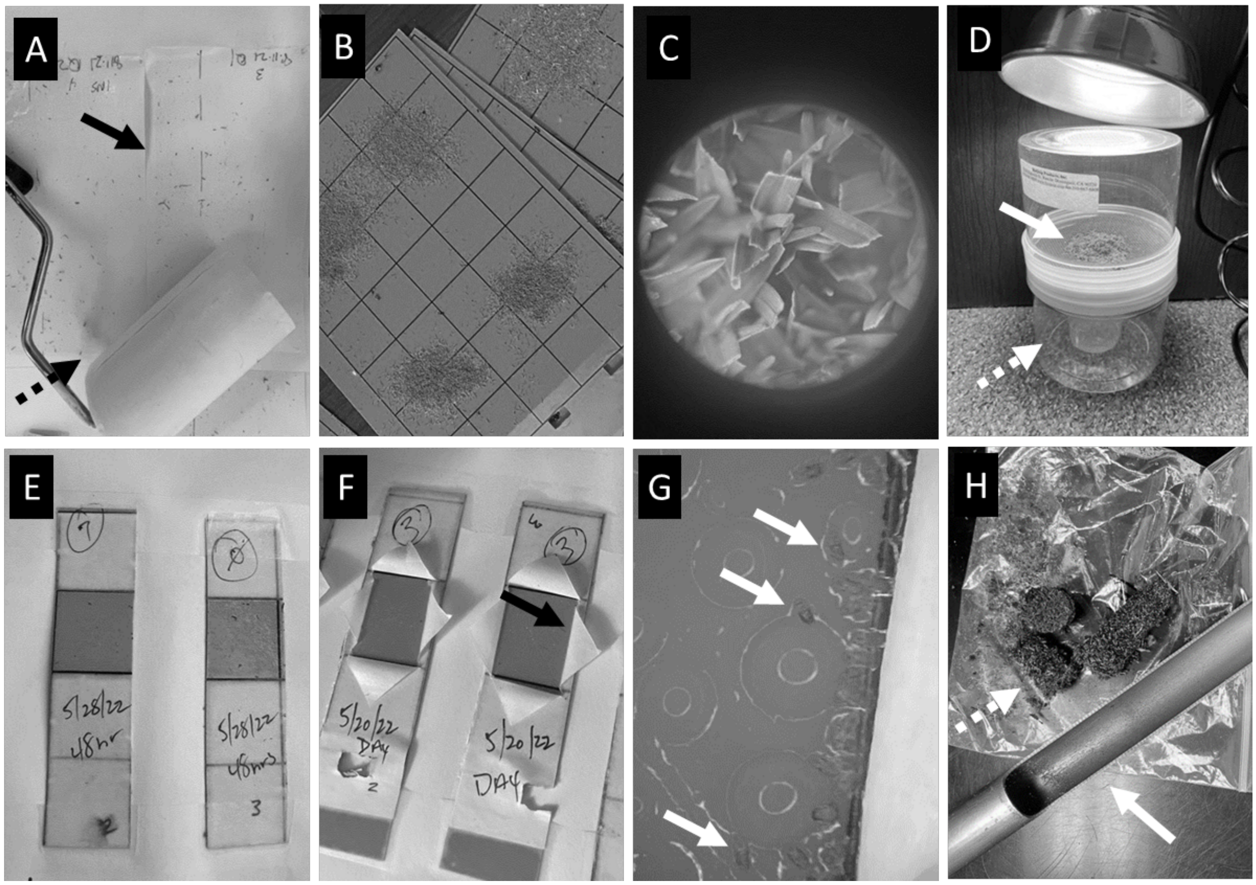

Figure 2.1. (A) sticky lint roller [solid black arrow, sticky roller coating; dotted black arrow, sticky paper with crawlers], (B) clippings on the yellow sticky card, (C) macro photo, (D) Berlese Funnel [solid white arrow, grass clippings; dotted white arrow, collection jar], (E) sticky card, (F) sticky card with paper rolled [black arrow, crawlers trapped on the edges], (G) crawlers trapped on sticky card margins [white arrows, crawlers trapped], and (H) plug samples with the plugger [solid white arrow, soil plugger; dotted white arrow, grass plugs].

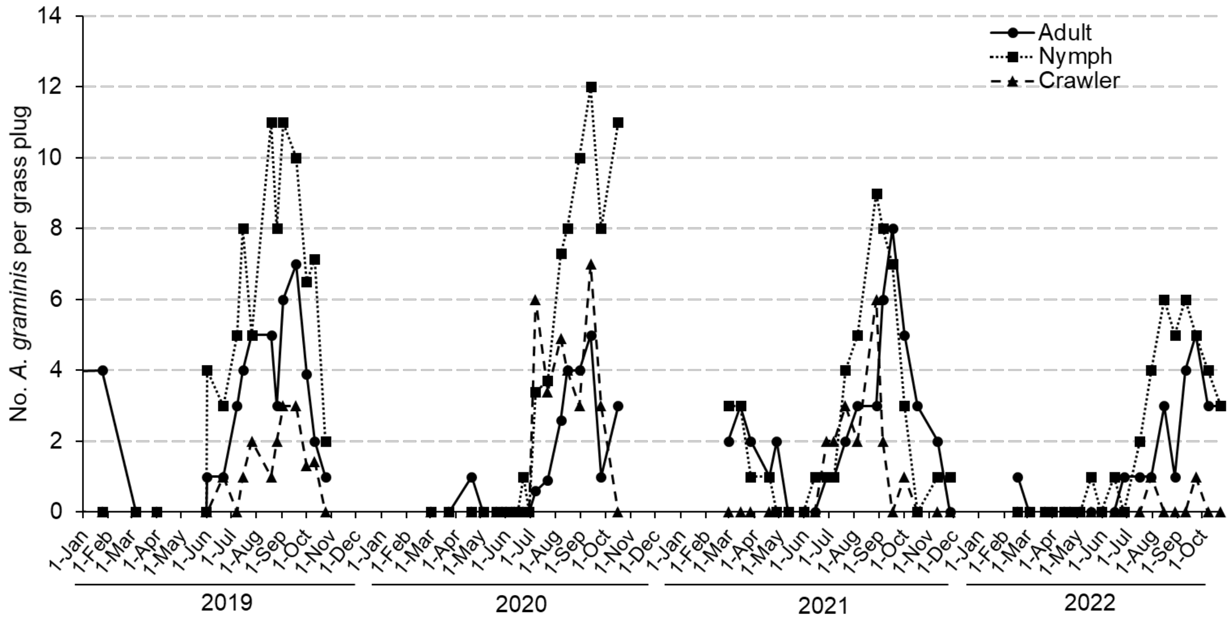

Figure 2.2. Mean densities of *A. graminis* life stages observed on ten turfgrass plug samples collected from a golf course green in Columbus, GA, from January 2019 to October 2022. At the beginning of the year, samples were collected at random intervals but later collected at biweekly intervals during the growing season.

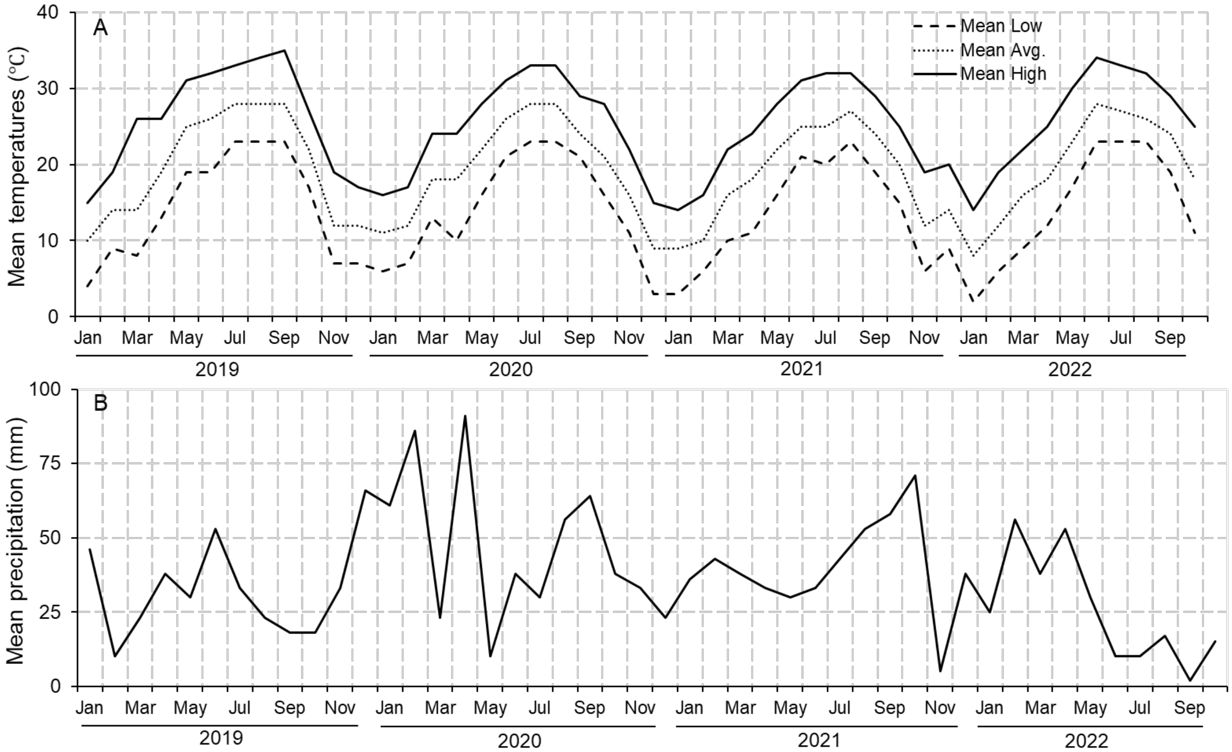

Figure 2.3. (A) Mean monthly high, average, and low temperatures, and (B) mean monthly precipitation. Weather data were obtained from www.underground.com and were updated from the Columbus Metropolitan Airport Weather Station 32.46 °N, 84.99 °W (which is < 1 km from the study site from 2019 to 2022.

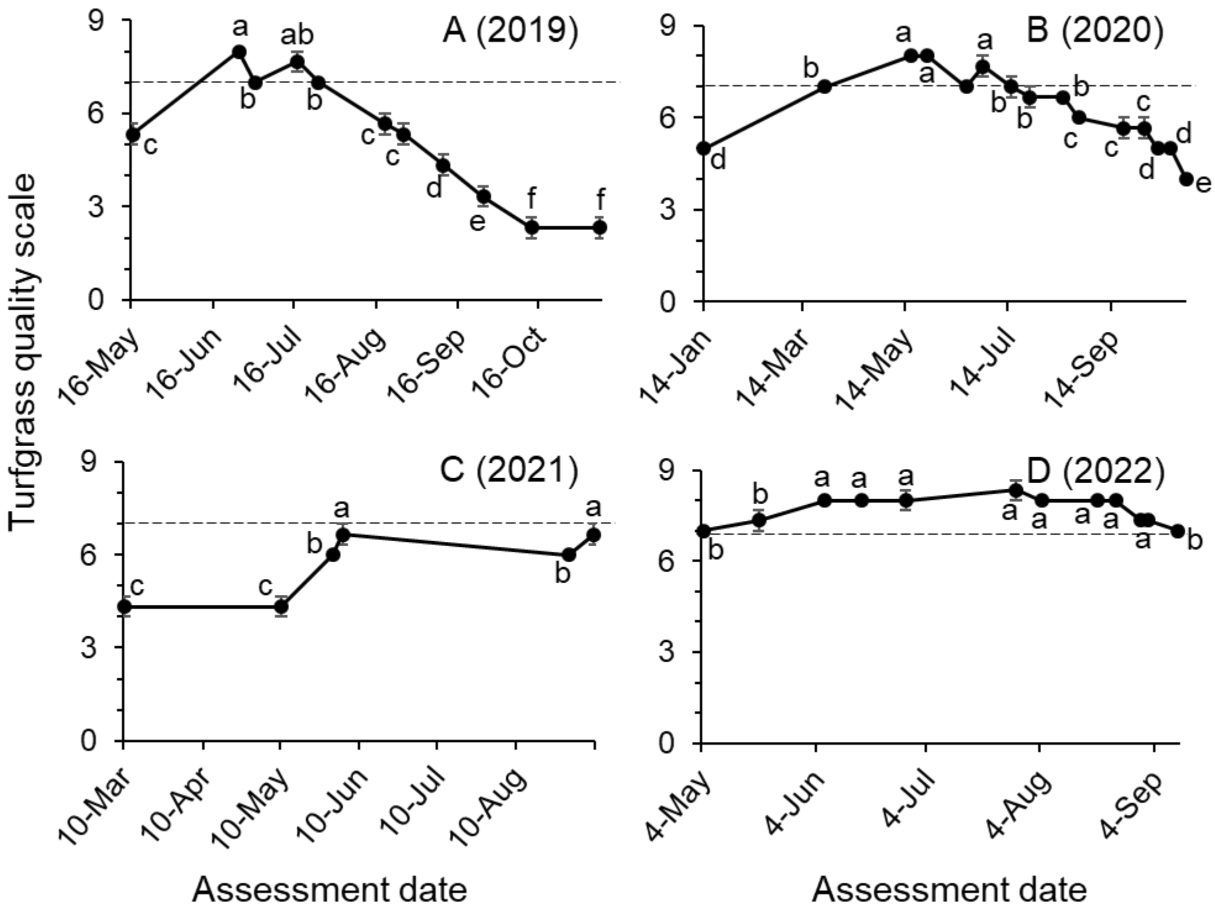

Figure 2.4. Mean (±SE) turfgrass quality score on (A) 2019, (B) 2020, (C) 2021, and (D) 2022 based on National Turfgrass Evaluation Program (NTEP). The putting green using plug sampling was photographed at various months of the year. Means ratings were calculated after evaluating three photos at any time of the year.

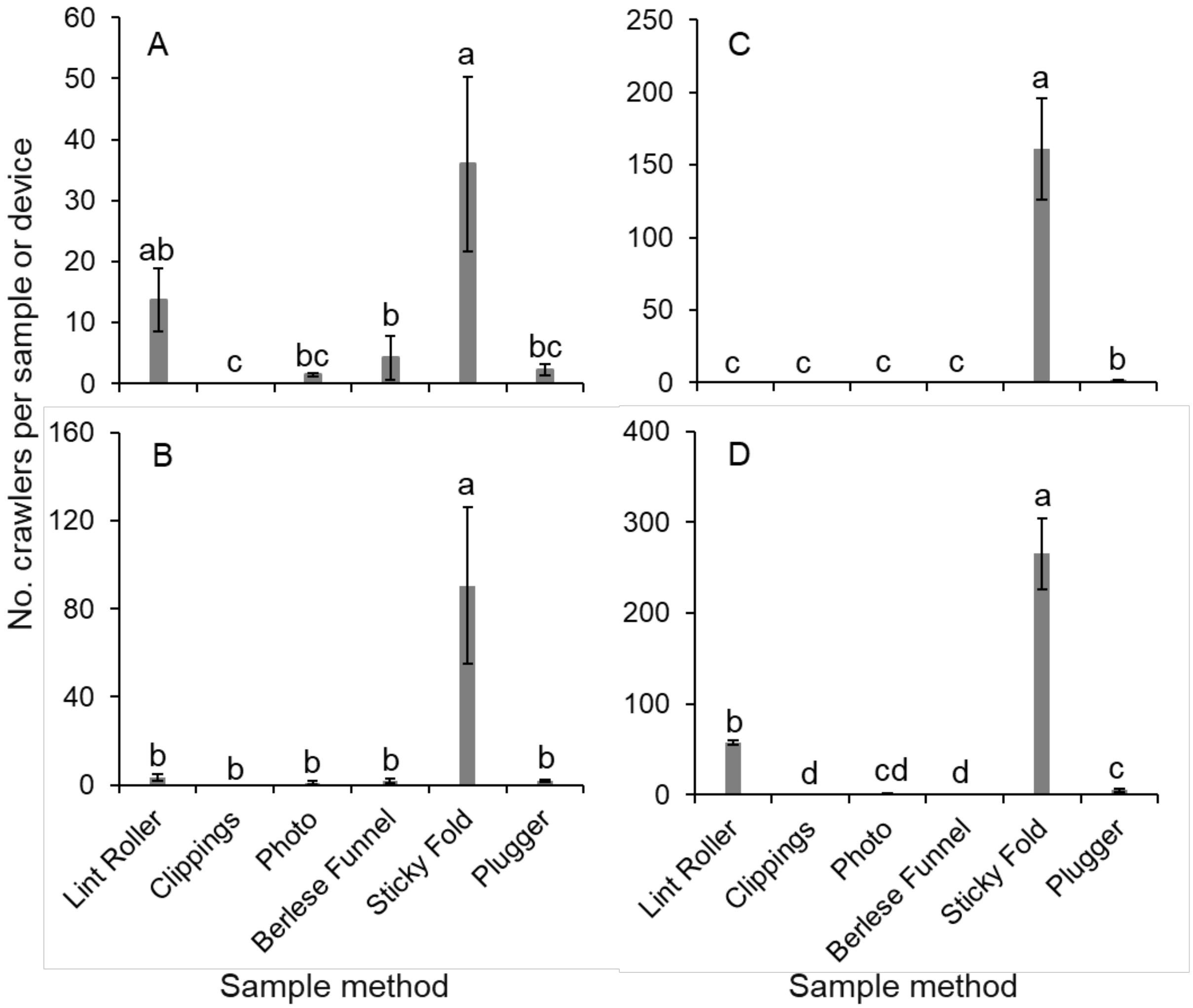

Figure 2.5. Mean (±SE) *A. graminis* crawlers per sample or tool for trials conducted on (A) 8 Aug [trial 1], (B) 11 August [trial 2], (C) 1 September [trial 3], and (D) 4 September, 2021 [trial 4]. Bars with the same letters are not significantly different (Tukey's HSD test, α = 0.05).

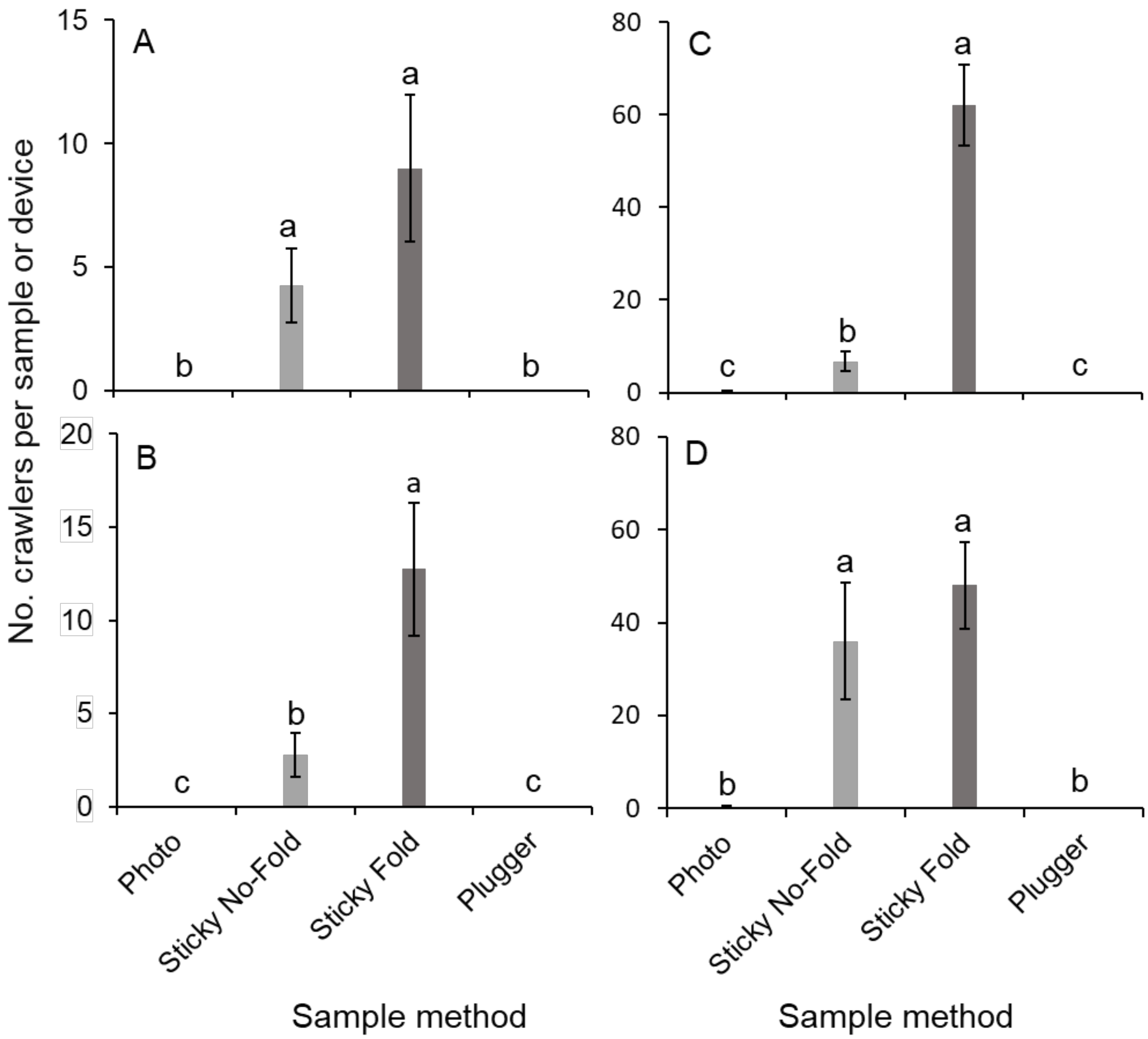

Figure 2.6. Mean (±SE) *A. graminis* crawlers per sample or tool for trials conducted on (A) 30 May [trial 1], (B) 6 June [trial 2], (C) 18 June [trial 3], and (D) 28 July, 2022 [trial 4]. Bars with the same letters are not significantly different (Tukey's HSD test, α = 0.05).

CHAPTER 3

EFFICACY OF SELECTED INSECTICIDES IN REDUCING RHODESGRASS MEALYBUG (HEMIPTERA: PSEUDOCOCCIDAE) DENSITY ON GOLF COURSE PUTTING GREENS

Shimat V. Joseph, Robert Wolverton, and Juang Horng Chong (2021)

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ABSTRACT Rhodesgrass mealybug, *Antonina graminis* (Maskell) (Hemiptera: Pseudococcidae), has long been a pest of warm-season grass species used for turf and hay. This species is benefiting from a recent resurgence as a pest of golf course putting greens. No efficacy information is currently available to aid in selecting insecticides for the management of rhodesgrass mealybug. This three-year study evaluated the efficacy of seven active ingredients (acephate, alpha-cypermethrin, cyantraniliprole, dinotefuran, flupyradifurone, imidacloprid, and thiamethoxam) applied at several concentrations to golf course putting greens in Georgia and South Carolina, United States. The goal of this study was to identify the most effective insecticides for rhodesgrass mealybug management. Acephate, flupyradifurone, imidacloprid and thiamethoxam achieved greater and more consistent reduction in rhodesgrass mealybug abundance than other insecticides in multiple experiments. Based on our results, long-term suppression of mealybug populations could only be achieved through repeated applications of these insecticides targeting crawlers or an integrated pest management program that complement chemical control. There are needs to further improve management efficacy against rhodesgrass mealybugs by identifying additional effective insecticides of different modes of action to complement acephate, flupyradifurone, imidacloprid and thiamethoxam, and methods by which the efficacy of these insecticides could be further improved.

KEY WORDS acephate, *Antonina graminis*, bermudagrass, flupyradifurone, thiamethoxam, imidacloprid

Rhodesgrass mealybug, *Antonina graminis* (Maskell) (Hemiptera: Pseudococcidae), is an invasive insect pest native to Asia and was first found in the United States in Texas in 1942 (Wood 1955). This species has now spread throughout the southern United States from southern North Carolina to southern California (Gracía Morales et al. 2016, Vittum 2020). Rhodesgrass mealybug can infest more than 100 grass species (Poaceae), including all warm-season grasses commonly used for pastures and turfgrass in the southern United States (Chada & Wood 1960, Baxendale & Shetlar 2012, Helms & Vinson 2000), such as bermudagrass (*Cynodon* spp.), St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze], centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.], buffalograss [*Bouteloua dactyloides* (Nutt.) J. T. Columbus], bahiagrass (*Paspalum notatum* Flüggé), and zoysiagrass (*Zoysia* spp.). It can also infest cool-season turfgrasses [such as tall fescue, *Lolium arundinaceum* (Schreb.) S. J. Darbyshire], grassy crops [such as sugarcane, *Saccharum officinarum* L., and sorghum, *Sorghum bicolor* (L.) Moench], and grassy weeds [such as large crabgrass, *Digitaria sanguinalis* (L.) Scop.].

Rhodesgrass mealybug populations in the southern United States had been successfully suppressed by the parasitoid *Neodusmetia sangwani* (Rao) (Hymenoptera: Encyrtidae), which was introduced from India as part of a classical biological control program against rhodesgrass mealybug in the 1950s through the 1970s (Riherd 1950, Schuster & Dean 1976, Dean et al. 1979, Filho et al. 2018). A native parasitoid (*Acerophagus* sp.) and an adventive parasitoid (*Pseudectroma* sp.) (both Hymenoptera: Encyrtidae) also contributed to population suppression (Chantos et al. 2009).

The pest status of rhodesgrass mealybug has experienced resurgence throughout the southern United States in the past ten years (Reinert & Vinson 2010). We also have noted increasing reports of rhodesgrass mealybug infestations and damage on turfgrass, particularly golf course putting greens (JHC & SVJ, personal observations). Although the precise reason for such resurgence is unknown, it may be related to the weakening of biological control. Chantos et al. (2009) reported that the recovery of *N. sangwani* was rather poor and sporadic, and the parasitism rate was very low from South Carolina to East Texas. The patchy distribution of *N. sangwani* may be related to the limited dispersal ability of the flightless parasitoid (thus, the parasitoid requires introduction), and that sod (which may contain both the mealybug and its parasitoid) from the original release sites had not been shipped throughout the distribution range of the mealybug (Chantos et al. 2009). The activity and effectiveness of *N. sangwani* may also be diminished by the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), which protects the honeydew-producing mealybugs (Helms & Vinson 2003, Chantos 2007, Chantos et al. 2009).

Rhodesgrass mealybug infestation causes yellowing, stunting, and thinning of bermudagrass putting greens on golf courses. The unreliability of biological control, extremely low tolerances for damage on golf course putting greens, and high susceptibility of bermudagrass to rhodesgrass mealybug necessitate the identification of effective insecticides for management programs on golf course putting greens. Many of the insecticides tested by Wene & Riherd (1950) and Richardson (1953) are no longer available for use on turfgrass. No study has evaluated the efficacy of insecticides against rhodesgrass mealybug since the 1950s. We conducted a series of field experiments in Georgia and South Carolina over three years to evaluate the efficacy of selected insecticides. The evaluated insecticides were selected because (1) they are either currently registered or may be registered for use on turfgrass, and (2) their active ingredients have been shown to be effective against other mealybug species on ornamental plants. Data gathered in these experiments will form the basis for building an effective insecticide management program against rhodesgrass mealybug on turfgrass.

Materials and Methods

Five experiments were conducted on golf course putting greens in Georgia (GA) and South Carolina (SC), United States, from 2018 to 2020 to evaluate the efficacy of various insecticides in reducing abundance in existing rhodesgrass mealybug populations. All treated greens were planted with a variety of ultradwarf hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. × *Cynodon transvaalensis* Burtt-Davy]. Insecticide treatments were made in August through September to target emerging crawlers.

Georgia; 2018-2020. Three experiments were conducted on infested "TifEagle" greens on one golf course in Columbus, GA, from 2018 to 2020. The putting greens were 4 yr old with a thatch layer that was approximately 2.5 cm deep. The putting greens are aerified annually, verticut once a month, and mowed to a height of 2.92 mm (0.11 in) 6 d per week. The putting greens were irrigated at least once daily, and the frequency of irrigation increased in the summer months.

Square plots (2.3 m^2 or 25 ft^2 in 2018 and 0.8 m^2 or 9 ft^2 each in 2019 and 2020) were randomly assigned to the insecticide treatments according to a randomized complete block design (RCBD) (Table 3. 1). Each treatment was replicated four times in 2018 and six times in 2019 and 2020. A modified vegetable oil surfactant, Dyne-Amic (Helena Chemical Company, Collierville, TN), was added to all insecticide solutions at the rate of 0.025% v/v in 2018, whereas no surfactant was used in 2019 and 2020. Broadcast sprays of insecticide solutions were applied thrice in 2018 (16 August, 16 September, and 16 October), once in 2019 (3 September), and twice in 2020 (11 and 25 September). Application volume was 0.09 L/m^2 (2.3 gallons/1000 ft^2) in 2019, and 0.08 L/m^2 (2 gallons/1000 ft^2) in 2018 and 2020. Applications were made with a hand-held compressed CO_2 sprayer (at 207 kPa or 30 psi) connected to a single spray wand fitted with one flat fan nozzle (TeeJet 8002VS; TeeJet Technologies, Springfield, IL). The experimental area was managed with a routine management schedule as indicated above except insecticides were not applied.

Four 4-cm diameter grass cores were collected randomly from each plot at 4, 8, and 10 wk after the first application (WAT) in 2018. Three 2-cm diameter grass cores were collected from each plot at pre-treatment and then weekly for 7 wk in 2019. Three 2-cm diameter grass cores were collected from each plot at pre-treatment and at 3, 4, 5, and 6 WAT in 2020. Grass cores were examined under microscopes at the Griffin Campus of the University of Georgia, and the total numbers of live mealybugs (adults and nymphs combined) were recorded for each plot on each sampling date.

South Carolina; 2019 and 2020. Two experiments were conducted in Florence, SC, on two separate "TifDwarf" bermudagrass putting greens constructed under specifications by the United States Golf Association (USGA 1989) approximately 28 yr ago. The turf was maintained at 3.5 mm (0.14 in) mowing height and irrigated nightly for 7 min (volume variable). The thatch layer was approximately 2.5 cm deep.

In both years, the golf course putting greens were divided into 14-m^2 plots (dimensions varied among plots because of the elliptical shape of the greens), and assigned to the treatments (Table 3. 1) under RCBD based on pre-treatment counts of adult mealybugs. Dyne-Amic was mixed in the solution of thiamethoxam (Meridian; Syngenta Crop Protection, Greensboro, NC) at the rate of 0.4% v/v in 2019, whereas surfactant was not used in other treatments in both years. Each treatment was replicated four times. No untreated alleys were prepared between the plots. Insecticide solutions were applied at an application volume of 0.08 L/m^2 (or 2 gallons/1000 ft^2)

twice in 2019 (9 August and 23 August) and 2020 (20 August and 3 September) to target emerging crawlers. Applications were made with a compressed CO_2 sprayer (at 207 kPa or 30 psi) connected to a hand-held 1.5-m (5-ft) spray boom fitted with four flat fan nozzles (TeeJet XR-8002VS). Care was taken not to broadcast insecticide solution into the adjacent plots. Treated putting greens were irrigated during the night of the application. No other insecticide was applied to the treated plots during the experimental period.

Three grass cores (5 cm diameter and 5 cm depth) were collected in a linear transect from the center of the treated plots before the treatment, and 4 and 8 WAT. The sampling period accounted for the entire mealybug life cycle of about 60 d per generation (Dale 2017). Each plug was taken about 30 cm from the next. The plug samples were put in a plastic container, stored on ice, and brought back to the laboratory for insect density assessment. The plug samples were washed clean of sand and organic matter before examination under microscopes. The total number of live mealybugs (adults and nymphs combined) on 10 randomly selected grass terminals (from the three grass cores) was recorded at each sampling date.

Statistical analyses. Adult, nymph, or total mealybug densities at each sampling date were $\log_{10}(x+1)$ transformed and analyzed with analysis of variance (ANOVA) under RCBD at $\alpha = 0.05$ (PROC GLM; SAS 2011). When a significant difference was detected, Fisher's LSD test was used to separate the raw means.

Results and Discussion

With the exception of dinotefuran (Zylam), all insecticide treatments applied to golf course putting greens in Georgia in 2018 significantly reduced the densities of live rhodesgrass mealybugs within 4 WAT (Table 3. 2). While flupyradifurone (Altus) achieved 75.3% reduction, and cyantraniliprole (Ference), thiamethoxam (Meridian) and their combination achieved 49.3-

69.9% reduction in mealybug densities at 4 WAT, no treatment achieved a significant reduction in mealybug densities when compared to the water-treated control at 8 and 10 WAT. The addition of cyantraniliprole to thiamethoxam might not be synergistic since the 4-WAT percentages of mealybug density reduction in thiamethoxam- and cyantraniliprole + thiamethoxam-treated plots were identical.

Foliar application of dinotefuran (e.g., Safari 20 SG) is one of the most effective management approaches in reducing mealybug populations infesting ornamental plants (e.g., Cabral & Hara 2015, Vafaie 2019, Vafaie & Pawlik 2020). In the Georgia 2018 experiment, however, dinotefuran (Zylam) achieved only 37% reduction in mealybug densities at 4 WAT. We suspect that the low efficacy in the 2018 experiment might have resulted from a lower-than-labeled application rate: tested application rate of 1169.2 ml/ha (or 16 fl oz/acre) vs. labeled rate of 5773.1 ml/ha (79 fl oz/acre). We used the label rate of dinotefuran in the Georgia 2019 experiment and compared its efficacy to alpha-cypermethrin (Fendona) and another commonly used neonicotinoid, imidacloprid (Merit). In this experiment, we did not observe a significant reduction in mealybug densities until 3 WAT, when dinotefuran and imidacloprid reduced the mealybug densities by 44% and 38%, respectively, when compared to the densities in the water-treated control (Table 3. 3). Alpha-cypermethrin did not achieve a significant reduction of mealybug densities when compared to those in the water-treated check at any time during this experiment. By 7 WAT, all treatments harbored mealybug densities that were similar to those in the water-treated check.

In the Georgia 2020 experiment, we evaluated a slightly lower rate of flupyradifurone and found that the insecticide treatment achieved 53.7% and 59.9% reduction in mealybug densities at 5 and 6 WAT, respectively (Table 3. 4). These percentages of density reduction are lower than those in the Georgia 2018 experiment. Acephate (Orthene), on the other hand, performed well in the Georgia 2020 experiment by reducing the densities of rhodesgrass mealybugs by 61.5% compared to the water-treated check at 3 WAT and continued to provide significant suppression of the mealybug population up to 6 WAT. It is important to note that acephate was applied at four times the label rates in the Georgia 2020 experiment. Acephate was evaluated at label rate 6101.6 g per ha (87.1 oz per acre) in experiments conducted in South Carolina in 2019 and 2020 (Table 3. 5 and 3. 6). We found that acephate at label rate (83.7% at 8 WAT in South Carolina 2020 experiment) performed as well as when the insecticide was applied at four times the label rate (79.2% at 6 WAT in Georgia 2020 experiment). The results suggest that acephate at label rate is sufficient in reducing mealybug densities, while likely has lower non-target impacts to natural enemies and users of the turfgrass system.

No significant reduction in rhodesgrass mealybug densities was observed in the South Carolina 2019 experiment because of low mealybug densities in the water-treated plots (Table 3. 5). It was not clear what caused the extent of reduction in the mealybug densities in the water-treated plots. The plots were not treated with insecticides before this experiment. While we expected a certain reduction in mealybug densities due to the naturally high mortality among crawlers, the reduction in the South Carolina 2019 experiment from month to month was greater than had been observed in other experiments in this study.

In the South Carolina 2020 experiment, we observed a significant reduction in mealybug densities in plots treated with imidacloprid and acephate (both applied at labeled rates) at 4 WAT (76.3% and 86.3% reduction, respectively) and 8 WAT (80.4% and 83.7% reduction, respectively) (Table 3. 6). Plots treated with dinotefuran and cyantraniliprole did not have significantly lower mealybug densities in this experiment.

The goal of this study was to identify insecticides that are effective in reducing rhodesgrass mealybug populations on golf course putting greens. Our experiments identified four insecticides with efficacy against rhodesgrass mealybug populations, namely acephate, flupyradifurone, imidacloprid and thiamethoxam. None of these products, however, achieved reduction of mealybug population that were sustained at a lower densities than the untreated plots in this 3-yr study. This observation suggests that long-term suppression of rhodesgrass mealybug population could only be achieved with repeated applications over multiple years or an integrated pest management program that complements insecticide use. Applications of contact insecticides should target crawlers, which have no wax deposits to protect them from contact with the insecticide solution. Acephate, flupyradifurone, imidacloprid and thiamethoxam are systemic insecticides and, therefore, they also impact mealybug survival through ingestion. It is not clear from this study if these insecticides act through the route of ingestion.

The necessity to make repeated insecticide applications results in a need to develop resistance management programs to delay insecticide resistance development in the rhodesgrass mealybug population. Among the products currently registered for turfgrass use, acephate is an organophosphate (IRAC Group 1B), and thiamethoxam and imidacloprid are neonicotinoids (IRAC Group 4A). Having only two modes of action severely limits our ability to develop a sustainable insecticide resistance management program. Although it is also identified as a potential management tool that may be registered for use on turfgrass in the United States, flupyradifurone (IRAC Group 4D) is of the same mode of action as the neonicotinoids, and therefore, it is not a suitable rotation partner to neonicotinoids. Additional studies will be needed to identify complementary insecticides with modes of action different from organophosphates and neonicotinoids. Some candidates for future evaluation include additional members of diamides (IRAC 28; such as chloratraniliprole, cyclaniliprole and tetraniliprole) and tetronic and tetramic acid derivatives (IRAC 23; such as spiromesifen and spirotetramat) that have already been registered for uses on turfgrass and/or ornamental plants. Alpha-cypermethrin and bifenthrin did not perform well against rhodesgrass mealybugs in this study and in our preliminary experiments (JHC and SVJ, unpublished data). These results suggest that pyrethroids are not a suitable management tool against rhodesgrass mealybug.

The effectiveness of dinotefuran in reducing rhodesgrass mealybug densities was inconsistent among the experiments; therefore, additional studies should be conducted to ascertain its efficacy. Similarly, additional studies are needed to further improve the efficacy of flupyradifurone, imidacloprid and thiamethoxam, potentially through improvement in application timing (targeting crawler emergence more precisely), frequency and rate, and the addition of a surfactant to the insecticide solution to improve solution coverage and absorption by plant tissues.

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Table 3. 1. Insecticides and application rates evaluated against rhodesgrass mealybug populations on golf course putting greens in Georgia and South Carolina in 2018 to 2020.

Active ingredient (a.i.)	Product (concentration of a.i.)	Manufacturer	Amount of product applied per ha (per acre)	Amount of active ingredient per ha (per acre)	State and year when product was evaluated
Acephate	Orthene TT&O WSP (750 g a.i. per kg)	AMVAC Chemical Corporation, Axis, AL	6101.6 g (87.1 oz) 24413.5 g (348.5 oz)	4576.2 g (4.08 lb) 18310.1 g (16.3 lb)	SC, 2019, 2020 GA, 2020
Alpha-cypermethrin	Fendona CS (30 g a.i. per L)	BASF Corporation, Research Triangle Park, NC	3186.2 ml (43.6 fl oz)	95.6 g (0.09 lb)	GA, 2019; SC, 2019
Cyantraniliprole	Ference (200 g a.i. per L)	Syngenta Crop Protection, Greensboro, NC	896.9 ml (12 fl oz) 1169.2 ml (16 fl oz)	175.5 g (0.16 lb) 234.0 g (0.21 lb)	GA, 2018 SC, 2020
Dinotefuran	Zylam Liquid (107 g a.i. per L)	PBI-Gordon Corporation, Shawnee, KS	1169.2 ml (16 fl oz) 5729.3 ml (78.4 fl oz) 5773.1 ml (79 fl oz)	124.7 g (0.11 lb) 611.1 g (0.54 lb) 615.8 g (0.55 lb)	GA, 2018 GA, 2019 SC, 2020
Flupyradifurone	Altus[1] (200 g a.i. per L)	BayerCrop Science, Saint Louis, MO	950.0 ml (13 fl oz) 1023.1 ml (14 fl oz)	190.1 g (0.17 lb) 204.7 g (0.18 lb)	GA, 2020 GA, 2018; SC, 2019
Imidacloprid	Merit 2F (240 g a.i. per L)	BayerCrop Science, Saint Louis, MO	1147.3 ml (15.7 fl oz) 1461.6 ml (20 fl oz)	275.0 g (0.24 lb) 350.3 g (0.31 lb)	GA, 2019 SC, 2020
Thiamethoxam	Meridian 25WG (250 g a.i. per kg)	Syngenta Crop Protection, Greensboro, NC	595.5 g (8.5 oz) 1190.9 g (17 oz)	148.9 g (0.13 lb) 297.7 g (0.26 lb)	GA, 2018 SC, 2019

[1]Altus (flupyradifurone) is not currently registered for use on turfgrass. The applications of Altus in the experiments represented off-label applications of the insecticide.

Table 3. 2. Georgia, 2018: Mean total number (± SEM) rhodesgrass mealybugs (adults + nymphs) per four 4-cm diameter grass cores.

Active ingredient	Application rate (per ha)	Mean density of live rhodesgrass mealybugs at weeks after the first application:		
		4	8	10
Water	-	36.5 ± 8.9 a	21.8 ± 6.6	20.0 ± 4.1
Cyantraniliprole	896.9 ml	18.5 ± 3.9 b	25.5 ± 4.3	18.3 ± 4.2
Thiamethoxam	595.5 g	11.0 ± 2.8 b	20.0 ± 3.7	13.3 ± 5.4
Cyantraniliprole + thiamethoxam	896.9 ml + 595.5 g	11.0 ± 1.2 b	14.0 ± 3.6	13.3 ± 4.0
Flupyradifurone	1023.1 ml	9.0 ± 2.5 b	17.3 ± 3.5	12.3 ± 5.5
Dinotefuran	1169.2 ml	23.0 ± 5.3 ab	24.0 ± 4.4	19.3 ± 11.8
Active ingredient[1]	*F*-value	4.13	2.33	1.19
	P-value	0.0147	0.0939	0.3586
Block	*F*-value	1.21	9.26	8.20
	P-value	0.3407	0.0010	0.0018

[1]$Log_{10}(x+1)$-transformed density data were analyzed with ANOVA under RCBD at $\alpha = 0.05$. Degree-of-freedom: d.f.(Active ingredient) = 5, d.f.(Block) = 3, and d.f.(Error) = 15. Means within the same column were separated with Fisher's LSD at $\alpha = 0.05$.

Table 3. 3. Georgia, 2019: Mean total number (± SEM) rhodesgrass mealybugs (adults + nymphs) per three 2-cm diameter grass cores.

Active ingredient	Application rate (per ha)	Mean density of live rhodesgrass mealybugs at weeks after the first application:					
		0	1	2	3	4	7
Water	-	10.2 ± 2.6	8.8 ± 1.4	12.2 ± 1.0	10.8 ± 1.6 ab	10.5 ± 2.0 a	4.7 ± 1.1
Alpha-cypermethrin	3186.2 ml	11.8 ± 3.6	7.7 ± 1.2	10.0 ± 1.7	12.3 ± 1.6 a	8.5 ± 1.0 ab	6.8 ± 1.4
Dinotefuran	5729.3 ml	7.2 ± 1.7	8.0 ± 1.9	7.7 ± 1.3	6.0 ± 1.0 b	6.7 ± 1.0 ab	5.0 ± 1.0
Imidacloprid	1147.3 ml	7.7 ± 1.9	6.3 ± 1.6	7.8 ± 1.9	6.7 ± 2.3 b	5.3 ± 1.1 b	5.5 ± 1.5
Active ingredient[1]	*F*-value	0.98	0.52	2.21	3.82	4.85	0.46
	P-value	0.4300	0.6737	0.1297	0.0324	0.0149	0.7162
Block	*F*-value	8.15	0.20	0.87	2.31	4.05	0.68
	P-value	0.0007	0.9568	0.5226	0.0955	0.0159	0.6488

[1]$Log_{10}(x+1)$-transformed density data were analyzed with ANOVA under RCBD at α = 0.05. Degree-of-freedom: d.f.(Active ingredient) = 3, d.f.(Block) = 5, and d.f.(Error) = 15. Means within the same column were separated with Fisher's LSD at α = 0.05.

Table 3. 4. Georgia, 2020: Mean total number (± SEM) rhodesgrass mealybugs (adults + nymphs) per three 2-cm diameter grass cores.

Active ingredient	Application rate (per ha)	Mean density of live rhodesgrass mealybugs at weeks after the first application:				
		0	3	4	5	6
Water	-	9.3 ± 1.4	14.8 ± 0.9 a	15.8 ± 2.6 a	27.0 ± 5.4 a	20.7 ± 2.2 a
Flupyradifurone	950.0 ml	9.8 ± 1.7	13.7 ± 0.6 a	10.5 ± 1.6 ab	12.5 ± 1.9 b	8.3 ± 0.6 b
Acephate	24413.5 g	14.7 ± 2.2	5.7 ± 0.7 b	6.2 ± 1.5 b	4.8 ± 1.0 b	4.3 ± 0.8 b
Active ingredient[1]	*F*-value	3.93	34.95	5.48	10.36	33.40
	P-value	0.0550	< 0.0001	0.0247	0.0037	< 0.0001
Block	*F*-value	4.20	0.63	0.46	0.82	0.45
	P-value	0.0256	0.6797	0.7983	0.5653	0.8058

[1]$Log_{10}(x+1)$-transformed density data were analyzed with ANOVA under RCBD at α= 0.05. Degree-of-freedom: d.f.(Active ingredient) = 2, d.f.(Block) = 5, and d.f.(Error) = 10. Means within the same column were separated with Fisher's LSD at α = 0.05.

Table 3. 5. South Carolina, 2019: Mean total number (± SEM) rhodesgrass mealybugs (adults + nymphs) on 10 randomly selected shoots.

Active ingredient	Application rate (per ha)	Mean density of live rhodesgrass mealybugs at weeks after the first application:		
		0	4	8
Water	-	39.8 ± 8.8	8.3 ± 3.7	3.3 ± 1.7
Flupyradifurone	1023.1 ml	31.0 ± 5.1	4.5 ± 2.1	2.5 ± 1.0
Acephate	6101.6 g	44.0 ± 14.4	4.3 ± 1.4	2.5 ± 0.3
Alpha-cypermethrin	3186.2 ml	34.3 ± 11.9	10.3 ± 3.5	4.0 ± 2.2
Thiamethoxam	1190.9 g	35.3 ± 10.6	4.3 ± 1.9	2.3 ± 0.9
Active ingredient[1]	*F*-value	0.08	0.04	0.07
	P-value	0.9855	0.8029	0.9894
Block	*F*-value	0.97	3.24	1.29
	P-value	0.4394	0.0603	0.3218

[1]$Log_{10}(x+1)$-transformed density data were analyzed with ANOVA under RCBD at $\alpha = 0.05$. Degree-of-freedom: d.f.(Active ingredient) = 4, d.f.(Block) = 3, and d.f.(Error) = 12. Means within the same column were separated with Fisher's LSD at $\alpha = 0.05$.

Table 3. 6. South Carolina, 2020: Mean total number (± SEM) rhodesgrass mealybugs (adults + nymphs) on 10 randomly selected shoots.

Active ingredient	Application rate (per ha)	Mean density of live rhodesgrass mealybugs at weeks after the first application:		
		0	4	8
Water	-	57.0 ± 26.2	35.0 ± 16.7a	24.5 ± 14.9 a
Cyantraniliprole	1169.2 ml	52.8 ± 17.4	26.3 ± 7.3 ab	28.8 ± 6.9 a
Dinotefuran	5773.1 ml	43.4 ± 15.2	21.3 ± 4.7 ab	16.0 ± 10.8 ab
Imidacloprid	1461.6 ml	39.5 ± 8.3	8.3 ± 2.3 b	4.8 ± 1.3 b
Acephate	6101.6 g	57.8 ± 24.2	4.8 ± 0.8 b	4.0 ± 1.8 b
Active ingredient[1]	*F*-value	0.57	18.38	4.15
	P-value	0.6899	< 0.0001	0.0245
Block	*F*-value	19.42	9.30	1.82
	P-value	< 0.0001	0.0019	0.1978

[1]$Log_{10}(x+1)$-transformed density data were analyzed with ANOVA under RCBD at α = 0.05. Degree-of-freedom: d.f.(Active ingredient) = 4, d.f.(Block) = 3, and d.f.(Error) = 12. Means within the same column were separated with Fisher’s LSD at α = 0.05.

CHAPTER 4

EFFECTS OF APPLICATION TIMING OF SYSTEMIC INSECTICIDE ON THE RHODESGRASS MEALYBUG (HEMIPTERA: PSEUDOCOCCIDAE) DENSITIES AND TURFGRASS QUALITY ON GOLF COURSE PUTTING GREENS

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Abstract Rhodesgrass mealybug, *Antonina graminis* Maskell (Hemiptera: Pseudococcidae) is an important insect pest on the putting greens of golf courses in the southern USA. *Antonina graminis* feeding damage appears as the yellowing of foliage, which gradually turns brown, affecting the aesthetics and playability of golf. Although systemic insecticides, such as flupyradifurone, have proven effective against *A. graminis*, optimal application timing during the growing season has not been determined. The objective of this study was to determine the effects of single and repeated applications of flupyradifurone on densities of *A. graminis* and turfgrass quality. The flupyradifurone treatments were applied at 1) June only, (2) July only, (3) August only, (4) June + July, (5) June + July + August, and (6) nontreated. In 2019 and 2021, the experiment was conducted on putting greens in Columbus, Georgia. A single application of flupyradifurone during the early summer (June or July only treatments) did not reduce *A. graminis* densities or improve turfgrass quality, whereas a single application in late summer (August only treatment) reduced *A. graminis* densities and improved turfgrass quality for at least 30 d post-application. Repeated applications of flupyradifurone until August (June + July + August treatment) significantly reduced densities of *A. graminis* and improved turfgrass quality. There was no significant difference between August only and June + July + August treatments on *A. graminis* densities and turfgrass quality. Thus, the late summer application of systemic insecticide is likely to provide adequate *A. graminis* control.

Key words: *Antonina graminis*, putting greens, flupyradifurone, systemic insecticide

Rhodesgrass mealybug, *Antonina graminis* Maskell (Hemiptera: Pseudococcidae) is an important insect pest of turfgrass putting greens in Florida, the southern region of Georgia, and the Gulf states of the USA (Joseph and Hudson 2019, Joseph et al. 2021). In 1954, *A. graminis* was first reported as a pest on golf courses (USGA 1954). Thereafter, there were many reports of *A. graminis* problems on golf courses in Florida and the southern belt of the Gulf coast region (Lawrence 1952, Watson Jr. 1953, Ferguson 1953, Bernard 1962). In the USA, golf courses were valued at $84 billion USD (NGF 2019), and in Georgia, the golf course industry was valued at $2.4 billion USD (GGE 2010). Any arthropod pests on the golf course, especially the putting greens, can seriously affect the aesthetics and playability. Adults and nymphs of *A. graminis* insert their mouthparts on nodes of turfgrass stems and consume photosynthates that flow through the vascular bundles (Watschke et al. 1995). On the putting greens, *A. graminis* feeding on turfgrass plants produces a yellow appearance by late summer, which eventually turns brown by the fall. As a result, these infested putting green surfaces become aesthetically unappealing and affect the playability of golf.

Antonina graminis undergoes parthenogenetic reproduction, where fertilized and unfertilized eggs develop into females and males, respectively. The *A. graminis* population is highly female-biased. Adult females produce 150-300 eggs (Chada and Wood 1960, Watschke et al. 1995, Joseph and Hudson 2019, Vittum 2020). The eggs of *A. graminis* ovoviviparously hatch and the newly born first instars wander and settle on the nodes of the stem beneath the leaf sheath. The first instars are referred to as crawlers and are the only mobile stage of *A. graminis*. Crawlers molt into a second sessile nymphal stage and lose all their legs. The second and third instars and adults produce a protective, bright-white, waxy cover around their purple bodies, which sometimes appear black-colored when sooty mold fungus grows on them. At 27 °C, the egg to adult development is approximately 52-60 d (Chada and Wood 1960).

In pasture grasses, *A. graminis* was primarily managed by introduced biological control agents, such as the encyrtid parasitoids *Anagyrus antoninae* Timberlake (Riherd 1950) and *Neodusmetia sangwani* (Rao) (Rao 1957, Dean and Schuster 1958, Schuster and Boling 1971, Schuster and Dean 1976, Dean et al. 1979). Populations of these parasitoid species are established on *A. graminis* on putting greens in southern Georgia. However, these introduced parasitoids rarely manage to reduce the overwhelmingly high densities of *A. graminis* on these surfaces and maintain them below the aesthetic threshold. Therefore, alternate management tactics, such as chemical control, are necessary, which are not fully developed against *A. graminis* on the putting greens.

As a chemical control tactic, the crawler stage of mealybug or scale insects, which lacks the wax covering, is susceptible to contact insecticides such as pyrethroids or horticultural oils. Thus, crawlers are specifically targeted with contact insecticide before their densities peak as a management strategy. However, predicting the initial emergence and peak activity of *A. graminis* crawlers is challenging on putting greens, and no method has been developed to date. Moreover, *A. graminis* nymphs settle under the leaf sheath, which is poorly accessible using contact insecticides. The nymphs and adults of *A. graminis* produce a wax covering that protects them from exposure to contact insecticide sprays. Thus, targeting the crawler stage using contact insecticide is likely effective for *A. graminis* management.

Organophosphates, such as chlorpyrifos, diazinon, and leptophos, were effective on *A. graminis* (Murdoch and Mitchell 1976), but these insecticides are no longer available for use in golf courses. In general, systemic insecticides, especially neonicotinoids, are effective on piercing and sucking insects (Camacho and Chong 2015), and neonicotinoids (imidacloprid and thiamethoxam) and flupyradifurone were effective on *A. graminis* (Joseph et al. 2021). However, because densities of nymphs and adults of *A. graminis* increase by June or July and continue to increase or remain at high densities until the late fall (Wolverton unpublished data), it is unclear when to use these insecticides during the growing season most effectively. Thus, the objectives of this study were to determine the effects of (1) single applications from the early peak of nymphs and adults in the summer leading to the fall and (2) repeated applications throughout the growing season. Ultimately, the aim was to determine limited early and late application of insecticide to develop an integrated pest management (IPM) strategy for *A. graminis* on putting greens.

Materials and Methods

Study site and *A. graminis* infestation. In 2019 and 2021, experiments were conducted on the putting greens of the golf course at the Key Golf Studio at Columbus State University, Columbus, Georgia. The turfgrass on the putting greens was 'TifEagle' bermudagrass [*Cynodon dactylon* CL. (Pers) × *C. transvaalensis* (Burtt-Davy)]. These putting greens were constructed in 2015 following the United States Golf Association's construction guidelines (USGA 1989) with sand and sphagnum peat mix (85: 15). Routine mowing began in March and continued through November each year. The bermudagrass was mowed six times each week during the experimental period with a triplex mower (TORO® Greensmaster 3150 triplex mower, Bloomington, MN) and maintained at 3 mm height. The bermudagrass putting greens were vertically mowed at 2 mm depth three times every year to reduce the accumulation of thatch. The putting greens were irrigated during nighttime and in the morning for six minutes using overhead sprinklers to prevent desiccation. Nitrogen fertilizer (Urea 46-0-0) was applied every two weeks from March to November for a total of 3.36 kg per ha N per year. The plant growth regulator, trinexapac-ethyl (Primo Maxx®, Syngenta, Basel, Switzerland) was applied at 0.15 L per ha at 14 d intervals during the growing season. Preventative fungicides, such as chlorothalonil (Manicure® 6 FL, Lesco, Cleveland, OH and Penthiopyrad (Velista®, Syngenta, Basel, Switzerland), were applied monthly at 9.4 L per ha and 1.61 L per ha respectively, on the putting greens for disease management. Two applications of foramsulfuron (Revolver® Bayer Environmental Science [Envu Environmental Science], Cary, NC) were applied in the spring at 0.7 L per ha to suppress grassy weeds. Insecticides were not applied on the putting greens other than those applied as part of the experiment on the designated plots. These putting greens were open to normal traffic of golfers five days a week in the spring and fall during both years. Trees and buildings surrounded the putting greens. The average temperatures from April to October were 28.1 °C and 22.1 °C, and from December to May were 14.8 °C and 14.3 °C in 2019 and 2021, respectively. In 2019, the average relative humidity (RH) in summer and fall was 67.2% and 71.4%, respectively. In 2021, the average RH from the winter to spring was 57.7% and 68.2%, respectively.

The putting greens at the Key Golf Studio were naturally infested with *A. graminis*. The problems from *A. graminis* population on the putting greens were first noticed in 2016 when the greens developed yellowing in the late summer and early fall and became brown in the late fall. The *A. graminis* infestation was detected on all the putting green surfaces on the golf course.

Experimental design and insecticide. To determine the effects of single and repeated applications of insecticide on *A. graminis* and turfgrass quality, insecticide timing treatments were developed. The treatments were: insecticide applied on (1) June only, (2) July only, (3) August only, (4) June + July, (5) June + July + August, and (6) nontreated. Insecticide was only applied once each month. The experiment was arranged in a randomized complete block design with four replications. The treatments were randomized within the block. The experiment was blocked as the *A. graminis* population could vary from one area to another within the same putting green. The experimental plot was a 2 m × 2 m area on the putting green. The plots were located ~1 m from the edge of the putting greens.

The insecticide used in the experiment was flupyradifurone (Altus™ [17.09%], Bayer CropScience, Research Triangle Park, NC). This insecticide product was selected based on its effectiveness against *A. graminis* (Joseph et al. 2021) and its minimal effects on beneficial insects. The insecticide was applied at 410 g ai per ha for one application treatment (June, July, or August only). The two application-treatment received 205 g ai per ha (June + July treatment). The three applications were made for June + July + August treatment (one application each month), where 205 g ai per ha was applied in the first month (June), then 103 g ai per ha during the second month (July), followed by 102 g ai per ha in the third month (August). The insecticide was sprayed using a CO_2-powered sprayer at 200 kPa with TeeJet® 11008 flat fan tip (TeeJet Technologies, Glendale Heights, IL). The insecticide was delivered at 7.6 L per m^2. The water volume used was 374.2 L per ha. No adjuvant was added to the insecticide solution. The insecticide treatments were applied on 29 June, 30 July, and 30 August 2019; and 16 June, 14 July, and 11 August 2021. The experiments were initiated in June each year once high densities of *A. graminis* crawlers and nymphs were detected in the ten random turfgrass plug samples collected weekly in June. The experiment was not conducted in 2020 because high densities of *A. graminis* crawlers and nymphs were not detected in June.

Evaluation. Samples were collected every two weeks using a 53.3 cm (length) × 1.9 cm (diameter) soil core probe (SiteOne® Landscape Supply, Roswell, GA) on 11 and 29 July, 16

August, 4, 10 and 17 September 2019; and 16, 23 and 30 June, 14 and 28 August and 9 September 2021. Three turfgrass plugs were randomly sampled from each plot and transferred into a labeled plastic bag. The plug samples were collected randomly from the central area of each plot. A plug consisted of turfgrass (leaves and stem), a thatch layer, and sandy soil (about 5 cm deep). The numbers of nymphs and adults within the turfgrass plugs were quantified under a dissecting microscope (AmScope, Irvine, CA) at 10× magnification. The *A. graminis* densities from all three plugs were combined as a sample per plot. The numbers of adults and nymphs were combined per sample for analysis purposes, as both the sessile nymphal stages and adults can cause feeding damage on the putting greens. The crawler stage was not considered to cause any direct feeding damage.

In addition, the quality of putting green surfaces within the experimental plots was visually rated by color and density on 25 June, 1, 9 and 23 July, 26 August and 15 September 2019; and 16 and 22 June, 4 July, 8, 11 and 30 August and 6 September 2021. National Turfgrass Evaluation Program (NTEP) criteria for turfgrass quality were used for the rating (Morris 2022). A rating score of at least seven or more was acceptable for the putting greens.

Statistical analyses. All statistical analyses were performed using SAS 9.4 (SAS Institute 2016). The numbers of *A. graminis* adults plus nymphs observed on the turfgrass plug samples were subjected to Analysis of Variance (ANOVA) using a generalized linear model procedure (PROC GLIMMIX) with log-link function and negative binomial distribution. The treatment was a fixed effect, whereas replication was a random effect. The ANOVA was performed on data collected on 4 (1 week after the last application [WAA]) and 17 September (3 WAA) 2019 and on 27 August (2 WAA) and 9 September (4 WAA) 2021. A repeated measure statement was added to the model. The means were adjusted using the Tukey-Kramer test ($P < 0.05$). To determine the effects of June only treatment, as well as June only, July only, and June + July treatments, the *A. graminis* data were subjected to ANOVA using a generalized linear model procedure (PROC GLIMMIX) with log-link function and negative binomial distribution. The treatment was a fixed effect, whereas replication was a random effect. The ANOVA was performed on data collected on 4 WAA (29 July) after the June application and 2 WAA (16 August) after the July application in 2019, as well as on 2 WAA (30 June) after the June application and 2 WAA (28 July) after the July application in 2021.

For the turfgrass quality data, the rating scores were subjected to ANOVA using the general linear model (PROC GLM) in SAS after log-transformation (ln[x +1]). Both treatment and replication were the fixed effects in the model. The analyses were performed on data collected on 26 August (0 week after the last application [WAA]) and 15 September (3 WAA) in 2019 and on 30 August (2 WAA) and 6 September (4 WAA) in 2021. The means were separated using the Tukey-HSD test ($P < 0.05$). Means and standard errors of treatments were calculated using the PROC MEANS procedure in SAS.

Results

Insecticide effects. In 2019, ~1 week after the last application (WAA), there were no significant differences in the numbers of *A. graminis* adults and nymphs among treatments ($F = 1.9$; df = 5, 15; $P = 0.158$; Figure 4. 1A). At 3 WAA, the numbers of *A. graminis* adults and nymphs were significantly lower for the June + July + August treatment than for the June only, July only, August only and June + July treatments ($F = 5.6$; df = 5, 15; $P = 0.004$; Figure 4. 1A).

In 2021, the numbers of *A. graminis* adults and nymphs were significantly lower for the August only, June + July, June + July + August treatments than for the nontreated treatment after 2 WAA ($F = 5.5$; df = 5, 15; $P = 0.005$; Figure 4. 1B). At 4 WAA, the densities of *A. graminis* adults and nymphs were significantly lower for the August only and June + July + August treatments than for the June only treatment (F = 5.6; df = 5, 15; P = 0.016; Figure 4. 1B).

In 2019, prior to the August application or after the June application, treatments were either compared between the June treatment and nontreated or among June only, July only, June + July, and nontreated treatments. At 4 weeks after the June application (sample collected on 29 July), there were no significant differences between the June only and nontreated treatments (F = 3.5; df = 1, 11; P = 0.087; Figure 4. 2A). Among June only, July only, June + July, and nontreated treatments, no significant differences were observed among each other (F = 3.2; df = 3, 13; P = 0.059; Figure 4. 2B). In 2021, there was no significant difference between June only treatment and nontreated treatment (F = 2.8; df = 1, 11; P = 0.125; Figure 4. 2C). At 2 weeks after the July application (samples collected on 16 August), significantly lower numbers of *A. graminis* adults and nymphs were found for the July only, June + July treatments than for the June only and nontreated treatments (F = 14.8; df = 3, 13; P < 0.001; Figure 4. 2D).

Turfgrass quality. In 2019, on the final application of treatment in August (0 WAA), there was no significant difference in turfgrass quality among treatments (F = 0.9; df = 5, 15; P = 0.462; Figure 4. 3A). At 2 WAA, the turfgrass quality was significantly greater for the June + July + August treatment than for the July only and nontreated treatments (F = 4.4; df = 5, 15; P = 0.011; Figure 4. 3A).

In 2021, at 2 WAA (after final application in August), there was no significant difference in turfgrass quality among treatments (F = 1.6; df = 5, 15; P = 0.232; Figure 4. 3B). At 4 WAA, the turfgrass quality was significantly greater for the August only and June + July + August treatments than for the nontreated treatment (F = 4.8; df = 5, 15; P = 0.008; Figure 4. 3B).

Discussion

We sought to understand the effects of an early or late single application or repeated applications against sessile nymphs and adults of *A. graminis* on the putting greens. The sessile nymphs and adults are destructive stages of *A. graminis.* The early or mid-summer single application of flupyradifurone did not adequately reduce *A. graminis* densities and damage symptoms on the putting greens. Although the exact reason is unclear, it could be partly related to low *A. graminis* population densities during the summer, as the population growth gradually responded to cooler temperatures in the spring or early summer. As the season progresses through the mid to late summer, *A. graminis* population increases and the honeydew secretions from *A. graminis* also increase. In response, the activity of other insects, such as specialized parasitoids of *A. graminis* and honey bees, wasps, and ants, increases through the growing season. Thus, the current data do not support the spring or early summer application of insecticides for *A. graminis* suppression. More research is warranted to develop strategies to enhance the suppression of *A. graminis* during the early summer.

When the *A. graminis* densities were high during the summer, flupyradifurone application effectively reduced their densities and damage symptoms. The effect of flupyradifurone lasted for approximately 30 d post-application on the putting greens. This result is consistent with previous research, where the effects of flupyradifurone were not evident beyond 30 d when applied during the late summer (Joseph et al. 2021). Flupyradifurone is fast acting, and the efficacy was observed within two weeks against *A. graminis*. In addition, the damage symptoms rapidly disappeared within two weeks post-flupyradifurone application. Because the effects of flupyradifurone do not last beyond 30 d, there are opportunities for beneficial insects to rebound if they were affected by flupyradifurone application. Because *A.*

graminis is a piercing and sucking pest and flupyradifurone has systemic rather than contact activity against *A. graminis*, the *A. graminis* densities would be selectively managed with minimal nontarget effects on beneficial insects, especially those with minimal plant feeding habit. Thus, future studies should be conducted to determine approaches to minimize the effects on nontarget beneficial insects while using a systemic insecticide.

Flupyradifurone is a systemic insecticide (Drotleff 2017) belonging to Butenolides and placed in Group 4D (IRAC 2022). Although flupyradifurone is not registered at this time for golf course use, it was selected for the current research because of its systemic activity and has proved effective against *A. graminis* (Joseph et al. 2021). In addition, flupyradifurone has minimal toxicity to bees and some beneficial insects (Nauen et al. 2015, Campbell et al. 2016, Joseph and Bolda 2016). Currently, flupyradifurone is only labeled on ornamental crops, with 410 g of ai, flupyradifurone allowed each year. In addition to *A. graminis*, it is effective against many other piercing and sucking pests, (Nauen et al. 2015, Ganjisaffar et al. 2019, Issa et al. 2022).

Repeated applications of flupyradifurone were effective in suppressing *A. graminis* densities and improving turfgrass quality. The repeated applications of systemic insecticide may help to keep the *A. graminis* population low and reduce turfgrass stress for a prolonged period. However, repeated applications may have detrimental effects on nontargets. Repeated exposure to insecticides from the same IRAC Group increases the risk of resistance development. The reduction of *A. graminis* density and improved turfgrass quality with repeated and single late-growing season applications were not different. Thus, a single application during the late growing season is advisable for month-long protection. However, multiple late-season applications may be necessary to maintain turfgrass quality for aesthetic and playability because *A. graminis* densities can rebound once the insecticide residues wear off.

In summary, a single late summer insecticide application effectively reduced *A. graminis* densities and improved turfgrass quality for approximately 30 d post-application. Single or multiple insecticide applications during early summer did not reduce the *A. graminis* densities for a prolonged period (or > 30 d). More research is warranted to determine the nontarget effects of insecticides during the late summer on parasitoids of *A. graminis* nymphs and adults and the predators and pollinators foraging on honeydew secretions from *A. graminis* nymphs and adults. Neonicotinoids, such as thiamethoxam and imidacloprid, were also effective against *A. graminis* (Joseph et al. 2021), and they are in Group 4A (IRAC 2022). However, the comparative effects of neonicotinoids and flupyradifurone on beneficial insects are unclear. The new information of the current study will be used to develop an IPM approach to combat *A. graminis* on the putting greens of golf courses.

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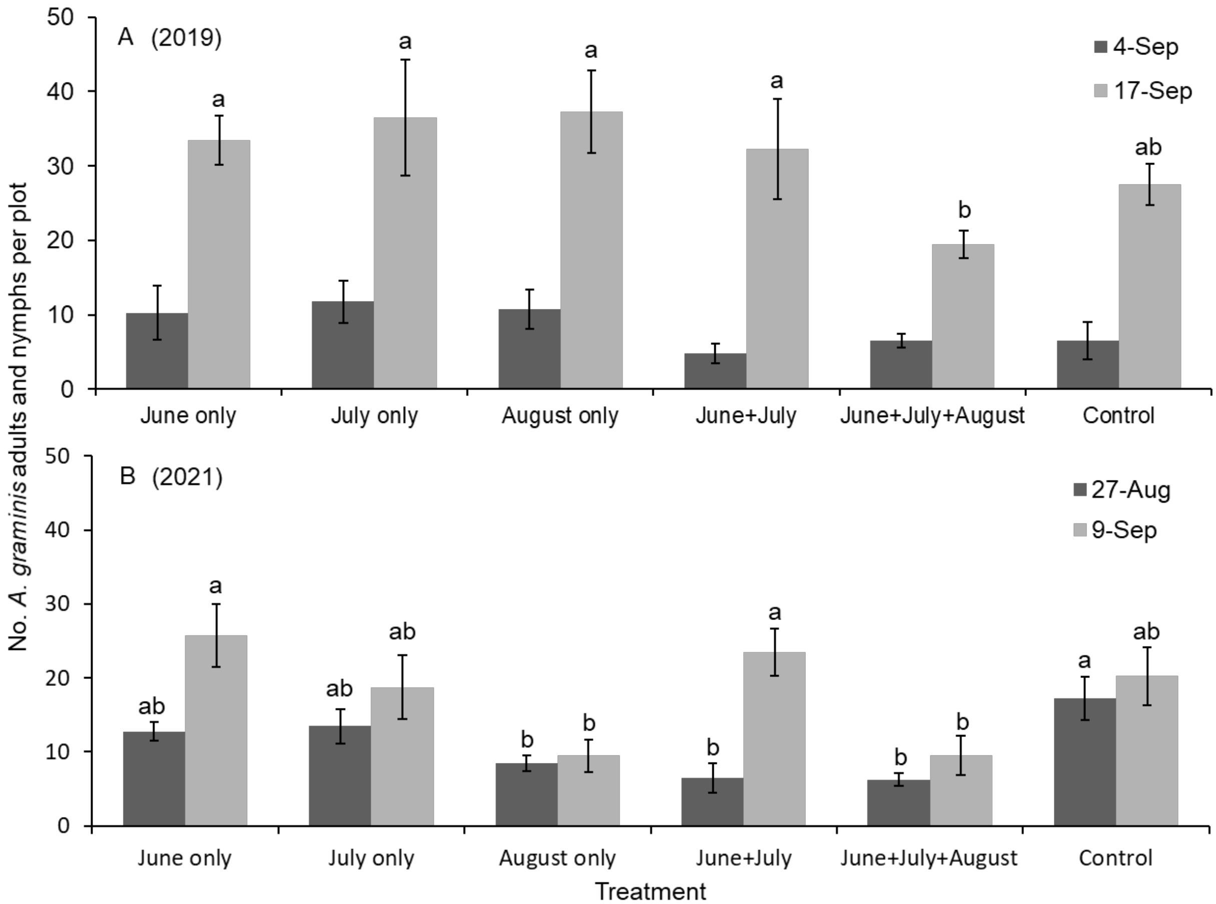

Figure 4. 1. Mean (±SE) adults and nymphs of *A. graminis* per plot after applying flurpyradifuron in June only, July only, and August only, June + July and June + July + August in (A) 2019 and (B) 2021. Bars with the same letters indicate no significant differences among treatments (Tukey-Kramer test, α = 0.05). The flurpyradifuron was applied on 29 June, 30 July, 30 August 2019; and 16 June, 14 July, and 11 August 2021. When not significantly different, letters were not provided.

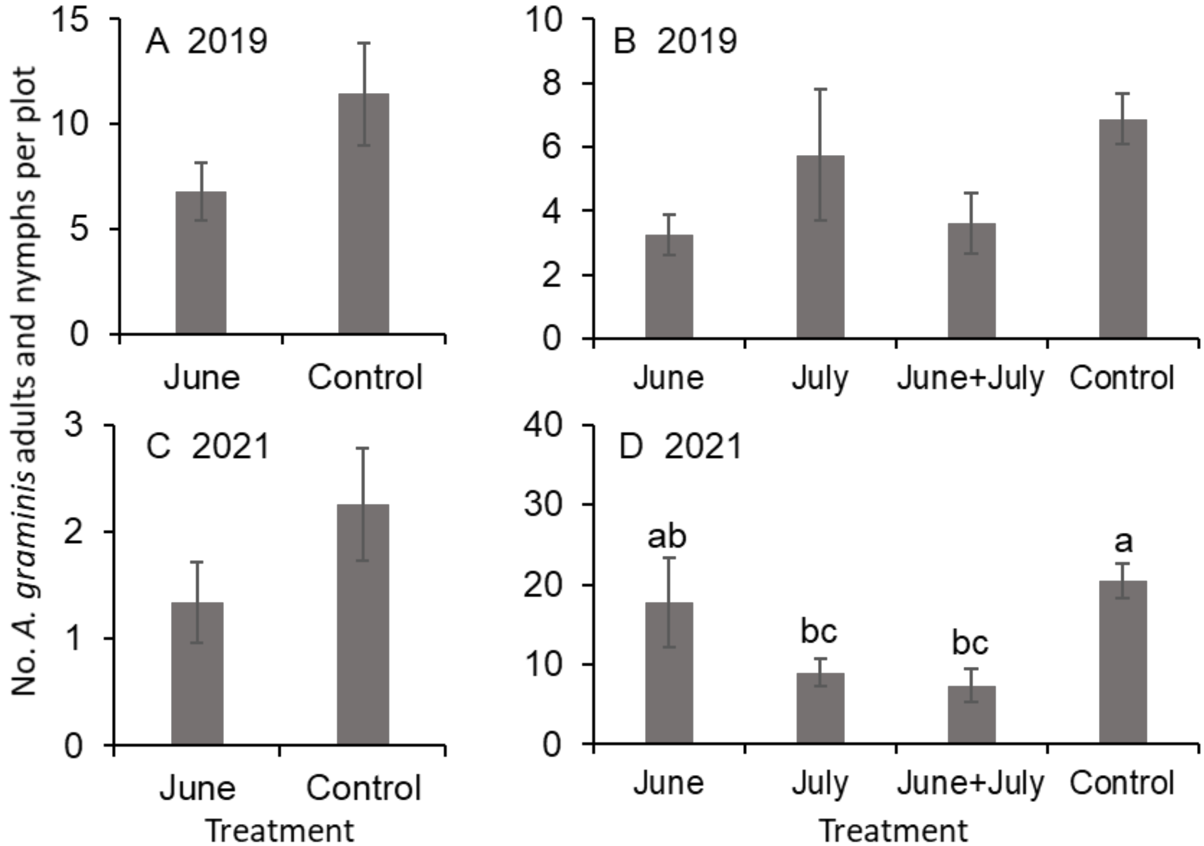

Figure 4. 2. Mean (±SE) adults and nymphs of *A. graminis* per plot after applying flurpyradifuron in (A) June only, (B) June only, July only, and June + July in 2019, and (C) June only, (D) June only, July only, and June + July in 2021. Bars with the same letters suggest no significant differences among treatments (Tukey-Kramer test, $\alpha = 0.05$). The data were collected on 4 (29 July) after the June application and 2 WAA (16 August) after the July application in 2019. In 2021, the data were collected on 2 (30 June) after the June application and 2 WAA (28 July) after the July application. When not significantly different, letters were not provided.

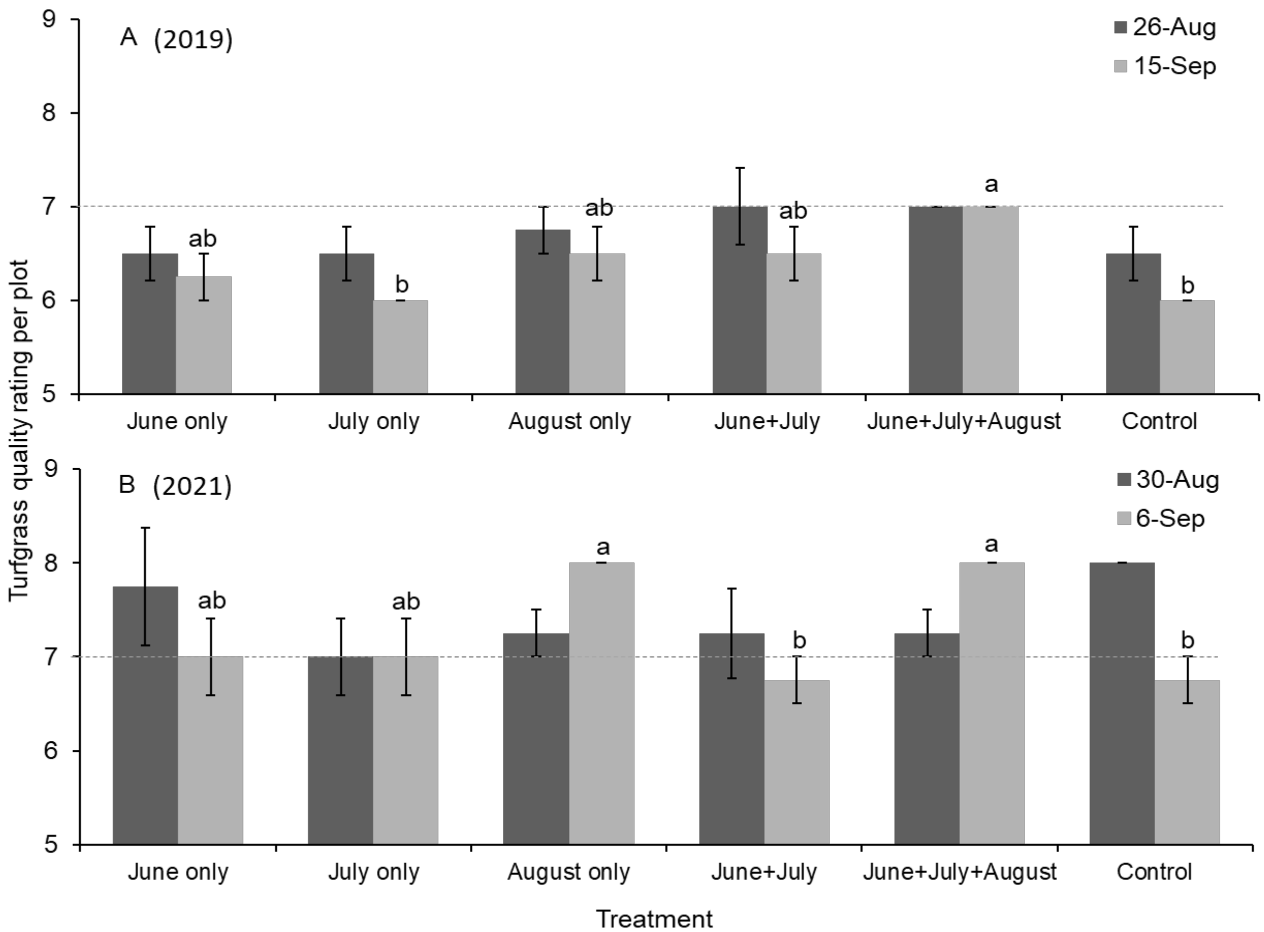

Figure 4. 3. Mean (±SE) turfgrass quality per plot after applying flurpyradifuron in June only, July only, and August only, June + July, and June + July + August in (A) 2019 and (B) 2021. Bars with the same letters suggest no significant differences among treatments (Tukey HSD test, α = 0.05). The flurpyradifuron was applied on 29 June, 30 July and 30 August 2019; and 16 June, 14 July and 11 August 2021. The dotted line indicates acceptable turfgrass quality of seven. When not significantly different, letters were not provided.

CHAPTER 5

EFFECTS OF FERTILIZER AND INSECTICIDE ON *ANTONINA GRAMINIS* (HEMIPTERA: PSEUDOCOCCIDAE) ON GOLF COURSE PUTTING GREENS

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Abstract Rhodesgrass mealybug, *Antonina graminis,* is a serious pest of ultradwarf hybrid bermudagrass (*Cynodon dactylon* × *C. transvaalensis*) on golf course putting greens. *A. graminis* feeding damage appears as extensive yellowing of turfgrass blades and heavy thinning from mid-to-late summer into fall. Putting greens are intensively managed areas of the golf course where fertilizers are routinely applied to maintain and enhance turfgrass quality, playability and aesthetics. We hypothesize that *A. graminis* populations can be minimized by reducing nitrogen (N) fertilizer, and then be effectively managed using systemic insecticides. The objective of this study was to determine the effects of various levels of N fertilizer and flupyradifurone on the *A. graminis* population and turfgrass quality on the golf course putting green. The treatments were low, medium, and high N fertilizer rates with and without insecticide (flupyradifurone). Applying a high dose of N fertilizer improved turfgrass quality without increasing *A. graminis* densities on the golf course green. Although flupyradifurone application reduced *A. graminis* densities regardless of N fertilizer treatments, suppression of *A. graminis* densities improved at the high fertilizer dose with flupyradifurone. Additionally, the turfgrass quality on the putting green improved with high N fertilizer alone, regardless of flupyradifurone application. Thus, *A. graminis* populations can be managed using moderate to high levels of N fertilizer and applying a systemic insecticide. The low nitrogen fertilizer did not effectively reduce the *A. graminis* densities on the putting green.

Key Words: Bermudagrass, Rhodesgrass mealybug, flupyradifurone

Rhodesgrass mealybug, *Antonina graminis* (Mask.) (Hemiptera: Pseudococcidae) is an important insect pest of warm-season turfgrasses in the southern states of the USA (Chada and Riherd 1950, Riherd 1950, Riherd 1954, Chada and Wood 1960, Reinhert et al. 2009). Native to Asia, *A. graminis* was accidentally introduced to the continental USA in 1942 (Riherd 1950) and is now distributed from Florida in the east to California in the west (Gracía Morales et al. 2016, Vittum 2020). The northward range of *A. graminis* is restricted by colder winter temperatures. *Antonina graminis* can infest more than 100 grass species commonly grown for pastures and turfgrass, which includes all warm-season grasses, such as bermudagrass (*Cynodon* spp.), bahiagrass (*Paspalum notatum* Flugg), and zoysiagrass (*Zoysia* spp.) (Chada and Wood 1960, Helms and Vinson 2000, Baxendale and Shetlar 2012). On golf courses, although the *A. graminis* occurs in the rough and fairways, severe feeding symptoms, such as yellowing and grass mortality, are more likely on putting greens (Dale 2017, Joseph and Hudson 2019). The ultradwarf hybrid bermudagrasses and kikuyugrass (*Pennisetum clandestinum* Hochst.), specifically bred for putting green surfaces, are highly susceptible to infestation (Reinhert and Vinson. 2010). . In 2018, the golf course industry was valued at $33 billion USD in the USA (NGF Report 2019). In Georgia, there are approximately 350 club golf courses (GSGA 2022), and the turfgrass industry contributed $2.4 billion USD to the state's economy in 2009 (GGE 2010). Damage from *A. graminis* infestations can be costly and are not easily remedied at this time.

Antonina graminis reproduces parthenogenetically, where most individuals are females (Chada and Wood 1960). A female can produce 150-300 eggs during her lifetime. The eggs hatch into crawlers, which settle on nodes between a leaf sheath and stem. They molt through two more nymphal stages before eclosion into adults. The second and third instars and females produce waxy coatings on their bodies and secrete honeydew. *A. graminis* populations do not undergo diapause and are susceptible to freezing temperatures, which causes a considerable population decline during winter, especially in transition zones. *A. graminis* populations are most active when temperatures are ~30 °C and become less active as temperatures rise above 37 °C (Chada and Wood 1960). The *A. graminis* population builds up through the growing season and can undergo up to five generations per year in the Gulf states of the USA (Chada and Wood 1960). On putting greens, high *A. graminis* populations cause extensive turfgrass mortality from late July to October if not managed using insecticide sprays with adequate fertilizer and irrigation applications.

In the USA, golf is played year-long along the southeast, Gulf, and Pacific southwest states. In this subtropical zone, bermudagrass, kikuyugrass, and seashore paspalum (*Paspalum vaginatum* Swartz) are typically planted on putting greens. The putting greens are the most intensively managed surfaces on any golf course. Superintendents devote most of their resources and time to ensuring that the conditions are right for grass growth, playability, and the aesthetics of putting green surfaces. Intensive management includes the regular application of pesticides (mostly fungicides and herbicides), nitrogen (N)-based fertilizer, and plant growth regulators. In addition, putting greens are subjected to intense cultural management, such as mowing, top dressing with sand, rolling at shorter intervals, and intermittent vertical mowing to remove thatch and for better aeration. The turfgrass growing on putting greens is under tremendous stress compared to any other region of the golf course.

To combat *A. graminis* populations in pastures, many species of parasitoids were introduced from the natural range in the Gulf states during the 1950s. Parasitic wasps, such as *Neodusmetia sangwani* (Subba Rao) and *Anagyrus antoninae* Timberlake, were established in

Gulf states, and they have provided consistent suppression of *A. graminis* populations during the past decades (Riherd 1950, Schuster and Dean 1976, Dean et al. 1979, Chantos et al. 2009, Filho et al. 2018). Although parasitoids are found in *A. graminis* individuals from turfgrass greens (R.W. unpublished data), they do not cause adequate suppression of *A. graminis* to reduce turfgrass mortality and to result in an acceptable turfgrass quality on putting greens. A recent survey exploring parasitic wasps in the Gulf states suggests that overall parasitism rates from the established parasitoid species were not adequately dispersed across the southern USA (Chantos et al. 2009). This suggests that alternative management strategies should be developed to manage *A. graminis* on putting greens. Nitrogen-based fertilizers are routinely used to ensure the quality of turfgrass on putting greens (Turgeon 1996, McCarty and Miller 2002). Insect pests often respond positively to increased N fertilizer with increased population growth and fecundity (Douglas 1993, 2006, Hogendorp et al. 2006), which can reduce their vulnerability to insecticide application (McKenzie et al. 1995). The hypothesis is that the population size of *A. graminis* established on turfgrass greens can be manipulated and optimized using a reduced rate of N fertilizer and adequately managed using effective systemic insecticide application. Flupyradifurone is a systemic pyropene insecticide effective against sucking pests (Nauen et al. 2015), including *A. graminis* (Joseph et al. 2021). It is in the 4D IRAC grouping under butenolides (IRAC 2022). Although flupyradifurone is not registered for turfgrass use, it is registered on other ornamental plants in nursery production and is a potential candidate for piercing and sucking pests in turfgrass. Flupyradifurone is a good fit for integrated pest management because it has minimal effects on various beneficial insects (Joseph and Bolda 2016, Barbosa et al. 2017, Koch et al. 2020). The objective of the study was to determine the effects of various levels of N fertilizer and flupyradifurone on the *A. graminis* population and turfgrass quality on golf course putting greens.

Materials and Methods

Study site and insects. Experiments were conducted at the Key Golf Studio at Columbus State University (32.4976332, -84.9320184) in 2019 and 2020. The putting greens at this facility were constructed with 30.5 cm deep sand and peat moss (17: 3) on a gravel layer for drainage, following the United States Golf Association's construction guidelines. The 'TifEagle' bermudagrass greens were irrigated twice (morning and night) daily with overhead irrigation and maintained at an ~ 0.25 cm grass height. The putting greens were mowed five times weekly using a riding mower (TORO® Greensmaster 3150 triplex, Bloomington, MN). The greens were aerified twice yearly for adequate root growth and development. To reduce grass thickness and remove excessive thatch, the putting green was vertically mowed monthly at a 2 mm depth. Coupled with vertical mowing, sand topdressing was applied on the green with 310 sand (Unimin Sand, Butler, GA) at ~ 7 kg per ha. To prevent the removal of *A. graminis* populations from the research area, vertical mowing (verticutting) was intentionally omitted during the experiment. In addition, the research plots were treated with routine preventative fungicides and wetting agents. Plant growth regulators and fertilizers were not administered in the experimental plots.

The experimental plots were naturally infested with *A. graminis*. The *A. graminis* population was first detected in Sept 2017 in the putting greens. The selected putting green had a uniform infestation of *A. graminis* on the entire putting green surface. The putting green used in the 2019 experiment was not used again in 2020.

Experimental design, fertilizer, and insecticide. The experiments were initiated on 16 May 2019 and 17 May 2020. The experiment was designed to determine the effects of various rates of N fertilizer with and without application of insecticide against *A. graminis*. The treatments were 1) low fertilizer rate without insecticide, 2) low fertilizer rate with insecticide, 3) medium fertilizer rate without insecticide, 4) medium fertilizer rate with insecticide, 5) high fertilizer rate without insecticide, and 6) high fertilizer rate with insecticide. Six replications of treatments were arranged in a randomized complete block design on the putting green. The experimental plot size was 2 × 2 m.

Sprayable fertilizer (Lesco Macron 20-20-20, Cleveland, OH) was used in both years. This fertilizer contains three forms of N derived from ammonium sulfate (7.9%), nitrate (5.9%), and urea (6.2%). The annual N rates for the low, medium and high treatments were 10, 20, and 30 g per m^2, respectively. A treatment with no fertilizer application was not included in the treatments because turfgrass is unlikely to survive without N fertilizer. Golf course superintendents typically use 20-25 g N per m^2 in Georgia. The fertilizer was applied at biweekly intervals using a CO_2-powered sprayer with a TeeJet® flat fan nozzle (XR11008) with a water volume of 800 L per ha at 219.9 kPa. In 2019, fertilizer was applied on 16 and 31 May, 17 and 28 Jun, and 17 and 30 Jul, whereas in 2020, fertilizer was applied on 17 and 29 May, 15 and 29 Jun, 8 and 21 Jul, and 16 Aug.

For insecticide, flupyradifurone (Altus®, Bayer Crop Science, Saint Louis, MO) was applied only once at 1 L per ha in both years. Flupyradifurone is a systemic insecticide (IRAC Group 4D) that is effective against *A. graminis*, although it is not yet registered for turfgrass use in the USA (Joseph et al. 2021). Flupyradifurone was applied using a CO_2-powered sprayer with a TeeJet® flat fan nozzle (XR11008) with a water volume of 800 L per ha at 219.9 kPa. The insecticide was applied on 19 Aug 2019 and 18 Sept 2020.

The phenology and densities of the *A. graminis* population varied between 2019 and 2020. The crawlers and nymphs were first active by May 2019, whereas in 2020, the activity of crawlers and nymphs was delayed, and they became active by mid-July (RW unpublished data). Thus, relative to 2019, the initial fertilizer and insecticide applications were delayed by 2-4 weeks in 2020.

Sampling and evaluation. To determine the effects of the treatments on the *A. graminis* population, three random 2-cm diameter soil core samples were collected from each plot using a 53 cm long tubular core sampler. The soil core samples were at least 5 cm below the soil surface so that the leaf sheath at the soil level was sampled. The three samples from each plot were bagged together and temporarily stored at 4 °C in a refrigerator. All the samples were evaluated within 4 d after collection. The numbers of *A. graminis* nymphs and adults were quantified under a 10× dissecting microscope (AmScope, Irvine, CA). Dead and live *A. graminis* were determined in each sample. An *A. graminis* individual was deemed alive if the red hemolymph oozed out of the body after poking with a needle. The samples were collected following flupyradifurone application on 19 and 26 Aug and 2 and 18 Sept 2019 and on 25 Sept and 2, 8, and 16 Oct 2020.

The putting green quality was visually assessed in both years. The visual ratings were recorded at biweekly intervals from each plot using a standard rating developed by NTEP (National Turfgrass Evaluation Program, Beltsville, MD) (Morris 2022). NTEP ratings are on a scale of 1 to 9, where 1 = poorest quality and 9 = outstanding quality. The ratings focused on color, turfgrass density, and percent living ground cover. Turfgrass texture was not recorded, as it would not reflect the treatment effects. Turfgrass ratings were performed on 15 Sept 2019 and 23 Oct 2020.

The fresh and dry weight (g) and plant nutrients from the treatment plots were determined in both years ~ one month after insecticide application. To determine fresh and dry weight from the plots, the turfgrass was not mowed 7 d before sample collection. A walking TORO® 1000 greensmower was used to cut the turfgrass clippings by making two passes to sample the entire plot, as the mower was 53 cm wide. The sampling height was set to the normal triplex mower setting and adjusted for fixed versus floating head compensation. The turfgrass clippings were collected in the bucket located at the base of the mower. The turfgrass clipping samples collected from each plot were transferred to paper bags and weighed immediately using a potable weighing balance. The samples were transported to the laboratory and oven-dried (Blue M Electric Company, model#POM-3240X, Blue Island, IL) at 115 °C for 48 h. After 48 h, the dry weights of the samples were recorded in the laboratory. To determine the foliar nutrients, the oven-dried turfgrass clipping samples were transported to the Plant and Soil Testing Laboratory (University of Georgia, Athens, GA). Nutrients (Mn, Fe, Al, B, Cu, Zn, Na, Pb, Cd, Ni, Cr, Mo, P, K, Ca, and Mg) were analyzed using an inductively coupled plasma emission spectrograph (Isaac and Johnson 1985, AOAC 1995). The combustion method was used to quantify the total percent N (Colombo and Giazzi 1982).

Statistical analyses. The statistical analyses for all data were conducted using SAS software (SAS Institute 2016). The numbers of *A. graminis* observed in the samples after insecticide applications in 2019 and 2020 were subjected to two-way ANOVA with interaction using the generalized linear model procedure (PROC GLIMMIX) with log link function and Poisson distribution. The *A. graminis* data analyses were conducted in a factorial design, where the factors fertilizer (three levels) and insecticide (two levels) with fertilizer × insecticide interaction. The treatments and replications were fixed and random effects, respectively. The means were separated by the Tukey–Kramer test ($P < 0.05$).

For the turfgrass quality data, the scale values from the plots were analyzed using nonparametric tests (PROC NPAR1WAY) in SAS, where Kruskal–Wallis chi-square tests were conducted to determine treatment effects. Analyses were adapted for the scale data because the scale values of turfgrass quality from the plots were in narrow ranges, and ANOVA may not provide meaningful outcomes. The treatment differences were separated using pairwise two-sided multiple comparison analysis with the Dwass, Steel, Critchlow-Fligner method (DSCF). The method produced DSCF and P values for every pair-treatment combination ($P < 0.05$).

The fresh and dry weight data of turfgrass clipping in 2019 were not transformed as they were normally distributed, whereas in 2020, the fresh and dry weight data were natural log-transformed ($\ln[x + 1]$). The normality of the residuals was checked after evaluating the histograms using the PROC UNIVARIATE procedure in SAS to determine before and after transformation. The data were subjected to two-way ANOVA using the general linear model procedure (PROC GLM) in SAS. The treatments and replications were fixed and random effects, respectively. The means were separated using the Tukey HSD method ($\alpha = 0.05$).

The plant nutrient data from tissue analysis were subjected to ANOVA after transformation. The percentage data of Ca, K, Mg, P, N, and S were arcsine square root transformed, whereas Al, B, Cu, Fe, Mn, and Zn were in mg and were natural log-transformed after checking for normality of residuals using the PROC UNIVARIATE procedure in SAS. Because the quantities of the nutrients Cd, Cr Na, Mo, Ni, and Pb were less than the detectable range from the tissue, they were not included in the analysis.

Results

Fertilizer and insecticide effects on *A. graminis*. On 19 Aug 2019, fertilizer treatments significantly affected the numbers of *A. graminis,* whereas insecticide treatment and their interaction had no significant effects on *A. graminis* densities (Table 5. 1). When the effects of fertilizer were analyzed by insecticide application status, the numbers of *A. graminis* were significantly greater for the high fertilizer treatment than for the low fertilizer treatment with insecticide ($F = 4.4$; df = 2,14; $P = 0.032$; Figure 5. 1A) but were not significantly different without insecticide ($F = 1.2$; df = 2,14; $P = 0.324$). There were no significant differences between insecticide and no insecticide treatments for the low ($F = 2.1$; df = 1,9; $P = 0.183$), medium ($F = 0.2$; df = 1,9; $P = 0.654$), and high fertilizer treatments ($F = 0.0$; df = 1,9; $P = 0.933$; Figure 5. 1A). On 26 Aug, fertilizer and insecticide had significant effects on the numbers of *A. graminis*, whereas the interaction between fertilizer and insecticide had no significant effect on *A. graminis* densities (Table 5. 1). When analyzed by insecticide application status, the numbers of *A. graminis* were not significantly different among fertilizer treatments (low, medium, and high) with ($F = 1.6$; df = 2,14; $P = 0.234$; Figure 5. 1B) or without insecticide ($F = 2.7$; df = 2,14; $P = 0.103$; Figure 5. 1B). The numbers of *A. graminis* were significantly lower for the insecticide than for the no insecticide treatments in the low ($F = 25.0$; df = 1,9; $P < 0.001$), medium ($F = 27.6$; df = 1,9; $P < 0.001$) and high fertilizer treatments ($F = 31.1$; df = 1,9; $P < 0.001$; Figure 5. 1B). On 2 Sept, fertilizer, insecticide and the interaction between fertilizer and insecticide treatments had significant effects on *A. graminis* densities (Table 5. 1). When the effects of fertilizer were analyzed by insecticide application status, the numbers of *A. graminis* were significantly greater for the medium fertilizer treatment than for the low fertilizer treatment, followed by the high fertilizer treatments ($F = 21.8$; df = 2,14; $P < 0.001$; Figure 5. 1C). In the absence of insecticide application, there were no significant differences in the numbers of *A. graminis* among treatments (F = 2.5; df = 2,14; P = 0.119; Figure 5. 1C). The *A. graminis* densities were significantly lower for the insecticide treatment than for the no insecticide treatment in the low (F = 67.4; df = 1,9; P < 0.001), medium (F = 41.4; df = 1,9; P < 0.001) and high fertilizer treatments (F = 136.7; df = 1,9; P < 0.001; Figure 5. 1C). On 18 Sept, the pattern of *A. graminis* densities was similar to the results from 2 Sept. The fertilizer, insecticide, and interaction between fertilizer and insecticide treatments significantly affected *A. graminis* densities (Table 5. 1). The numbers of *A. graminis* were significantly greater for the low and medium fertilizer treatments than for the high fertilizer treatment (F = 24.7; df = 2,14; P < 0.001; Figure 5. 1D) with insecticide. There were no significant differences in the numbers of *A. graminis* among treatments (F = 0.4; df = 2,14; P = 0.683; Figure 5. 1D) in the absence of insecticide. The *A. graminis* densities were significantly lower for the insecticide treatment than for the no insecticide treatments in the low (F = 32.5; df = 1,9; P < 0.001), medium (F = 12.0; df = 1,9; P = 0.007) and high fertilizer treatments (F = 18.7; df = 1,9; P < 0.001; Figure 5. 1D).

On 25 Sept 2020, fertilizer, insecticide, and their interaction significantly affected the numbers of *A. graminis* (Table 5. 1). When the effects of fertilizer were analyzed by insecticide application status, the numbers of *A. graminis* were significantly greater for the low and medium fertilizer treatments than for the high fertilizer treatment with insecticide (F = 15.9; df = 2,14; P < 0.001; Figure 5. 2A) but were not significantly different from that with no insecticide (F = 2.7; df = 2,14; P = 0.099). Although there were no significant differences between insecticide and no insecticide treatments for the low (F = 0.9; df = 1,9; P = 0.355) and medium fertilizer treatments (F = 2.3; df = 1,9; P = 0.002) on the numbers of *A. graminis* collected, significantly lower densities of *A. graminis* were found for the insecticide treatment than for the no insecticide treatment in the high fertilizer treatment (*F* = 18.7; df = 1,9; *P* = 0.933; Figure 5. 2A). Similarly, on 2 Oct, fertilizer, insecticide, and their interaction had significant effects on the numbers of *A. graminis* collected (Table 5. 1). The numbers of *A. graminis* were significantly lower for the medium fertilizer treatment than for the low fertilizer treatment with insecticide (*F* = 4.6; df = 2,14; *P* = 0.029; Figure 5. 2B), but there were no significant differences between low and high or medium and high fertilizer treatments. In the absence of insecticide, significantly higher numbers of *A. graminis* were found for the low and medium fertilizer treatments than for the high fertilizer treatment (*F* = 7.1; df = 2,14; *P* = 0.007; Figure 5. 2B). The numbers of *A. graminis* were significantly lower for the insecticide treatment than for the no insecticide treatment only in the medium fertilizer treatment (*F* = 16.7; df = 1,9; *P* = 0.003) but were not significant in the low (*F* = 2.4; df = 1,9; *P* = 0.159) and high fertilizer treatments (*F* = 0.1; df = 1,9; *P* = 0.798; Figure 5. 2B). On 8 Oct, fertilizer and insecticide treatments but not their interaction had significant effects on *A. graminis* densities (Table 5. 1). The numbers of *A. graminis* were significantly greater for the low and high fertilizer treatments than for the medium fertilizer treatment with insecticide (*F* = 7.9; df = 2,14; *P* = 0.005; Figure 5. 2C). When insecticide was not applied, the numbers of *A. graminis* were significantly greater for the low and medium fertilizer treatments than for the high fertilizer treatment (*F* = 4.6; df = 2,14; *P* = 0.029; Figure 5. 2C). The *A. graminis* densities were significantly lower for the insecticide treatment than for the no insecticide treatment in the low (*F* = 6.3; df = 1,9; *P* = 0.033) and medium (*F* = 22.9; df = 1,9; *P* = 0.001) fertilizer treatments and were not significantly different in the high fertilizer treatment (*F* = 4.2; df = 1,9; *P* = 0.072; Figure 5. 2C). On 16 Oct, fertilizer, insecticide, and their interaction had significant effects on the numbers of *A. graminis* (Table 5. 1). The numbers of *A. graminis* were significantly more abundant for the low fertilizer treatment than for the medium and high fertilizer treatments with (F = 11.4; df = 2,14; P = 0.001; Figure 5. 2D) or without insecticide (F = 13.8; df = 2,14; P < 0.001; Figure 5. 1B). The numbers of *A. graminis* were significantly lower for the insecticide treatment than for the no insecticide treatment in the low (F = 65.2; df = 1,9; P < 0.001), medium (F = 54.6; df = 1,9; P < 0.001) and high fertilizer treatments (F = 52.1; df = 1,9; P < 0.001; Figure 5. 2D).

Turfgrass quality. In 2019, the fertilizer and insecticide combination treatments had significant effects on the quality of turfgrass (χ^2 = 30.1; df = 5; P < 0.001; Figure 5. 3A). The scale values were significantly greater for the insecticide plus high fertilizer treatment than for the medium and low fertilizer with and without insecticide treatments (Figure 5. 3A). The high fertilizer without insecticide treatment and medium fertilizer with and without insecticide treatments resulted in significantly greater turfgrass quality than the low fertilizer with and without insecticide treatments. There were no significant differences in turfgrass quality with and without insecticides for the low, medium, and high fertilizer treatments (Figure 5. 3A). Based on NTEP standards, the acceptable treatments were high and medium fertilizer with and without insecticide in 2019.

Similarly, in 2020, the fertilizer and insecticide combination treatments had significant effects on the quality of turfgrass (χ^2 = 27.1; df = 5; P < 0.001; Figure 5. 3B). Significantly greater scale values of turfgrass quality were recorded for the high fertilizer with insecticide treatment than for the medium fertilizer without insecticide and low fertilizer with and without insecticide treatments (Figure 5. 3B). There were no significant differences among the high fertilizer with and without insecticide and medium fertilizer with insecticide treatments. Based on NTEP standards, the only acceptable treatment was high fertilizer with insecticide treatment in 2020.

Turfgrass biomass. In 2019, the fresh and dry weights of the turfgrass clippings were significantly greater for the high fertilizer treatment than for medium fertilizer treatment, followed by the low fertilizer treatment (Table 5. 2; Figures 5. 4A and 5. 4C). The insecticide treatments did not affect the biomass of the turfgrass clipping (Table 5. 2; Figures 5. 5A and 5. 5C). The interaction between fertilizer and insecticide treatments was not significantly different on the fresh and dry biomass (Table 5. 2).

In 2020, the fresh and dry weights of the turfgrass clippings were significantly greater for the high and medium fertilizer treatments than for the low fertilizer treatment (Table 5. 2; Figures 5. 4B and 5. 4D). The treatments that received insecticide had significantly higher fresh and dry weights than the no insecticide treatment. The interaction between fertilizer and insecticide treatments was not significantly different for the fresh and dry biomass of the turfgrass clippings (Table 5. 2).

Foliar nutrient content. In 2019 and 2020, the percentage of N content in the foliage was significantly greater for the high fertilizer treatment than for the medium fertilizer treatment, followed by the low fertilizer treatments (Table 5. 3; Figures 5. 6A and 5.6C). Although there was no significant difference in the foliar percentage N content between the insecticide treatment and no insecticide treatment in 2019, a significantly higher percentage of N was detected for the insecticide treatment than for the no insecticide treatment (Table 5. 3; Figures 5. 6B and 5. 6D). The interaction between fertilizer and insecticide treatments was not significantly different in foliar percentage N content in 2019 and 2020 (Table 5. 3).

In 2019, foliar percentage K was significantly greater for the high fertilizer treatment than for the medium fertilizer treatment, followed by the low fertilizer treatment (Supplementary Table 5. 1). Other nutrients, such as P, S, and Cu, were more abundant for the high and medium fertilizer treatments than for the low fertilizer treatment. In contrast, significantly higher amounts of Al and Fe were detected for the low fertilizer treatment than for the medium and high fertilizer treatments. The only nutrient affected by insecticide treatment was Zn, which was more abundant for the no insecticide treatment than for the insecticide treatment (Supplementary Table 5. 1). The interaction between fertilizer and insecticide treatments was not significantly different for any foliage nutrient in 2019.

In 2020, the foliar percentages of Ca and Mg were significantly greater for the high fertilizer treatment than for the medium and low fertilizer treatments (Supplementary Table 5. 2). Likewise, the B and Zn levels were significantly higher for the high fertilizer treatment than for the low fertilizer treatment. Among the nutrients, Ca, Mg and Zn were more abundant for the insecticide than for the no insecticide treatments (Supplementary Table 5. 2). The interaction between fertilizer and insecticide treatments was not significantly different for any foliage nutrient in 2020.

Discussion

We sought to determine whether optimized fertilizer and insecticide applications could reduce *A. graminis* densities and maintain acceptable turfgrass quality for golf courses. The results show that applying a high dose of N fertilizer improved turfgrass quality without an overall increase in *A. graminis* densities on golf course green. This result was consistent with results reported by Chau et al. (2005), in which *Aphis gossypii* Glover populations inconsistently responded to increased N fertilizer doses on chrysanthemum (C*hrysanthemum* spp.). Numerous studies with piercing and sucking insect pests routinely show that N fertilizer inputs increase insect density, body weight, body size, and fecundity (Nevo and Coll 2001, Joseph et al. 2011, Fernandes et al. 2012, Camacho and Chong 2015, Iskra et al. 2018). Although the exact reason for the inconsistent effects of increased N fertilizer on *A. graminis* densities is unclear, it is likely a function of turfgrass growth and population increase of *A. graminis* in summer. The growth of turfgrass is predetermined through the use of plant growth regulators and frequent mowing. However, the rate of population increase of *A. graminis* is exponential, as greater numbers of crawlers hatch through overlapping generations, and they are more likely to occupy all the available nodes evenly. Thus, it is possible that the ratio of numbers of *A. graminis* individuals to occupied nodes and the density of the nodes would remain constant. This scenario could be reflected by the data, as no consistent differences in *A. graminis* densities were observed across the N fertilizer treatments. Because turfgrass is only at the vegetative growth stage, nutrient resources are likely allocated solely for vegetative growth, unlike other plants that switch plant physiology from vegetative to reproductive stages (Reekie and Bazzaz 1987, Saulnier and Reekie 1995).

The use of flupyradifurone consistently reduced the *A. graminis* densities regardless of fertilizer doses and improved the turfgrass quality. This result was consistent with previous research on *A. graminis,* where flupyradifurone reduced the densities of *A. graminis* in turfgrass greens (Joseph et al. 2021). Interestingly, when flupyradifurone was applied, the reduction in *A. graminis* densities improved with the increase in N fertilizer dose (Figures 5. 1C and 5. 1D, 5. 2C and 5.2D). This effect was not evident in the absence of flupyradifurone. The foliar N content increased in response to the N fertilizer dose applied. This suggests that a high dose of N fertilizer systemically improved the uptake and movement of flupyradifurone within turfgrass and reduced *A. graminis* densities, although applying a high dose of N fertilizer did not necessarily favor a surge in the *A. graminis* population. Previously, growth and N uptake in cotton (*Gossypium hirsutum* L.) was enhanced following the application of the systemic insecticide aldicarb (Ragab 1981). This suggests that turfgrass with adequate access to plant nutrients, especially N, could efficiently move flupyradifurone within the turfgrass system and be more detrimental to *A. graminis* compared to nutrient-deficient turfgrass. More research is warranted to understand the relationship between N updating and the systemic movement of insecticides within turfgrass.

Turfgrass quality improved with high N fertilizer alone, regardless of flupyradifurone application. Based on NTEP standards, the turfgrass quality rating of seven is reasonably acceptable for the playability and aesthetics of the golf course green (Clint Waltz, personal communications). The study aimed to determine if a moderate population of *A. graminis* could be sustained using a reduced N fertilizer dose and if this moderate population could be manageable using a systemic insecticide applied at the minimum frequency for an extended period. However, a moderate *A. graminis* population was not achieved with an incremental increase in N fertilizer dose, as there was no consistent increase in *A. graminis* populations across N fertilizer dose treatments in the current study. Flupyradifurone application certainly reduced the *A. graminis* densities, but this reduction improved turfgrass quality at medium N fertilizer doses (Figures 5. 1-3). For high N fertilizer doses, flupyradifurone application did not necessarily improve turfgrass quality. The current and previous (Joseph et al. 2021) studies on turfgrass greens suggest that the effectiveness of flupyradifurone application against *A. graminis* densities faded after a month, suggesting that flupyradifurone may not provide longer-term control beyond six weeks and warrants reapplication. Additionally, the biomass data generated from various N fertilizer treatments suggest that increased amounts of turfgrass clippings were produced as the dose of N fertilizer increased. Generation of turfgrass clippings at an increased rate can be unfavorable for maintaining turfgrass quality and increase the risk of unintended scalping of golf course green if the mowing operations are inappropriately timed.

The N fertilizer and insecticide effects on *A. graminis* densities were not consistent between 2019 and 2020. This variation between years was likely caused by the disruption of routine mowing operations in the 2020 season affected by persistent rain. The turfgrass clippings for biomass assessment were collected two weeks prior to and four weeks after insecticide application in both years. The growth of turfgrass during these periods varied between 2019 and 2020. In 2020, persistent and frequent rain events disrupted scheduled mowing operations; thus, turfgrass growth was greater than normal. Precipitation during the final four weeks of sampling was < 2.5 cm in 2019, whereas in 2020, it was 6.35 cm in Columbus, GA (Weather Underground 2020). In addition, hurricane Sally dumped 16.35 cm of rain in 2020. As a result, the turfgrass was taller than normal before mowing operations could be administered, which caused excessive scalping of the golf course green. That excessive scalping possibly removed the adults and nymphs of *A. graminis* and reduced the population size. The adults and nymphs of *A. graminis* settle at the nodes of the turfgrass crown (Chada and Wood 1960). In summer, because the population size is very large, multiple individuals colonize every node of the turfgrass crown. These events impacted the outcome of the *A. graminis* density data in the 2020 season (Figures 5. 1 and 5. 2), especially those treatments that received the high N fertilizer. The high N fertilizer treatment recovers quickly and regains acceptable quality within three weeks compared to lower N fertilizer treatments.

In summary, a reduction in N fertilizer application did not directly decrease the overall density of *A. graminis* populations while providing acceptable turfgrass quality in either year (Figure 5. 3). Superintendents are required to keep putting greens at acceptable or higher standards at all times (Burton and Powell 1971) or otherwise potentially risk losing their employment. The current study shows that the high N fertilizer application maintained acceptable turfgrass quality as long as the mowing operations were adequately timed to avoid unintended scalping. Insecticide use, especially systemic insecticide use, reduced the *A. graminis* densities regardless of the N fertilizer dose. Moreover, the reduction in *A. graminis* densities improved with increasing N fertilizer dose. This suggests that systemic insecticide can drastically reduce *A. graminis* densities for a period, and when used with fertilizer, it can enhance the efficacy and maintain the quality of turfgrass in golf course putting greens. In southern GA, because temperatures during winter contribute to reducing the *A. graminis* population, they develop into damaging population levels by June or July the following year and signs of turfgrass decline appear by mid-to-late summer. Thus, the use of systemic insecticide application might be critical (RW unpublished data) to maintain turfgrass quality above acceptable levels through the late summer and fall seasons. In the current study, the low N fertilizer treatment negatively impacted the turfgrass, as the grass blades turned brown and were unacceptable for putting green quality. This suggests that a medium to high dose of N fertilizer application is necessary to maintain the turfgrass putting green quality. More research is warranted to optimize N fertilizer applications to maintain acceptable putting green quality and reduce the frequency of insecticide applications.

Moreover, parasitoid species introduced to the Gulf region decades ago for *A. graminis* population control in pasture grass (Riherd 1950, Schuster and Dean 1976, Dean et al. 1979) are still active on the golf course green in the current study. They offer *A. graminis* control but are slow to establish and provide acceptable *A. graminis* control (Watschke et al., 1995, Vittum 2020). Additionally, the honeydew produced by the adults and nymphs of *A. graminis* attracts pollinators, such as *Apis mellifera*, and other predatory wasps (Watschke et al. 1995, RW unpublished data). Systemic insecticides, primarily neonicotinoids, have been implicated in causing negative effects on pollinators (Blacquière et al. 2012, Larson et al. 2013, Pisa et al. 2015), calling for more research to develop a management program that reduces impacts on nontarget organisms on putting green surfaces. The current study suggests that proper fertilizer application through the season and late season insecticide use are essential for *A. graminis* control and maintenance of acceptable turfgrass quality of golf course greens. This information can be incorporated into integrated pest management programs in golf courses infested with *A. graminis*.

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Table 5. 1. Effects of fertilizer and insecticide on *A. graminis* adults and nymphs (combined) in 2019 and 2020.

2019[a]					2020[b]				
Sampling date	Treatment	*F*	df	*P*	Sampling date	Treatment	*F*	df	*P*
19-Aug	Fertilizer[c]	5.1	2,29	0.013	25-Sept	Fertilizer	16.9	2,29	0.001
	Insecticide[d]	1.2	1,29	0.283		Insecticide	17.9	1,29	< 0.001
	Fertilizer × Insecticide	0.7	2,29	0.531		Fertilizer × Insecticide	4.6	2,29	0.019
26-Aug	Fertilizer	3.9	2,29	0.030	2-Oct	Fertilizer	8.3	2,29	0.001
	Insecticide	83.0	1,29	< 0.001		Insecticide	11.2	1,29	0.003
	Fertilizer × Insecticide	0.0	2,29	0.980		Fertilizer × Insecticide	3.7	2,29	0.036
2-Sept	Fertilizer	16.8	2,29	< 0.001	8-Oct	Fertilizer	10.2	2,29	< 0.001
	Insecticide	242.5	1,29	< 0.001		Insecticide	30.0	1,29	< 0.001
	Fertilizer × Insecticide	16.9	2,29	< 0.001		Fertilizer × Insecticide	2.9	2,29	< 0.069
18-Sept	Fertilizer	20.3	2,29	< 0.001	16-Oct	Fertilizer	22.0	2,29	< 0.001
	Insecticide	124.2	1,29	< 0.001		Insecticide	162.9	1,29	< 0.001
	Fertilizer × Insecticide	15.9	2,29	< 0.001		Fertilizer × Insecticide	1.8	2,29	0.189

[a]Fertilizer was applied on 16 and 31 May, 17 and 28 June, and 17 and 30 July; insecticide was applied on 19 Aug; [b]fertilizer was applied on 17 and 29 May, 15 and 29 June, 8 and 21 July, and 16 Aug; insecticide was applied on 18 Sept; [c] low (10 g N per m^2), medium (20 g N per m^2), and high (30 g N per m^2) (20:20:20 [N:P:K, Lesco Macron] in 2019 and 2020); [d]flupyradifurone [Altus®] was applied with 1 L product per ha .

Table 5. 2. Effects of fertilizer and insecticide on fresh and dry weight of turfgrass clippings in 2019 and 2020.

	2019*				2020*			
Parameter	Treatment	*F*	df	*P*	Treatment	*F*	df	*P*
Fresh weight								
	Fertilizer[a]	170.3	2,30	< 0.001	Fertilizer	14.3	2,30	< 0.001
	Insecticide[b]	2.9	1,30	0.094	Insecticide	5.6	1,30	0.025
	Fertilizer × Insecticide	0.7	2,30	0.528	Fertilizer × Insecticide	0.1	2,30	0.906
Dry weight								
	Fertilizer	100.3	2,30	< 0.001	Fertilizer	9.6	2,30	0.001
	Insecticide	1.2	1,30	0.279	Insecticide	4.5	1,30	0.042
	Fertilizer × Insecticide	0.4	2,30	0.673	Fertilizer × Insecticide	0.3	2,30	0.776

*Samples were collected on 1 Oct 2019 and 20 Oct 2020. Insecticide (flupyradifurone) was applied on 19 Aug 2019 and 18 Sept 2020. Fertilizer was applied on 16 and 31 May, 17 and 28 Jun, and 17 and 30 Jul 2019 and on 17 and 29 May, 15 and 29 June, 8 and 21 July, and 16 Aug 2020. [a]The fertilizer treatments were low (10 g N per m^2), medium (20 g N per m^2), and high (30 g N per m^2). [b]flupyradifurone [Altus®] was applied with 1 L product per ha.

Table 5. 3. Effects of fertilizer and insecticide on the percentage nitrogen in turfgrass clipping in 2019 and 2020.

2019*				2020*			
Treatment	*F*	df	*P*	Treatment	*F*	df	*P*
Fertilizer[a]	53.3	2, 25	< 0.001	Fertilizer	23.7	2, 25	< 0.001
Insecticide[b]	0.3	1, 25	0.600	Insecticide	10.1	1, 25	0.004
Fertilizer × Insecticide	3.4	2, 25	0.051	Fertilizer × Insecticide	0.2	2, 25	0.832

*Samples were collected on 1 Oct 2019 and 20 Oct 2020. Insecticide (flupyradifurone) was applied on 19 Aug 2019 and 18 Sept 2020. Fertilizer was applied on 16 and 31 May, 17 and 28 Jun, and 17 and 30 Jul 2019, and on 17 and 29 May, 15 and 29 Jun, 8 and 21 Jul, and 16 Aug 2020. [a]The fertilizer treatments were low (10 g N per m^2), medium (20 g N per m^2), and high (30 g N per m^2).
[b]flupyradifurone [Altus®] was applied with 1 L product per ha.

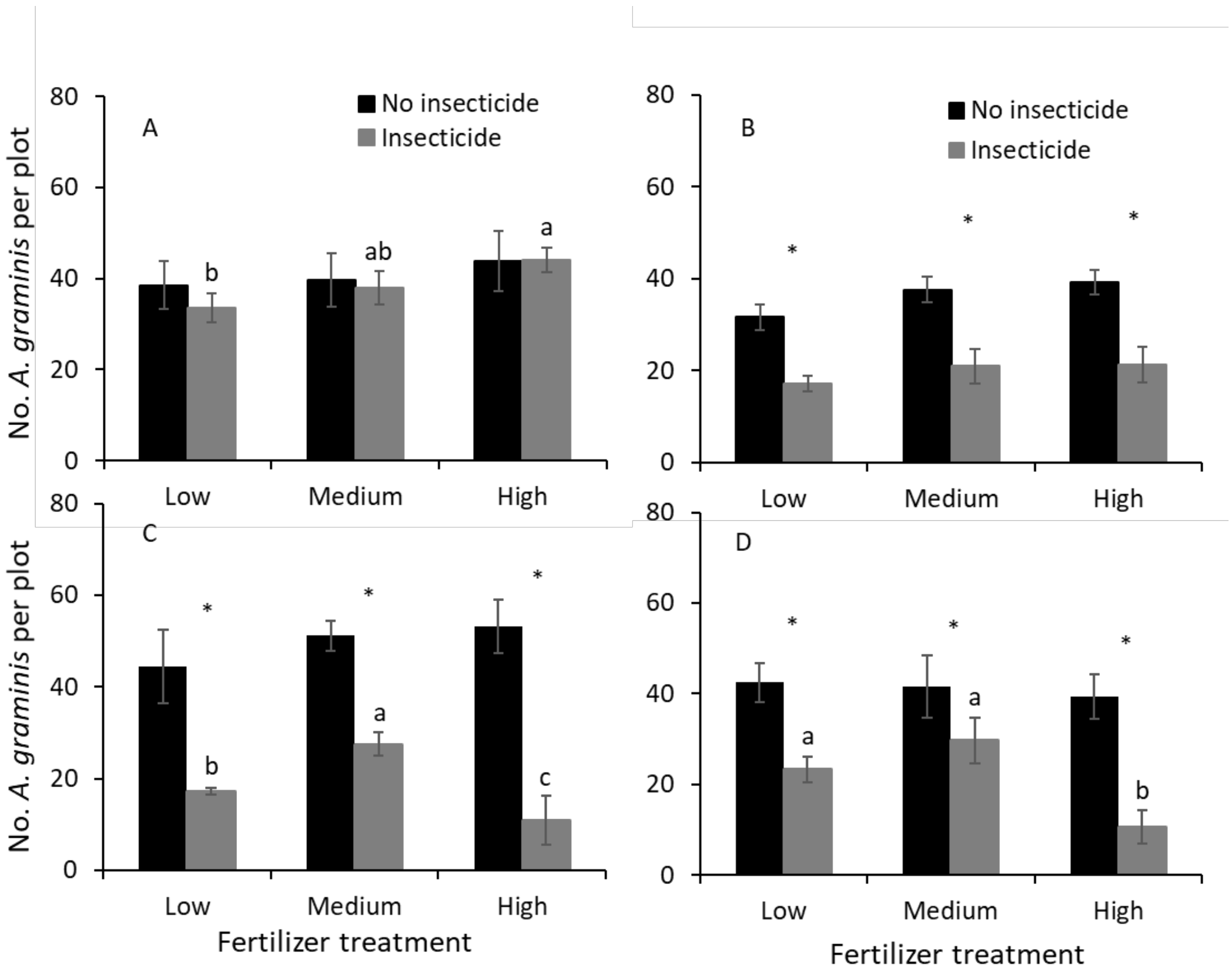

Figure 5. 1. Mean (± SE) numbers of *A. graminis* adults plus nymphs after fertilizer and insecticide treatment at (A) 0, (B) 7, (C) 14, and (D) 30 d post-insecticide application in 2019. Insecticide (flupyradifurone [Altus®]) was applied 19 Aug. Fertilizer treatments were applied on 16 and 31 May, 17 and 28 Jun, and 17 and 30 Jul. The same letters on the same-colored bars within each figure indicate no significant difference among fertilizer treatments (Tukey–Kramer Test, $\alpha = 0.05$). The absence of asterisks between insecticide and no insecticide treatments for each fertilizer level indicates no significant difference (Tukey–Kramer Test, $\alpha = 0.05$). Where no differences were observed between fertilizer and insecticide treatments, no letters or asterisks, respectively, are shown.

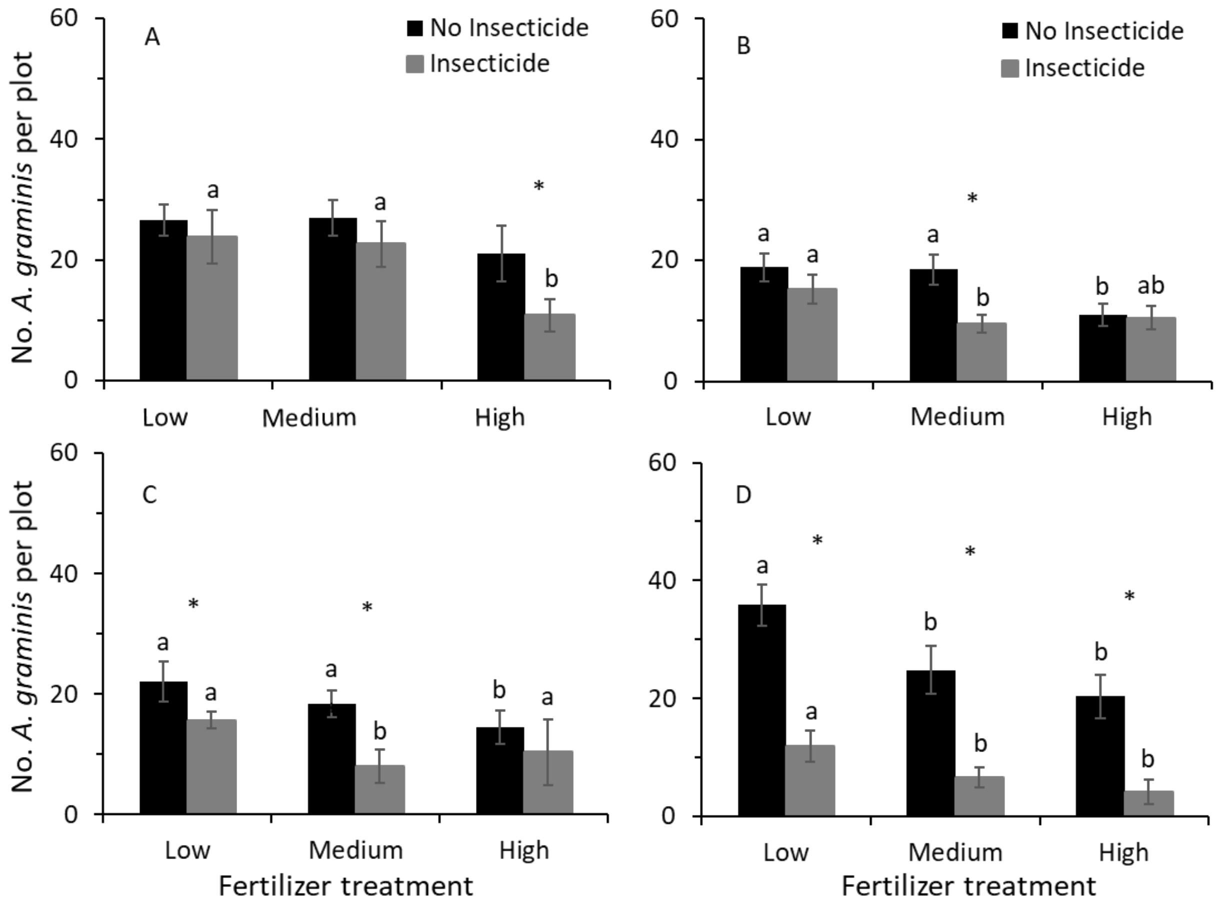

Figure 5. 2. Mean (± SE) numbers of *A. graminis* adults plus nymphs after fertilizer and insecticide treatment at (A) 7, (B) 14, (C) 20, and (D) 28 d post-insecticide application in 2020. Insecticide (flupyradifurone [Altus®]) was applied on 18 Sept. Fertilizer treatments were applied on 17 and 29 May, 15 and 29 June, 8 and 21 July, and 16 Aug. The same letters on the same-colored bars within each figure indicate no significant difference among fertilizer treatments (Tukey–Kramer test, $\alpha = 0.05$). The absence of asterisks between insecticide and no insecticide treatments for each fertilizer level indicates no significant difference (Tukey–Kramer Test, $\alpha = 0.05$). Where no differences were observed between fertilizer and insecticide treatments, no letters or asterisks, respectively, are shown.

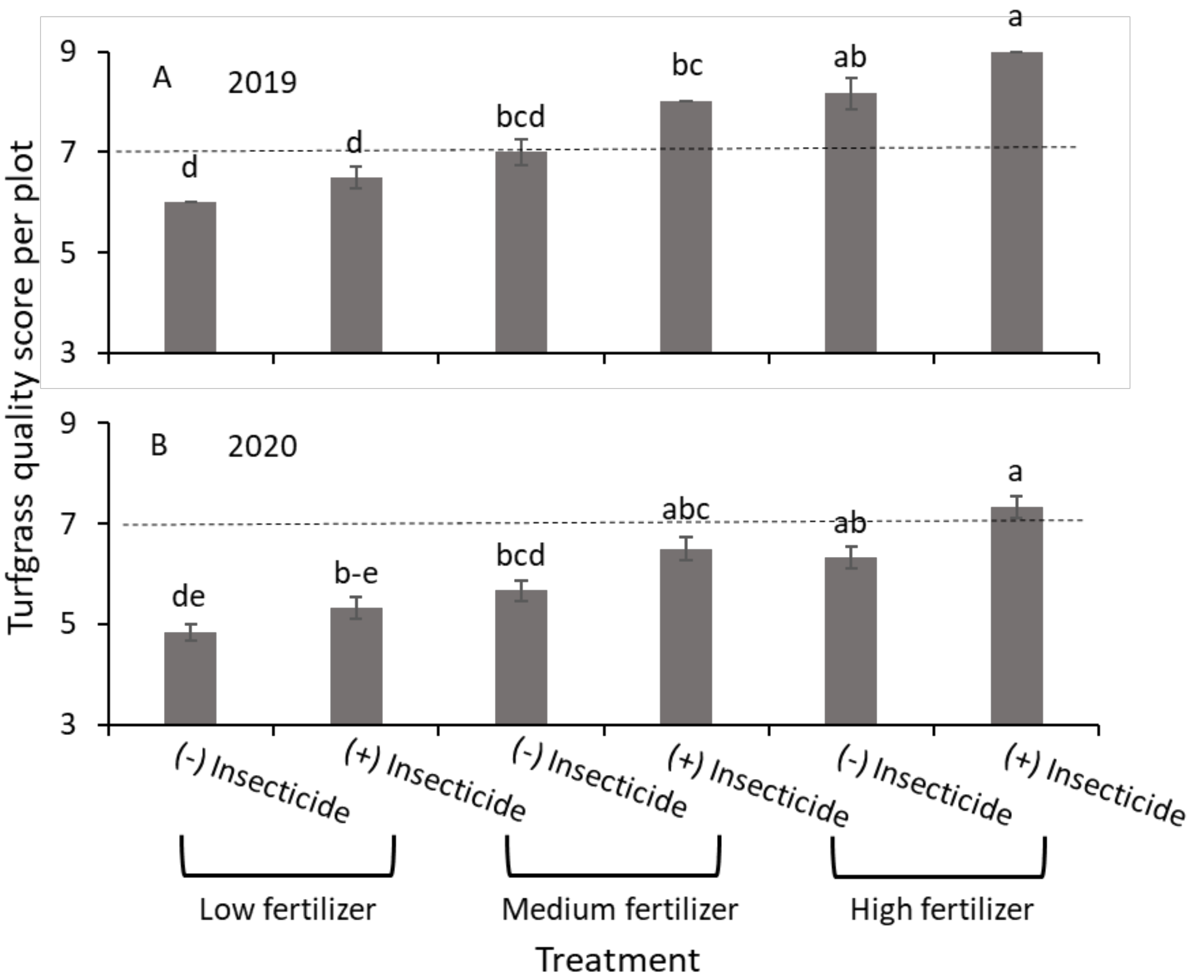

Figure 5. 3. Mean (± SE) turfgrass quality rating on (A) 15 Sept 2019 and (B) 23 Oct 2020 after application of treatments. The ratings were developed by the National Turfgrass Evaluation Program (NTEP). Insecticide (flupyradifurone [Altus®]) was applied on 19 Aug 2019 and 18 Sept 2020. Fertilizer treatments were applied on 16 and 31 May, 17 and 28 Jun, 17 and 30 Jul 2019 and on 17 and 29 May, 15 and 29 June, 8 and 21 July, and 16 Aug 2020. The dotted lines within the figures indicate an acceptable NTEP rating for turfgrass. The same letters on the bars within the figures indicate no significant difference among fertilizer-insecticide combination treatments. The treatment differences were separated using a pairwise two-sided multiple comparison analysis with the DSCF test ($\alpha = 0.05$).

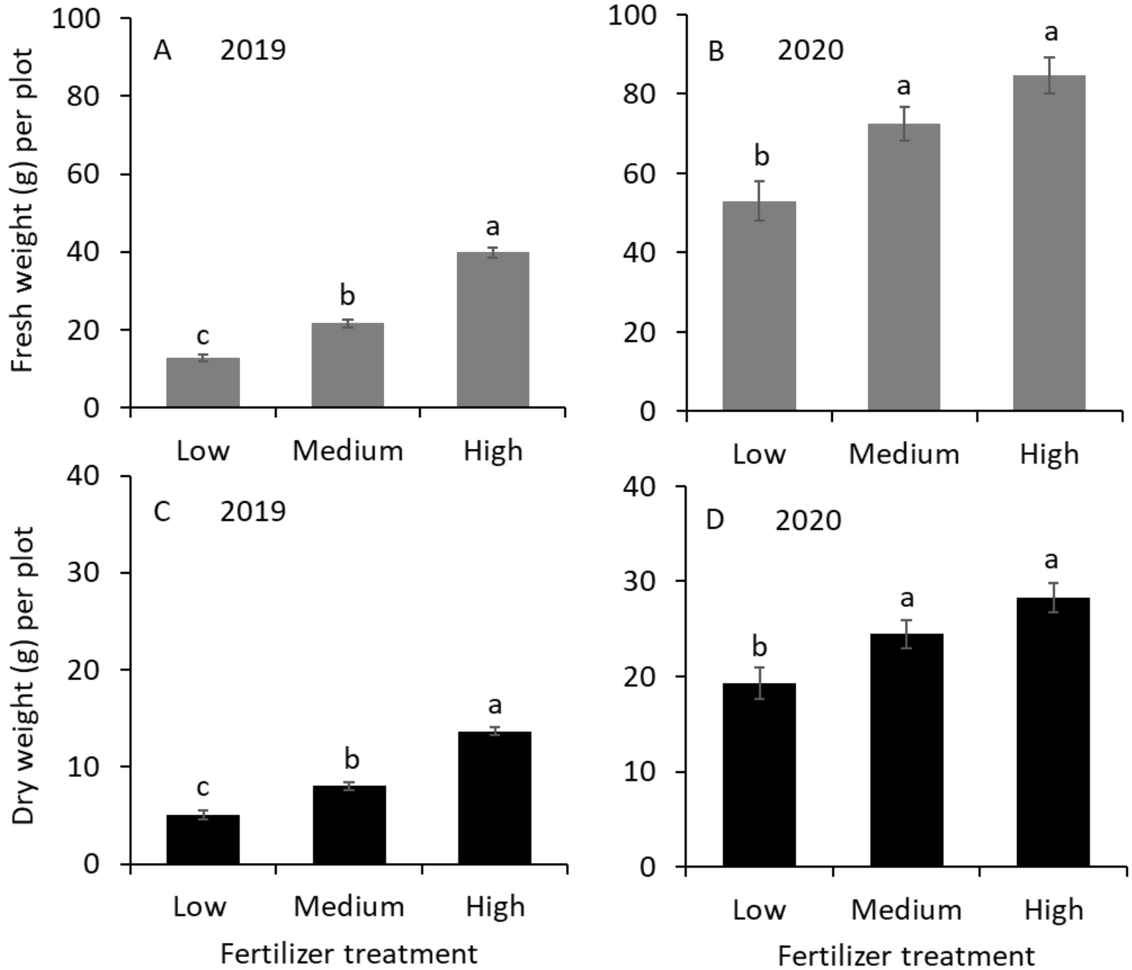

Figure 5. 4. Mean (± SE) fresh and dry weights (g) of turfgrass clippings (A and C) on 1 Oct 2019 and (B and D) on 18 Oct 2020 after application of treatments. Insecticide (flupyradifurone [Altus®]) was applied on 19 Aug 2019 and 18 Sept 2020. Fertilizer treatments were applied on 16 and 31 May, 17 and 28 Jun, and 17 and 30 Jul 2019 and on 17 and 29 May, 15 and 29 June, 8 and 21 July, and 16 Aug in 2020. The same letters on the bars within the figures indicate no significant difference among fertilizer treatments (Tukey's HSD test, $\alpha = 0.05$).

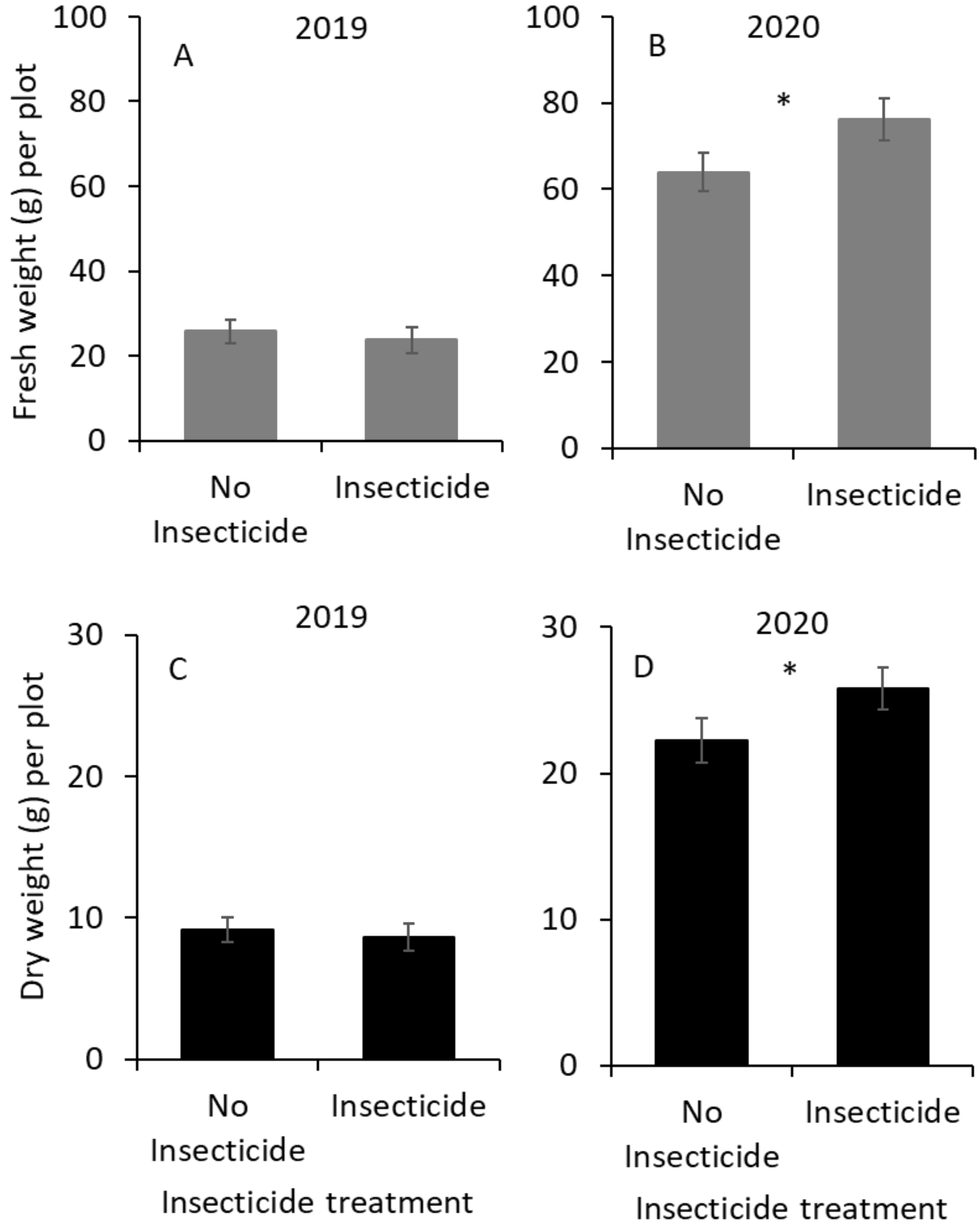

Figure 5. 5. Mean (± SE) fresh and dry weights (g) of turfgrass clippings (A and C) in 2019 and (B and D) in 2020 after application of treatments. Insecticide (flupyradifurone [Altus®]) was applied on 19 Aug 2019 and 18 Sept 2020. Fertilizer treatments were applied on 16 and 31 May, 17 and 28 Jun, 17 and 30 Jul 2019 and on 17 and 29 May, 15 and 29 June, 8 and 21 July, and 16 Aug 2020. The absence of asterisks between bars within the figures indicates no significant differences among insecticide treatments (Tukey's HSD test, $\alpha = 0.05$).

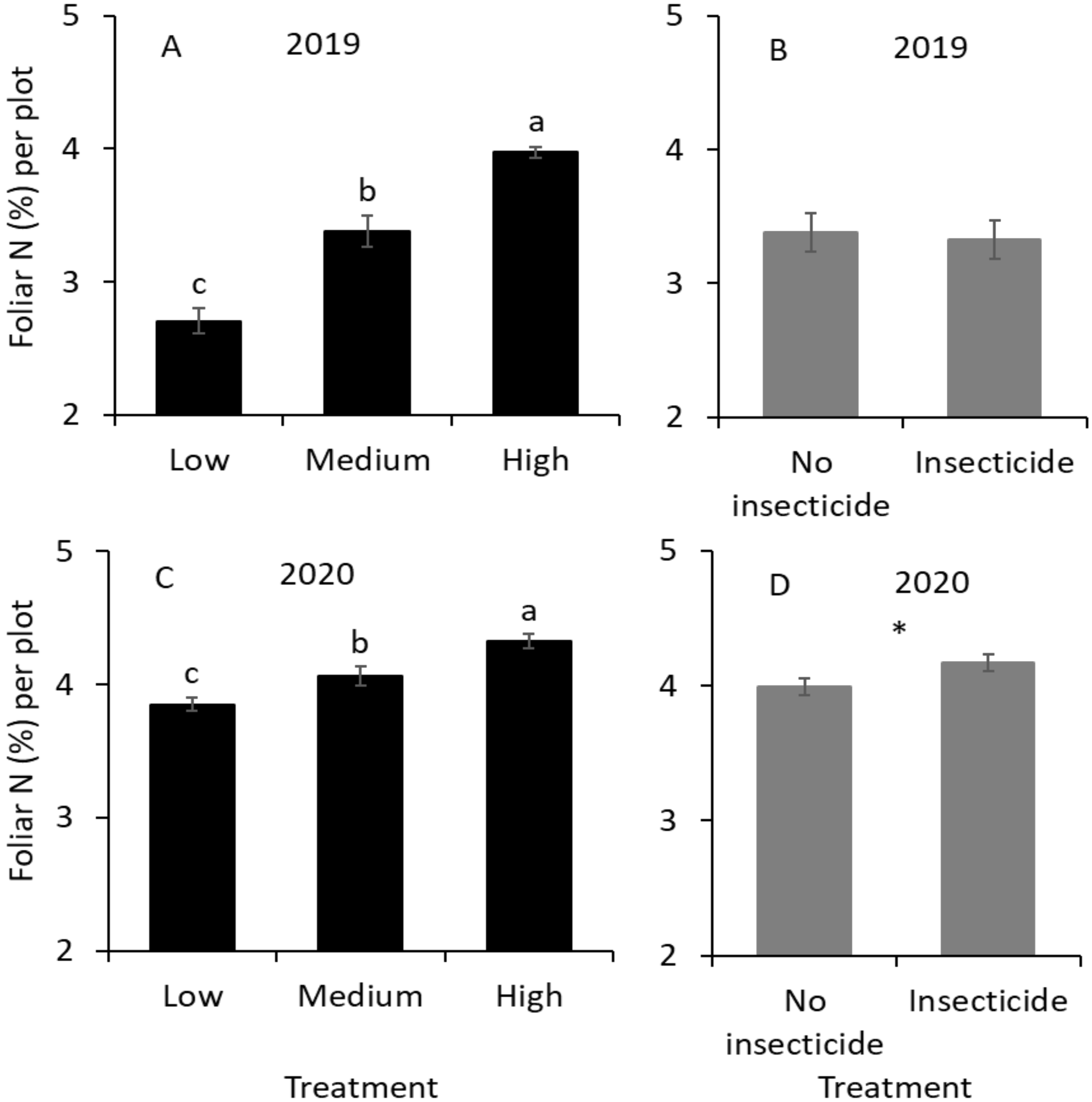

Figure 5. 6. Mean (± SE) foliar N (%) of turfgrass clippings by (A) fertilizer and (B) insecticide treatments in 2019 and (C) fertilizer and D) insecticide treatments in 2020. Insecticide (flupyradifurone [Altus®]) was applied on 19 Aug 2019 and 18 Sept 2020. Fertilizer treatments were applied on 16 and 31 May, 17 and 28 Jun, 17 and 30 Jul 2019 and on 17 and 29 May, 15 and 29 June, 8 and 21 July, and 16 Aug 2020. The same letters on the bars within the figures indicate no significant difference among fertilizer treatments (Tukey's HSD test, $\alpha = 0.05$). The absence of an asterisk between bars within the figures indicates no significant difference between insecticide treatments (Tukey's HSD test, $\alpha = 0.05$).

Supplementary Table 5. 1. Mean (± SE) foliar nutrient content of turfgrass clippings treated with three rates of fertilizer (N = 12) and insecticide (N = 18) in Oct 2019.

Foliar nutrient	Fertilizer treatment			F (df$_1$, df$_2$) $^{P\ \text{value status}}$	Insecticide treatment		F (df$_1$, df$_2$) $^{P\ \text{value status}}$
	Low	Medium	High		No	Yes	
Ca	0.4 ± 0.02	0.4 ± 0.02	0.4 ± 0.02	2.9 (2,25)^{NS}	0.4 ± 0.02	0.4 ± 0.02	0.4 (1,25)^{NS}
K	1.2 ± 0.06c	1.6 ± 0.08b	1.9 ± 0.07a	58.3 (2,25)***	1.6 ± 0.09	1.7 ± 0.09	1.9 (1,25)^{NS}
Mg	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.0	3.3 (2,25)^{NS}	0.2 ± 0.01	0.2 ± 0.01	0.9 (1,25)^{NS}
P	0.5 ± 0.03b	0.6 ± 0.03a	0.6 ± 0.02a	11.2 (2,25)***	0.6 ± 0.02	0.6 ± 0.03	0.2 (1,25)^{NS}
S	0.3 ± 0.01b	0.4 ± 0.02a	0.4 ± 0.02a	18.5 (2,25)***	0.4 ± 0.01	0.3 ± 0.01	0.6 (1,25)^{NS}
Al	47.9 ± 8.2a	36.8 ± 9.4ab	17.9 ± 2.3b	8.9 (2,25)**	30.3 ± 4.4	38.1 ± 8.1	0.2 (1,25)^{NS}
B	3.6 ± 0.1	3.7 ± 0.2	3.8 ± 0.2	0.2 (2,25)^{NS}	3.6 ± 0.1	3.8 ± 0.2	0.2 (1,25)^{NS}
Cu	14.9 ± 0.6b	17.4 ± 0.7a	18.6 ± 0.7a	11.9 (2,25)***	16.5 ± 0.7	17.5 ± 0.6	2.4 (1,25)^{NS}
Fe	129.1 ± 13.0a	105.3 ± 7.4ab	95.6 ± 6.1b	3.5 (2,25)*	105.3 ± 6.7	114.7 ± 9.4	0.6 (1,25)^{NS}
Mn	42.3 ± 2.5	42.7 ± 2.6	42.3 ± 4.6	0.3 (2,25)^{NS}	43.8 ± 2.9	41.1 ± 2.5	1.2 (1,25)^{NS}
Zn	42.1 ± 2.5	43.0 1.5	44.8 ± 2.1	1.2 (2,25)^{NS}	45.5 ± 1.9a	41.1 ± 1.3b	6.9 (2,25)*

Not significant, NS; $P < 0.05$, *; $P < 0.01$, **; and $P < 0.001$, ***. Insecticide (flupyradifurone) was applied on 19 Aug 2019. Fertilizer was applied on 16 and 31 May, 17 and 28 Jun, and 17 and 30 Jul 2019. The fertilizer treatments were low (10 g N per m^2), medium (20 g N per m^2), and high (30 g N per m^2). Flupyradifurone [Altus®] was applied with 1 L product per ha.

Supplementary Table 5. 2. Mean (± SE) foliar nutrient content of turfgrass clippings treated with three rates of fertilizer (N = 12) and insecticide (N = 18) in Oct 2020.

Foliar nutrient	Fertilizer treatment			F (df$_1$, df$_2$) $^{P \text{ value status}}$	Insecticide treatment		F (df$_1$, df$_2$) $^{P \text{ value status}}$
	Low	Medium	High		No	Yes	
Ca	0.273 ± 0.009b	0.283 ± 0.009b	0.312 ± 0.008a	6.4 (2,25)**	0.277 ± 0.008b	0.302 ± 0.007a	7.4 (1,25)*
K	1.4 ± 0.05c	1.5 ± 0.05b	1.6 ± 0.08a	1.4 (2,25)^{NS}	1.5 ± 0.04	1.5 ± 0.06	0.1 (1,25)^{NS}
Mg	0.178 ± 0.008b	0.183 ± 0.003b	0.198 ± 0.004a	12.2 (2,25)***	0.183 ± 0.004b	0.191 ± 0.003a	5.3 (1,25)*
P	0.5 ± 0.01	0.5 ± 0.01	0.5 ± 0.01	0.4 (2,25)^{NS}	0.5 ± 0.01	0.5 ± 0.01	0.4 (1,25)^{NS}
S	0.3 ± 0.0	0.3 ± 0.01	0.3 ± 0.0	0.3 (2,25)^{NS}	0.3 ± 0.0	0.3 ± 0.0	1.6 (1,25)^{NS}
Al	44.9 ± 3.3	45.5 ± 4.3	50.4 ± 4.1	0.5 (2,25)^{NS}	47.4 ± 2.9	46.5 ± 3.4	0.1 (1,25)^{NS}
B	2.5 ± 0.1b	3.1 ± 0.3ab	3.3 ± 0.08a	6.4 (2,25)**	2.8 ± 0.1	3.1 ± 0.2	1.2 (1,25)^{NS}
Cu	13.0 ± 0.3	13.3 ± 0.3	13.6 ± 0.4	0.5 (2,25)^{NS}	13.0 ± 0.3	13.6 ± 0.2	2.3 (1,25)^{NS}
Fe	151.3 ± 10.1	140.1 ± 7.2	156.9 ± 5.9	1.5 (2,25)^{NS}	148.4 ± 7.5	150.5 ± 5.5	0.5 (1,25)^{NS}
Mn	94.5 ± 4.8	90.4 ± 4.8	104.2 ± 6.7	1.5 (2,25)^{NS}	92.8 ± 4.6	99.9 ± 4.5	1.4 (1,25)^{NS}
Zn	35.7 ± 1.2b	37.7 ± 1.0ab	40.8 ± 1.3a	5.5 (2,25)*	36.7 ± 1.0b	39.4 ± 0.9a	4.4 (2,25)*

*Samples were collected on 20 Oct 2020. Insecticide (flupyradifurone) was applied on 18 Sept 2020. Fertilizer was applied on 17 and 29 May, 15 and 29 Jun, 8 and 21 Jul, and 16 Aug 2020. The fertilizer treatments were low (10 g N per m^2), medium (20 g N per m^2), and high (30 g N per m^2). Flupyradifurone [Altus®] was applied with 1 L product per ha.

CHAPTER 6

EFFECTS OF LIGHTWEIGHT ROLLING, SAND TOPDRESSING, AND INSECTICIDE ON THE RHODESGRASS MEALYBUG (HEMIPTERA: PSEUDOCOCCIDAE) ON GOLF COURSE PUTTING GREENS

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Abstract Rhodesgrass mealybug, *Antonina graminis* Maskell (Hemiptera: Pseudococcidae) is an important pest on golf course putting greens. *Antonina graminis* feeding causes extensive yellowing and browning that causes turfgrass mortality. Lightweight rolling and sand topdressing are standard cultural practices on the golf course; however, it is unclear whether they can reduce *A. graminis* densities and provide additional suppression when combined with insecticide. Thus, the objectives of the study were to determine the effects of (1) lightweight rolling and sand topdressing and (2) when combined with a systemic insecticide, thiamethoxam, on *A. graminis* densities on golf course putting greens. In 2021 and 2022, experiments were conducted on putting greens in a split-plot design where lightweight rolling was mainplot treatment and sand topdressing, insecticide, sand topdressing + insecticide, and nontreated were subplot treatments. The numbers of *A. graminis* were not significantly affected by rolling treatment in the 2021 and 2022 experiments. Similarly, the sand topdressing alone had no significant effect on the *A. graminis* densities on the putting greens. The numbers of *A. graminis* were significantly lower for the insecticide (thiamethoxam) and combination of sand topdressing + insecticide treatment than for the nontreated treatment.

Keywords lightweight rolling, sand topdressing, thiamethoxam, *Antonina graminis*

Rhodesgrass mealybug, *Antonina graminis* (Mask.) (Hemiptera: Pseudococcidae) is an important pest of golf course putting greens in subtropical regions of the USA (Chada and Wood 1960). The golf courses, ranging from Myrtle Beach, South Carolina, along the coast, throughout Florida, and Texas along the Gulf of Mexico, and westward into California (GGE 2010, NGF 2019), are vulnerable to *A. graminis* infestation and feeding damage. *Antonina graminis* was first observed causing damage on the golf course in 1949 at Houston Country Club (Henry 1950), followed by others courses in Florida and Louisiana in the early 1950s (Sander 1953, Watson 1953, Ferguson 1953, Lawrence 1952, Ferguson 1954). *Antonina graminis* damage to golf courses has been reported worldwide, especially in Australia, Israel, and Korea (Brimblecombe 1968, Berlinger and Barak 1981, Gyu-Yul and Kim 1994). *Antonina graminis* can attack more than 100 species of grasses (Poaceae) but prefers turfgrass on the putting greens (Reinhert et al. 2009, 2010). In the southern USA, golf is played year-round, as the climate remains warm during the winter. Golf enthusiasts travel from the north (USA) to courses in the southern states. However, these courses are susceptible to *A. graminis* infestations and affect the playability of golf. On the putting greens, severe *A. graminis* feeding causes yellowing and browning of turfgrass, and the affected turfgrass may die within weeks. These damage symptoms affect the aesthetic appearance and playability of golf.

Antonina graminis females are 2-3 mm long insects. They are parthenogenetic, with each female capable of producing 150-300 eggs (Chada and Wood 1960). The eggs hatch within the female, and crawlers are born live (Joseph and Hudson 2019). Crawlers are the only mobile stage of *A. graminis*. They settle on the nodes under the leaf sheath close to the soil and molt through two sessile nymphal stages before molting into adults. It can take about 50-60 d for a female *A. graminis* to reach reproductive maturity. Adult longevity is up to 100 d (Chada and Wood 1960).

The putting greens are a highly maintained golf course area for aesthetics and performance. Many cultural practices are routinely administered on the putting greens to achieve aesthetic and performance goals. The turfgrass on the putting greens is mowed multiple times each week and maintained at ~2.3 mm height sometimes for the entire year. In addition to mowing, lightweight rolling is performed to improve smooth ball roll on the putting green surfaces. The lightweight rollers are specifically designed for putting green surfaces, typically weighing less than 500 kg and exerting 24 to 50 kPa ground pressure (Turgeon and Kaminski 2019). To prevent excessive shoot growth, which could later build up as thick thatch deposition, a 0.5-1 mm thin layer of sand is topdressed on the putting greens and incorporated into the thatch layer to maintain firmness and uniform soil structure. In addition, the putting greens are irrigated as needed to prevent turfgrass from desiccation. Fertilizers and plant growth regulators are regularly applied to promote turfgrass growth, and pesticides are prophylactically applied to prevent pathogen, insect, and weed pest attacks. Although all these cultural practices are available to golf superintendents to achieve aesthetic and performance goals of the putting greens, not all practices are adopted due to economic constraints.

Cultural control is an important tactic within integrated pest management (IPM) principles, where the production or cultural practices are modified to reduce the incidence or abundance of pests or their damage (Flint 2012). Previous observations showed that vertical mowing reduced *A. graminis* densities on the putting green surfaces (Ferguson 1954). Modern putting green management techniques encourage frequent sand topdressing and lightweight rolling in addition to routine mowing operations. These practices enhance the firmness and smoothness of the putting green surfaces for improved playability. Little is known whether frequent lightweight rolling and sand topdressing would reduce the *A. graminis* densities on the putting green surface. The assumption was that the angular edges of the sand grains applied as topdressing and the rolling would abrasively exert additional pressure on females and enhance *A. graminis* mortality. In addition, it is unclear whether these cultural practices combined with effective insecticide can provide additional *A. graminis* control on the putting greens. Previously, systemic insecticides, especially thiamethoxam and flupyradifurone have shown promise in reducing *A. graminis* densities (Joseph et al. 2021). Thus, the objectives of the study were to determine the effects of (1) lightweight rolling and sand topdressing and (2) when they were combined with insecticide on *A. graminis* densities on the golf course putting greens.

Materials and Methods

Study site, general methods, insects. In 2021 and 2022, the experiments were conducted on the putting greens at the Columbus State University, Key Golf Studio in Columbus, GA, USA. Two separate putting greens 15 m apart were selected for the study. In 2015, the putting greens were constructed in the golf facility based on the United States Golf Association's construction guidelines (USGA 2018) using sand and sphagnum peat mix (85:15). The turfgrass on the putting greens was 'TifEagle' bermudagrass [*Cynodon dactylon* CL. (Pers) × *C. transvaalensis* (Burtt-Davy)]. The putting greens were naturally infested with *A. graminis* before the trials, and the entire greens were infested with *A. graminis*. From March to November 2021 and 2022, the putting greens were mowed using a walk-behind mower (TORO® Greensmaster 1000, Bloomington, MN) with a bench height of 2.3 mm. The putting greens received nitrogen fertilizer every week as liquid urea at 3.36 kg per ha per year. The plant growth regulator, trinexapac-ethyl (11.3%) at 30-59 mL per ha (Primo Maxx®, Syngenta, Basil, Switzerland), was applied weekly depending on the growth rate of turfgrass. Preventative fungicides, such as chlorothalonil (Manicure 6FL, Lesco, Cleveland, OH) at 9.5 L per ha and penthiopyrad (Velista, Syngenta, Basil, Switzerland) at 1.53 kg per ha were applied when disease pressure was high. In the spring, foramsulfuron (Revolver®, Bayer Environmental Science [Envu Environmental Science], Cary, NC) was applied at 0.7 L per ha to suppress grassy weeds. No insecticides were applied previously on the putting greens before the start of the experiments. In addition, except for mowing, all mechanical practices, such as aerification, vertical mowing, rolling, and sand topdressing, were suspended on the selected putting greens.

Experimental design. The treatments were arranged in a split-plot design where lightweight rolling was mainplot treatment and sand topdressing, insecticide, a combination of sand topdressing and insecticide, and nontreated were subplot treatments. The treatments were replicated four times. The individual plot was 1.5 m × 1 m. The experiments were initiated on 31 August 2021 and 5 August 2022, as *A. graminis* populations increased through the summer. The rolling treatment was conducted using a TruTurf RB48 Golf Greens Roller (TruTurf, Gold Coast, Australia) with a 1.2 m width, 321 kg weight (plus 84 kg of the operator), and exerting 24 kPa ground pressure. The rolling treatment was conducted six times each week of the experiment, where one rolling each was performed on Mondays and Wednesdays and two rollings each on Fridays and Saturdays. For the sand topdressing treatment, 1.25 kg of 45 mesh, 0.35 mm, subangular silica sand (Covia Holdings Corp., Junction City, GA) was added thrice to each designated plot at 0, 14 and 28 d post-initiation of the experiment. The sand was applied by hand and incorporated with a 61 cm wide palmyra bristled push broom (Grainger®, Lake Forest, IL) from both directions. For the insecticide treatment, thiamethoxam (Meridian® 25WG, Syngenta, Basil, Switzerland) was applied once at 224 g product per ha using a CO_2-powered sprayer with a TeeJet® flat fan nozzle (XR11008). The water volume used for the application was 813 L per ha at 219.9 kPa.

Evaluation. In 2021 and 2022, three turfgrass plugs were collected from each plot using a 1.9 cm stainless steel soil core probe (soil probe portion: 53.3 cm length × 1.91 cm diameter, SiteOne® Landscape Supply, Roswell, GA). The plug samples were collected in plastic bags, transported into the laboratory, and stored at 10 °C for ~2 d. The samples were examined for live *A. graminis* individuals under a dissecting microscope (AmScope, Irvine, CA) at 10× magnification. Individual *A. graminis* were examined for alive or dead by piercing with a needle as alive *A. graminis* individuals exuded a reddish-brown colored fluid, whereas dead ones exuded yellow colored fluid or none. In 2021, the sampling for *A. graminis* was conducted at 0, 7, 15, 21, 31, 39, and 45 d post-initiation of the experiment, whereas in 2022, the sampling was conducted at 0, 7, 14, 21, 28, and 35 d post-initiation of the experiment. The sampling dates were 31 August, 7, 15, and 22 September and 1, 9, 15 October 2021, and 5, 12, 19, and 26 August, and 2 and 9 September 2022.

Statistical analyses. All statistical analyses were conducted using SAS 9.4 (SAS 2016). The data sets were log-transformed (ln[x+2]) after checking the normality of residuals using the PROC UNIVARIATE procedure in SAS. To determine the effects of the mainplot, subplot, and their interaction for *A. graminis* density, a two-way analysis of variance (ANOVA) was conducted using the PROC MIXED procedure in SAS. The fixed effects were the mainplot (rolling) and subplot (topdressing, insecticide, topdressing + insecticide, and nontreated) treatments. The random effects were the replication and replication × mainplot treatment. The sample date was included in the model as a repeated measure. To understand further, one-way ANOVA was conducted by sampling date on *A. graminis* density data using the PROC MIXED procedure in SAS. The fixed effects were mainplot (rolling) and subplot (topdressing, insecticide, topdressing + insecticide, and nontreated). The random effects were as the replication and replication ×

mainplot treatment. The least-square means for the mainplot and subplot treatments were separated using the Tukey-Kramer ($P < 0.05$) test in SAS. Means and standard errors of the mainplot and subplot treatments were calculated using the PROC MEANS procedure in SAS.

Results and Discussion

In 2021 and 2022, there were no significant differences between rolling versus nonrolling treatments for the *A. graminis* densities (Table 6. 1; Figure 6. 1), suggesting that the lightweight rolling failed to reduce the *A. graminis* densities on the putting greens. The lightweight roller is primarily used on the putting greens to smooth the turfgrass putting surface without causing soil compaction. The results show that despite multiple rollings of lightweight rolling at 24 kPa, it did not exert enough ground pressure to crush the *A. graminis* densities physically. This is the first report showing the use of a lightweight roller to reduce arthropod pests on the putting green surfaces. However, a previous study showed that severity of anthracnose and dollar spot was reduced after lightweight rolling (Inguagiato et al. 2009). The current study was not conducted beyond 6 weeks. It is unclear if prolonged rolling and sand topdressing treatments would reduce *A. graminis* densities in the longer term.

The subplot treatments were significantly different for the *A. graminis* densities (Table 6. 1). However, the interaction between mainplot and subplot treatments was not significantly different for the *A. graminis* densities (Table 6. 1). Because there were significant effects for the subplot treatments, one-way ANOVA was conducted by sampling dates. In 2021, there were no significant differences for the *A. graminis* densities among subplot treatments at 0 and 7 d post-initial treatment. At 15, 21, 31, and 39 d post-initial treatment, significantly lower numbers of *A. graminis* were observed for the insecticide and insecticide + topdressing treatments than for the topdressing alone and nontreated treatments (Table 6. 1; Figure 6. 2A). There were no significant differences between insecticide and insecticide + topdressing treatments for these sampling dates (Figure 6. 2A). At 45 d, the numbers of *A. graminis* were significantly lower for the insecticide and insecticide + topdressing treatments than for the topdressing treatment (Table 6. 1; Figure 6. 2A).

In 2022, there were no significant differences for the *A. graminis* densities among subplot treatments at 0, 7 and 14 d after initial treatment (Table 6. 1; Figure 6. 2B). At 21 d, the numbers of *A. graminis* were significantly lower for the insecticide + topdressing treatment than for the nontreated treatment (Table 6. 1; Figure 6. 2B). At 28 d, the *A. graminis* densities were significantly lower for the insecticide and insecticide + topdressing treatments than for the topdressing only and nontreated treatments (Table 6. 1; Figure 6. 2B). There were no significant differences between insecticide and insecticide + topdressing treatments for these sampling dates (Figure 6. 2B). At 35 d, the numbers of *A. graminis* were significantly lower for the insecticide treatment than for the topdressing and nontreated treatments (Table 6. 1; Figure 6. 2B). Although the application of sand topdressing firmed up the turfgrass canopy (RW personal communication), topdressing application did not reduce *A. graminis* densities on the putting greens. Some studies have shown that initiating sand topdressing on the putting green surface increased the incidence of disease outbreaks as the sand created more wounds on the turfgrass blades. However, after repeated applications, sand topdressing improved turfgrass quality and a reduction in disease severity was observed (Inguagiato et al. 2012). In the current study, a single application of thiamethoxam effectively reduced *A. graminis* densities within four weeks and was consistent with the previous study (Joseph et al. 2019).

In summary, the results showed that cultural practices, such as lightweight rolling and sand topdressing, were ineffective in reducing *A. graminis* densities on the putting greens. These practices were only administered for a short period in the current study, and an extended application of these cultural tactics may produce suppression of *A. graminis* densities, which warrants further investigation. Developing sustainable management tactics for *A. graminis* on putting greens, in conjunction with compatible, reduced-risk insecticides, should be further evaluated along with other strategies, such as biological control. Only chemical control strategies effectively reduced *A. graminis* densities (Joseph et al. 2019) despite the active biological control agents on the putting greens (RW personal observation). Previous studies show that enhanced use of nitrogen fertilizer can improve the turfgrass quality and mitigate the damage to a certain extent (RW unpublished data); however, insecticidal suppression of *A. graminis* densities is critical for the longer-term management of *A. graminis* populations on the golf course putting greens.

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Table 6. 1. The effects of mainplot (rolling) and subplot (insecticide, topdressing, topdressing + insecticide and nontreated) on *A. graminis* densities on golf course putting greens in 2021 and 2022.

Treatment	2021				2022			
	Post-treatment intervals (d)[c]	*F*	df	*P*	Post-treatment intervals (d)[c]	*F*	df	*P*
	Combined				Combined			
Mainplot[a]		1.9	1, 3	0.261		1.8	1, 3	0.268
Subplot[b]		14.1	3, 210	< 0.001		8.6	3, 178	< 0.001
Mainplot × Subplot		0.6	3, 210	0.618		0.7	3, 178	0.530
	0				0			
Mainplot		0.0	1, 3	0.932		1.9	1, 3	0.254
Subplot		0.1	3, 18	0.955		0.5	3, 18	0.723
Mainplot × Subplot		1.2	3, 18	0.344		0.3	3, 18	0.814
	7				7			
Mainplot		0.9	1, 3	0.420		0.4	1, 3	0.597
Subplot		1.9	3, 18	0.158		0.1	3, 18	0.933
Mainplot × Subplot		0.4	3, 18	0.755		1.0	3, 18	0.412
	15				14			
Mainplot		1.0	1, 3	0.384		3.1	1, 3	0.175
Subplot		16.6	3, 18	< 0.001		3.1	3, 18	0.053
Mainplot × Subplot		0.6	3, 18	0.640		0.2	3, 18	0.913
	21				21			
Mainplot		0.7	1, 3	0.461		4.1	1, 3	0.137
Subplot		28.19	3, 18	< 0.001		9.4	3, 18	0.001
Mainplot × Subplot		0.73	3, 18	0.550		2.3	3, 18	0.115
	31				28			
Mainplot		1.3	1, 3	0.335		2.5	1, 3	0.215
Subplot		23.7	3, 18	< 0.001		8.4	3, 18	0.001
Mainplot × Subplot		1.3	3, 18	0.298		2.1	3, 18	0.141
	39				35			
Mainplot		2.9	1, 3	0.187		0.0	1, 3	0.920
Subplot		18.0	3, 18	< 0.001		3.8	3, 18	0.028

Mainplot × Subplot		1.5	3, 18	0.260	0.3	3, 18	0.860
	45						
Mainplot		0.2	1, 3	0.656	-	-	-
Subplot		15.9	3, 18	< 0.001	-	-	-
Mainplot × Subplot		1.1	3, 18	0.369	-	-	-

[a] The mainplot treatments were rolled and nonrolled.
[b] The subplot treatments were insecticide only, top dressing only, top dressing + insecticide, and nontreated.
[c] The sampling was conducted on 31 August, 7, 15, and 22 September and 1, 9, and 15 October 2021, and 5, 12, 19, and 26 August, 2, and 9 September 2022.

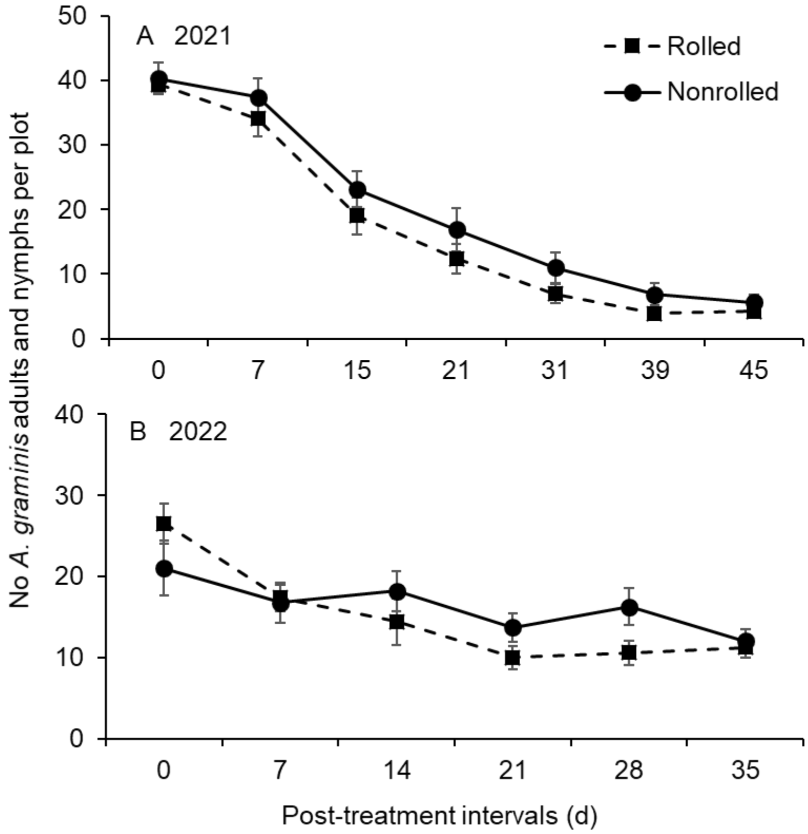

Figure 6. 1. Mean (± SE) numbers of *A. graminis* densities collected from mainplot (rolling) treatment on golf course putting greens at various sampling dates after the initial application of treatments in 2021 and 2022. No letters or symbols are provided at each sampling date as treatment effects were not significantly different at $\alpha = 0.05$.

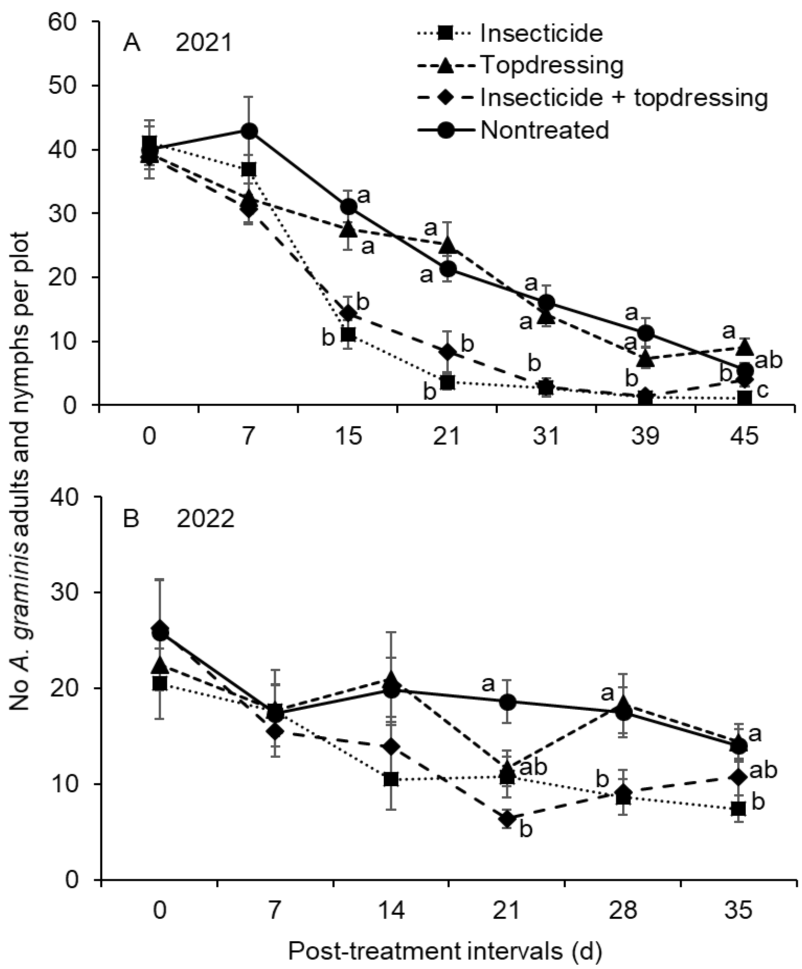

Figure 6. 2. Mean (± SE) numbers of *A. graminis* densities collected from subplot (insecticide, topdressing, topdressing + insecticide and nontreated) treatments on golf course putting greens at various sampling dates after the initial application of treatments in 2021 and 2022. The same letters with sampling dates indicate no significant differences among treatments (Tukey – Kramer test, $\alpha = 0.05$). No letters are provided for sampling dates when none of the treatments significantly differed at $\alpha = 0.05$.

Chapter 7

Summary

Rhodesgrass mealybug, *Antonina graminis* Maskell (Hemiptera: Pseudococcidae) is resurging as a pest of turfgrass, particularly on golf course putting greens. In 2017, a severe population of *A. graminis* was identified in Columbus, Georgia, on the putting greens associated with Columbus State University. This is the first *A. graminis* infestation reported this far inland in Georgia. It was possible that *A. graminis* may have been spread from other golf courses and was misdiagnosed because their feeding symptoms resemble general turfgrass stress symptoms. Unfortunately, there was very little information available to effectively manage this pest at the time. Cultural practices, such as vertical mowing and overseeding with cool-season grasses in the fall, were employed to suppress the *A. graminis* feeding damage from becoming noticeable. Honey bees, wasps and ants were seen foraging on the honeydew secretions from *A. graminis*. Initially, systemic insecticide (dinotefuran) was applied, but it did not suppress *A. graminis* population and feeding damage was evident on 50% of the entire putting green surfaces in the golf course. There was an urgent need to develop management strategies for *A. graminis* on golf course putting greens.

Since *A. graminis* was not studied on putting greens in Georgia, the phenology of *A. graminis* on putting greens was poorly understood. Thus, studies were conducted from 2019 to 2022 with turfgrass sampling at biweekly intervals. The results showed that *A. graminis* densities increased gradually in the spring and became detectable by June or July on the putting greens. Multiple generations of *A. graminis* were observed in the summer and fall. By mid to late

October, *A. graminis* densities declined. The *A. graminis* feeding damage was evident by the late summer and fall. Because no sampling methods were developed for crawlers in the putting greens, six trap-type designs were investigated. The results showed that strips of yellow sticky cards with or without a folded paper trapped more densities of crawlers than any other trap-types, such as Berlese funnel, photo, etc.

When the *A. graminis* infestation and feeding damage were observed, limited information was available on an effective insecticide for *A. graminis* densities. Four insecticides emerged as effective on *A. graminis* with 65-75% efficacy compared to nontreated and they were flupyradifurone, thiamethoxam, imidacloprid and acephate. Besides flupyradifurone, all other insecticides were labeled for use on golf courses. Currently, flupyradifurone is not labeled on golf courses but is specifically for use in ornamental production.

To determine the best timing for the use of systemic insecticides on *A. graminis*, experiments were conducted in 2019 and 2021, where insecticide applications were made once in June, July and August and they were compared with repeated applications made in June plus July and June plus July plus August. The results showed that late application of insecticide effectively reduced the *A. graminis* densities. The efficacy of repeated three applications and late application in the fall were not different, although the efficacy did not last for more than a month.

It was not clear if lower doses of nitrogen fertility could be used optimize the *A. graminis* densities, which could be managed using systemic insecticide. Results showed that moderate to high doses of nitrogen fertilizer improved turfgrass quality. The systemic insecticide effectively reduced *A. graminis* densities on putting greens. Moreover, high dose of nitrogen fertilizer plus systemic insecticide provided the greatest suppression of *A. graminis* densities than the remaining nitrogen fertilizer and insecticide combinations. Moderate to high nitrogen fertilizer doses along with insecticide outperformed low nitrogen fertilizer and nontreated.

Practices, such as lightweight rolling and sand topdressing were evaluated on *A. graminis* densities to develop cultural control options. The assumption was that the angular edges of the sand grains along with pressure would crush the soft bodies of *A. graminis*. In addition, these cultural practices with insecticide (thiamethoxam) would enhance the efficacy on *A. graminis* suppression. Results showed that lightweight rolling and sand topdressing methods were not effective in reducing *A. graminis* densities on the putting green surfaces. Comparatively, insecticide was effective on *A. graminis*.

Clearly, these studies showed some important information about the phenology, sampling for crawlers, effective insecticides, application timing, and integrating insecticide tactic with nitrogen fertilizer, sand topdressing and lightweight rolling on putting greens of golf course. This new information will be integrated into integrated pest management for *A. graminis* on putting greens.