# IMPROVING INTEGRATED PEST MANAGEMENT STRATEGIES FOR THE FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) IN TURFGRASS

by

# **GURJIT SINGH**

(Under the Direction of SHIMAT V. JOSEPH)

# **ABSTRACT**

Evaluation of sweep, vacuum, and pitfall trap samples revealed that the abundance of certain arthropods was influenced by the turfgrass genotype, height, density, and thatch thickness in the sod fields. Pitfall traps captured most of the arthropods including 89% and 96% of all the predatory arthropods and parasitic Hymenoptera. When bermudagrass 'TifEagle' was treated with varied ratios of N: K, the survival of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) was found significantly lower in 0:1, 1:2, 1:0, and 0:0 than in 2:1 and 1:1 treatment in 2018, whereas in 2019, it was similar among all the treatments beyond 6d post-introduction. The development of *S. frugiperda* larvae was significantly greater on 2:1 treatment than 1:1 both years. Using a newly developed resistance index, 'Zeon' zoysiagrass was found most resistant, and the performance of '13-T-1032', 'T-822', '11-T-510', '11-T-56', '09-T-31' and '11-T-483' lines was comparable to 'TifTuf' bermudagrass, suggesting antibiosis as the underlying resistance mechanism.

INDEX WORDS: *Spodoptera frugiperda*, host plant resistance, antibiosis, predatory arthropods, grass height, grass density, thatch thickness, turfgrass

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# **GURJIT SINGH**

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**GURJIT SINGH** 

Major Professor: Shimat V. Joseph

Committee: G. David Buntin

David G. Riley

Electronic Version Approved:

Ron Walcott Interim Dean of the Graduate School The University of Georgia August 2020

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# TABLE OF CONTENTS

	Pa	age
ACKNO	WLEDGEMENTS	. iv
СНАРТЕ	R	
1	INTRODUCTION AND LITERATURE REVIEW	1
2	EFFECTS OF GRASS PARAMETERS AND THATCH THICKNESS ON	
	INCIDENCE AND ABUNDANCE OF ARTHROPODS IN THE CENTRAL	
	GEORGIA SOD FARMS.	37
3	EFFECTS OF POTASSIUM AND NITROGEN ON SURVIVAL AND	
	DEVELOPMENT OF FALL ARMYWORM (LEPIDOPTERA:	
	NOCTUIDAE)	.74
4	SCREENING FOR HOST PLANT RESISTANCE AGAINST FALL ARMYWOR	M
	(LEPIDOPTERA: NOCTUIDAE) USING NEWLY DEVELOPED	
	BERMUDAGRASS LINES	00
5	SUMMARY 1	30

### CHAPTER 1

### INTRODUCTION AND LITERATURE REVIEW

Grasses are essential to human beings as they are the primary source of food and fiber. Cereal grains are the primary food source for humans and feed for the ruminant animals.

Bamboo, which is the tallest of all the grasses, is an excellent building material (Beard and Green 1994). The turfgrass species that we see today were evolved over millions of years and have been utilized by humans for more than ten centuries (Beard 1973). Turfgrass meets our functional, recreational, and aesthetic needs. Turfgrass provides land cover to prevent soil erosion. It also improves the water-holding capacity of the soil and water infiltration rates, which helps in recharging the groundwater levels. A large number of hazardous pollutants such as Pb, Cd, Cu, Zn, and other hydrocarbon compounds were found in runoff water, and they pose health risks if not cleared from the soil and contaminate groundwater resources.

The turfgrass ecosystem inhabits diverse communities of soil invertebrates and microorganisms, helping to decompose harmful pollutants. Turfgrass can accelerate the soil restoration process by improving soil physical, chemical, and biological properties through the addition of organic matter (Gould 1968). Moreover, turfgrass provides a safe and inexpensive venue for several outdoor sports and recreational activities. Additionally, turfgrass, when combined with other landscape trees and shrubs, gives enhanced aesthetic properties to the area (Beard and Green 1994).

The turfgrass ecosystem provides food and shelter to diverse arthropod communities, including herbivores, predators, and decomposers (Streu 1973, Cockfield and Potter 1984a,

1985, Potter et al. 1985). Many of these organisms play a pivotal role in controlling the insect pests (Reinert 1978, Cockfield and Potter 1984b), improving soil physical properties (Turgeon et al. 1975), and aid in the thatch decomposition (Randell et al. 1972, Potter et al. 1985). Due to increased litigation and rising insurance costs, loss of registrations, and local ordinances restricting pesticide use, growers always seek alternative control options (Potter and Braman 1991). However, the major constraint towards the development of alternative options is a lack of understanding of biology and ecology of beneficial arthropods in the commercial settings (Potter and Braman 1991). More research is needed towards the turfgrass faunal composition and seasonality to develop effective pest management strategies.

# Importance of bermudagrass

Bermudagrass (*Cynodon dactylon* (L.) Pers.) is a major pasture and turfgrass species in the southern U.S. It is comparable to the Kentucky bluegrass (*Poa pratensis* L.) in the northeastern and northcentral regions of the U.S. (Juska and Hanson 1954). Although there is no record denoting when bermudagrass was introduced to the U.S., it is widely believed that it was introduced from India or Africa during the colonial era. In Georgia, Governor Henry Ellis introduced bermudagrass in Savannah in 1751. In the U.S., it is distributed from New Jersey and Maryland to Florida in the South and Kansas and Texas in the west. However, bermudagrass is not well adapted to the southern Appalachian highlands. Under adequate irrigation, bermudagrass can grow well in New Mexico, Arizona, and California (Juska and Hanson 1954).

Bermudagrass is a warm-season perennial grass that grows best when temperatures are above 24°C. Its growth declines when the temperature falls below 18°C (Juska and Hanson 1954). Moreover, it grows well on various types of soils with adequate soil fertility.

Bermudagrass has high N and K demand when compared to other warm-season grass species. It

can tolerate frequent flooding but cannot thrive under water-logged conditions (Juska and Hanson 1954). It can tolerate drought in high humid conditions but cannot survive in arid regions without irrigation. In terms of quality and texture, improved varieties of bermudagrass are among the finest of all the warm-season grasses (Juska and Hanson 1964).

# **Bermudagrass breeding**

In 1946, Dr. Glenn Burton led a breeding program at University of Georgia experimental station in Tifton, GA, to develop better bermudagrasses that could replace common or seeded varieties. Researchers collected accessions of bermudagrass genotypes after exploring several golf courses throughout the southeastern U.S. These accessions were vegetatively propagated in greenhouses and later planted in field plots to develop bermudagrass cultivars with superior horticultural attributes (Burton 1991, Baxter and Schwartz 2018). The bermudagrass cultivars released from this program laid the foundation of the turfgrass industry in Georgia. Several cultivars were developed from the University of Georgia experimental station at Tifton, and the cultivars were Tifway released in 1960, TifSport released in 1995, TifEagle released in 1997, TifGrand released in 2009, and TifTuf released in 2014. These cultivars are widely planted and have set high standards for turfgrass breeding programs across the U.S. and the world (Baxter and Schwartz 2018).

# Bermudagrass 'TifEagle'

In the 1990s, the focus of research at Tifton shifted towards the development of dwarf cultivars. Researchers induced mutations in the established cultivars of bermudagrass using Cobalt-60 gamma radiation to increase genetic diversity (Hanna and Elsener 1999). 'TifSport' and 'TifEagle', which were commercially released in 1995 and 1997, were the results of this program. These were the first two bermudagrass hybrids patented from Tifton (Hanna and

Elsener 1999). 'TifEagle' was a fine-textured and dense grass with high tolerance towards tawny mole crickets. The primary benefit of this cultivar is its tolerance against shallow mowing heights (at 0.3 cm). Moreover, it makes excellent putting surface because it produces fewer seed heads and a higher number of stolons than the previous 'TifDwarf' (Hanna and Elsener 1999, Baxter and Schwartz 2018, McCarty and Miller 2002).

# The turfgrass industry

In 2002, the turfgrass industry's estimated value was \$57.9 billion USD and generated 822,849 jobs in the U.S. (Haydu et al. 2006). The sod production was worth\$1.3 billion USD, generated 17,028 jobs (Haydu et al. 2006). Based on revenue generation, Georgia sod production was ranked fourth, and sixth for employment, and impact, respectively, in 2002 in the U.S. (Haydu et al. 2006). Based on a survey in 2002, Georgia is one of the major sod producing states in the U.S (Haydu et al. 2006). The turfgrass has been ranked 23rd in 2018 Georgia Agricultural Commodity Rankings. Moreover, based on recent estimates in the 2018 Farm Gate Value Report, sod farms are valued at \$118.3 million USD with sod produced on more than 26,651 acres in Georgia.

# Fall armyworm

Despite the continuous pest management efforts, animal pests (comprising of vertebrates and arthropods) are the second most important factor (after weeds) contributing to the loss of food and cash crops around the world, causing potential yield losses of 17.6% (Oerke and Dehne 2004). The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (FAW) is a severe but sporadic pest of turfgrass in Georgia (Luginbill 1928, Braman et al. 2000). This insect pest attacks turfgrass on sod farms, golf courses, and residential and public lawns. In sod farms, detection of early stages of FAW larvae can be challenging as small-sized young instars

hide within turfgrass canopy during the day. Because a typical sod farm covers a vast area (at least 100 acres), scouting for FAW becomes a real challenge.

The FAW is a peculiar lepidopteran pest, in the sense that it lacks a diapausing mechanism (Luginbill 1928, Sparks 1979). It overwinters in the mild climates of Florida and Texas where the hosts are present throughout the year, and temperatures usually do not fall below 10□ in winters. The next growing season, it migrates northwards and spread in the eastern and central US and southern Canada. The migration rates have been estimated to be up to 300 miles per generation (Sparks 1979). In Georgia, it usually starts re-infesting the hosts by the end of April each year (Pair et al. 1986). The adults of FAW are nocturnal, and their feeding initiates shortly after the onset of darkness up to two hours. After feeding, the virgin females emit pheromones in the air to attract males for mating.

Females mate only once in a night. Oviposition usually begins with the feeding period in the night and almost always overlaps with the mating period up to mid-night (Sparks 1979). The females of FAW are known to oviposit on the structures, including trees, shrubs, flag posts in the golf course, sheds near the farm, window panes, carts, etc. These structures are usually near turfgrass. Eggs are laid in clusters of a few to hundreds covered in the scales of moth. The oviposition period may last from 4 to 17 days, depending on the temperature (Sparks 1979). The eggs generally hatch within 2-4 days in the warm temperature conditions. After hatching, the 1st instar larva molts five times before pupating in the soil. The pupation period may vary from 7 to 37 days, depending upon the soil temperature. Adult emergence begins 2-3 h after the sunset and lasts up to midnight. In the overwintering regions, one generation of FAW may last from four weeks in the summers to 12 weeks in the winters (Luginbill 1928, Vickery 1929, Sparks 1979).

The outbreaks of FAW depend, to a large extent, on the weather conditions in winters in the overwintering sites. Cool-weather conditions with abundant rainfall provide favorable conditions for the FAW and check the populations of natural enemies. Later in the spring, if the humid weather conditions continue to prevail, the FAW populations build up in enormous numbers that migrate northwards and cause economic losses to the summer crops in Georgia (Luginbill 1928, Sparks 1979). The FAW has been described as a damaging pest in Georgia by Smith and Abbott (1797). Luginbill (1928) reported multiple outbreaks of FAW between 1856 to 1928. After 1928, several outbreaks occurred but not well documented. During the FAW outbreak of 1975-1977, the estimated economic losses reached as high as \$61.2 million, \$31.9 million, and \$137.5 million USD in 1975, 1976, and 1977, respectively, in Georgia alone.

In fall, homeowners often complain that newly planted sod is infested with FAW larvae. The FAW problem can originate at the homeowner site or from the sod producers, but often sod producers are blamed for selling FAW infested sod. The ecology of FAW in sod farm environments is poorly understood. Sod farms are often surrounded by vegetation, including weed hosts. The understanding of naturally occurring enemy communities in sod farms may help to determine how resilient the system is to tackle FAW infestation. Currently, sod producers use back-to-back applications of insecticides on entire farms starting in July to prevent potential FAW infestation.

# Abundance and diversity of arthropods in turfgrass

Diversity and seasonal occurrence of several selected groups of turfgrass inhabitants have been studied such as Collembola (Rochefort et al. 2005), Carabidae and Staphylinidae (Braman and Pendley 1993a, b, Rochefort et al. 2006). Predatory insects such as ground beetles (Carabidae), rove beetles (Staphylinidae), spiders (Araneae), bigeyed bugs (*Geocoris* spp.), and

ants (Formicidae), are particularly well documented (Braman et al. 2002). Studies focused on beneficial arthropods, particularly turfgrass genotype such as buffalograss, *Buchloe dactyloides* (Nuttall) Engelmann, have improved our knowledge about the potential natural enemies of major insect pests (Heng-Moss et al. 1998). In this study, beneficial arthropods including predatory ants, spiders, ground beetles, rove beetles, bigeyed bugs, and several species of hymenopterous parasitoids were collected from buffalograss evaluation plots and vegetatively established buffalograss lawns at the University of Nebraska. Ants and spiders (84% of the total beneficial arthropods captured) were found to be the most abundant beneficial arthropods collected. In the same study, families of parasitic Hymenoptera captured on sticky traps over the 2-yr sampling period included Scelionidae, Encyrtidae, Mymuridae, and Trichogrammatidae. Mymarids and trichogrammatids were the most abundant, representing 76.8% of the total parasitoids collected. Also, the pitfall traps were found to be a more effective technique than sod plugs, collecting >2.5 times as many (16,094) beneficial arthropods as sod plug samples (6,054) (Heng-Moss et al. 1998).

Beneficial arthropods, including predators and hymenopteran parasitoids, are well documented in the managed turfgrass systems (Cockfield and Potter 1983,1985, Braman and Pendley 1993, Heng-Moss et al. 1998, Braman et al. 2000b). The intricate role of plant taxa and other plant parameters such as height and density on the occurrence and abundance of arthropods in residential turfgrass has been documented (Joseph and Braman 2009). Joseph and Braman (2009) showed that predatory heteropterans, including Anthocoridae, Lasiochilidae, Geocoridae, and Miridae were found to be most abundant in St. Augustinegrass and zoysiagrass. Moreover, the numbers of herbivorous insects such as Blissidae, Delphacidae, Cicadellidae, and Cercopidae were higher in the taller grasses. However, the abundance of predatory Heteroptera did not

change with grass density (Joseph and Braman 2009). However, little is known about the incidence and abundance of arthropods in commercial production settings such as sod farms. The incidence, abundance, and diversity of arthropods could vary in the commercial sod farms as they are intensively managed using pesticides and fertilizer applications. Earlier studies carried out in large (1-4 ha) institutional lawns showed that the population of many predators decreased at sites that received commercial lawn care for several years (Cockfield and Potter 1985).

Insecticides can play a very crucial role in determining the predatory arthropod communities in managed turfgrass systems. Short-term effects of chlorpyrifos, bendiocarb, trichlorfon, and isofenphos insecticidal applications on predaceous arthropods and oribatid mites in Kentucky bluegrass were assessed where chlorpyrifos and isofenphos considerably reduced predators for at least 6 weeks (Cockfield and Potter 1983). In the same study, they found that the residual effects of bendiocarb and trichlorfon were milder on predators, and most of the insecticides did not influence oribatid mite populations. Similarly, another study was conducted on a 2 ha Kentucky bluegrass (*Poa pratensis* L.) lawn in Lexington, Ky, where the predators consumed 75% of the sod webworm (*Crambus* and *Pediasia* spp.) eggs within 48 h after introduction (Cockfield and Potter 1984). Cockfield and Potter (1984) showed that a single application of chlorpyrifos reduced the rate of predation on sod-webworm eggs for at least three weeks post-treatment. Moreover, the insecticide residues on the turfgrass reduced the abundance of generalist predators such as ants and spiders (Cockfield and Potter 1984).

Previously, Braman et al. (2002) showed that populations of foliar dwelling spiders and bigeyed bugs increased considerably when a wildflower mix with 15 flower species was planted. In another two-year study, all the wildflower mixes evaluated disproportionately enhanced the abundance of beneficial arthropods. Most beneficial arthropods were collected from the

wildflowers, which suggest beneficial insects can find refuge in wildflowers when insecticides are applied to suppress pests on turfgrass (Braman et al. 2002).

The generalist predators control pest populations through direct consumption as well as through a myriad of non-consumptive effects. Overall predation of all billbug life stages, changes in the adult behavior, mating, and oviposition were measured in the lab and field study to evaluate the consumptive and non-consumptive effects of predators on billbugs (Dupuy and Ramirez 2019). Predatory arthropod samples were collected from the golf courses in Utah and Idaho through linear pitfall traps and consisted mainly of carabids and spiders (60% and 28% of all the predators, respectively). Predation on adult billbugs was found low (below 6%) in both the lab and field assays. Moreover, predators readily consumed billbug larvae when kept in the petri dish arenas, but predation was reduced when billbug larvae were at 1 cm deep in the soil. Overall, after exposure to predators, the hunting billbug activity and mating were reduced by 56% and 28%, respectively (Dupuy and Ramirez 2019).

Cultural practices can also affect the suitability of turfgrass as a food source for herbivores. Turfgrass characteristics, such as height and density, can influence arthropod communities (Hull et al. 1994). Hull et al. (1994) showed that taller and denser grasses have better shading, cooling, and moisture retention properties that can disproportionately influence arthropod abundance (Hull et al. 1994). A previous study also showed that levels of alkaloids present in endophyte-infested tall fescue and perennial ryegrass could be varied with the mowing height (Salminen et al. 2003). When densities of root-feeding white grubs in the soil were evaluated after restricted irrigation before the Japanese beetle flight, a study showed, tall turfgrass with aluminum sulfate residues reduce white grub infestations (Potter et al. 1996). Another study showed that populations of certain spiders and rove beetles were greater in the

taller than shorter turfgrass. However, the overall rate of predation remained constant regardless of mowing height, which could be related to ants' activity on turfgrass maintained at various mowing heights (Dobbs and Potter 2014). Low mowing also promotes mounding by ants (Maier and Potter 2005). Mowing height is also associated with the population dynamics of insect-pests and predatory arthropods. In a study conducted in a golf course in Michigan, consisting of perennial ryegrass, *Lolium perenne* L., it was found that the numbers of scarab grub pest, *Ataenius spretulus* Haldeman, were found consistently higher in the grass plots mowed to fairway height the previous year, regardless of the mowing height on the current year. In contrast, the numbers of staphylinids were found higher in the rough mowed plots, regardless of the turf height in the previous years (Rothwell and Smitley 1999).

Braman et al. (2004) showed that when resistant zoysiagrass was treated with the low rates of chlorpyrifos, the survival of FAW larvae was lower relative to bermudagrass or seashore paspalum. However, when the same zoysiagrass was treated with spinosad, the larval survival was either equal or greater than that of susceptible bermudagrass or paspalum. Moreover, low halofenozide rates reduced the survival of larval at 7 d but not at 14 d post-exposure (Braman et al. 2004). This shows that the effectiveness of insecticides is influenced by turfgrass genotype and residual activity.

# **Turfgrass:** A unique system

The turfgrass industry had undergone rapid growth since the 1970s when large areas of land were started to develop to accommodate growing suburban populations (Held and Potter 2012). Turfgrass in sod farms is a low-cut, evenly mowed monoculture of the same grass species, expanding over large swaths of land, which provides harborage to a large number of insect-pests (Layton and Held 2005). Insect control in sod farms is primarily insecticide driven. This is

because the integrated pest management (IPM) programs designed for the field crops cannot be directly implemented in the turfgrass system. The pest control decisions in the turfgrass mainly depend on the aesthetics rather than the traditional metrics like yield loss (Potter 2005, Potter and Braman 1991, Held and Potter 2012). Turfgrass in the golf courses, private properties, athletic fields, and public landscapes come from the commercial sod farms. Turfgrass is intensely managed in the sod farms, and there is a very low tolerance for insect damage (Layton and Held 2005). Sod farm managers emphasize keeping the grass look attractive, lush-green, and free of weeds. These conditions may lead to the population build-up of plant-feeding arthropods (Busey and Snyder 1993, Davidson and Potter 1995) and decrease the alternate hosts for the natural enemies (Braman et al. 2002, Frank and Shrewsbury 2004). Fertilizers like nitrogen and iron are used to maintain the cosmetics of the grass. However, greener grasses not only attract buyers alone but herbivorous insects as well (Busey and Snyder 1993). Nitrogen fertilizers promote faster development of grass-feeding insects. The researchers at the University of Kentucky found that the development of FAW larvae significantly enhanced when fed the grass treated with medium or high fertilizer rates as compared to the larvae fed on unfertilized tall fescue. The green bugs, Schizaphis graminum Rondani, preferred fertilized, endophyte-free tall fescue rather than the unfertilized, endophyte-free grass. They also noticed faster development of bird cherryoat aphids, Rhopalosiphum padi (L.) on fertilized, endophyte-free tall fescue (Davidson and Potter 1995).

# **Prospects for conservation biological control**

Several studies have shown the parasitism of major turfgrass pests by the parasitic hymenopterans. Parasitism of FAW larvae by the braconid, *Aleiodes laphygmae* Viereck, was found to be different among turfgrass genotypes and cultivars. Other parasitic wasps like *Cotesia* 

marginiventris Cresson and Meteorus sp. were also reared from the FAW larvae in the same experiment (Braman et al. 2004b). Similarly, the larvae of black cutworms, Agrotis ipsilon Hufnagel (Lepidoptera: Noctuidae), were found to be parasitized by the parasitic wasps in the families, braconidae, encyrtidae, and tachinidae (Bixby-Brosi and Potter 2010). Moreover, the application of soil insecticides intended to control white grubs can affect their biological control by parasitic hymenopterans. Application of imidacloprid has been shown to interfere in the biological control of Japanese beetle, Popillia japonica Newman, larvae by Tiphia vernalis Rohwer, an ectoparasitic wasp (Rogers and Potter 2003). Moreover, the rhodesgrass mealybug, Antonina graminis Maskell, is biologically controlled by parasitic wasps like Neodusmetia sangwani, Acerophagus sp., and Pseudectroma sp. (Chantos et al. 2009).

# Importance of plant nutrients

Fertilizers and pesticides are responsible for high economic and environmental costs in modern agriculture. In the U.S. alone, farmers spend ~ \$20 billion USD on fertilizers and pesticides every year. Still, as per the estimation, it represents only 80% of the actual costs because additional costs like damages to environmental resources and human health are not usually considered (Pimentel 2005, Tegtmeier and Duffy 2004). For sustainable agriculture, we have to ensure that the usage of chemicals is efficient and optimized. Bermudagrass is the most common turfgrass type grown in Georgia. It is highly responsive to N fertilizers but also requires a considerable amount of K fertilizer for optimum growth. Some amount of P is also required for optimum plant growth; however, it needs to be applied only once in a year because it does not leach down the soil like K. Bermudagrass uses considerable amounts of nutrients from the soil for growth. Every 6 tons of bermudagrass hay removes 136 kg of N, 114 kg of K, and 38 kg of P nutrients from the soil (Lee et al. 2017). Soil K could deplete without adequate K fertilizer inputs

at a faster rate under bermudagrass sod production, especially in sandy soils where the K reserves are usually limited and susceptible to leaching. A previous study showed that K levels in soil declined from 160 to 50 ppm in three years, with bermudagrass hay production (Nelson et al. 1983).

In Georgia, available soil K is highly prone to leaching, especially in the sandy soils, and soils can be deficient of potash (Mikkelsen 2007). Thus, supplementing the soil with K fertilizer is critical as it improves plant health with a better root system and helps to resist biotic and abiotic stress, including disease and high salt (Wang et al. 2013). Because K does not directly yield plant growth benefits relative to N, turfgrass managers rarely supplement K fertilizer regularly (Miller and Dickens 1996). Fertilizers used by turfgrass industry are usually N driven as it mostly produces profound grass growth. Inadequate available K can contribute to nutrient imbalance (Miller 1999) and increases susceptibility to arthropod infestation (Walter and Difonzo 2007, Amtmann et al. 2008). Potassium plays a crucial role in determining the health of plants by affecting metabolic, physiological, and hormonal pathways (Blatt 1988, Baker and Weatherley 1969). These processes are crucial for the susceptibility of the plant to pathogens and insect pests. Production and distribution of various primary plant metabolites are profoundly affected by the K availability in the plant tissues, which determines the incidence of insect pests and diseases (Amtmann et al. 2008). The activity of ~60 enzymes in plants depends on the availability of K, mostly involved in the metabolism of sugar and N (Jones and Pollard 1983). Levels of K influence the opening and closure of guard cells of stomata, hence regulating the transpiration rates in plant tissues (Blatt 1988). It also affects the flow of water, and other organic and inorganic solutes in the xylem vessels in plants (Baker and Weatherley 1969).

# Plant nutrients and arthropods

A previous study showed that the density of soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) was greater on the soybean plants having K deficiency symptoms than on the plants that did not have K deficiency symptoms (Walter and Difonzo 2007). The phloem exudation samples from the same study showed that the percentage of asparagines, a vital amino acid that favor aphid survival was found to be increased as available K in soil decline (Walter and Difonzo 2007). Several studies showed that the levels of soluble sugars, organic acids, and amino acids increased in plant tissues when K is deficient (Amtmann et al. 2008). Deficiency in K levels provides an opportunity for insect pests and pathogens to feed and develop (Amtmann et al. 2008).

The plasma membrane enveloping plant tissues is sensitive to foreign invaders (Amtmann et al. 2008). Change in membrane potential and ion fluxes after an attack triggers an increase in cytoplasmic Ca. Cytoplasmic Ca function as a messenger as it triggers a cascade of events, including the defense signals for H<sub>2</sub>O<sub>2</sub> production. Changes in the concentration of K outside the plasma membrane significantly influence the ionic potential around it because K has a greater ability for conductance than any other ion. Hence, K nutrition regulates the production of cytoplasmic Ca (Amtmann et al. 2005, 2008).

Many hormones are involved in the plant defense against pathogens and herbivorous insects, including Salicylic acid (SA) and Jasmonic acid (Shah 2003; Turner et al. 2002). Salicylic acid is known to be involved in the hypersensitive reactions and systemically acquired resistance (SAR) mechanisms against viruses, bacteria, and biotrophic fungi. Jasmonic acid involves the induction of secondary metabolites against necrotrophic fungi and insect pests (Glazebrook 2005, Thomma et al. 1998). Research has shown that K affects the hormonal

defensive pathways in plants. Also, K applications are often used to increase winter hardiness in bermudagrass. However, applying K more than required for optimum growth does not provide enhanced winter hardiness benefits (Miller and Dickens 1996).

### Plant nutrients and resistance

Plant nutrition affects plant resistance against pests by altering the plant's growth and changing the composition of various defensive compounds in the plant tissues (Huber et al. 2012). Fertilizer application also modifies the microbial activity in the soil and rhizosphere, which indirectly impact the plant's resistance towards the root and shoot pests/diseases. Different macronutrients have different roles in the plant's innate resistance. For example, Ca and B strengthen cell walls and membranes, Si and Mn play an essential role in the plant's defensive reaction against pest attacks. In contrast, N and K affect the composition of soluble compounds in the plant tissue (Huber and Wilhelm 1988, Huber et al. 2012). Plant's resistance towards pest infestations can be either increased or decreased depending upon the type of pest, nutrient, plant species, and nutritional status of the plant. Generally, a balanced nutrient supply optimal for plant growth ensures optimal plant resistance (Huber et al. 2012).

Potassium is known to increase fiber strength and quality in plants (Read et al. 2006). As an enzyme activator, K plays a vital role in various plant physiological processes such as photosynthesis, respiration, carbohydrate metabolism, translocation, and protein synthesis (Pettigrew 2008). Data from field and greenhouse trials show the effects of plant nutrients on its susceptibility to insect pests and disease incidence. Perrenoud (1990) reviewed more than 2000 studies and concluded that K fertilizer application tends to reduce the incidence of diseases and insect pests in many cases. About 70 and 69% of the studies reported a decrease in fungal and bacterial diseases, respectively, and a 63% reduction in arthropods. The K fertilizer application

in rice has been shown to improve its tolerance to various abiotic and biotic stresses, including insect pests (Tiwari 2002). Studies also demonstrate that K fertilizer inputs influence the effectiveness of applied N (Haby et al. 2008, Xu et al. 2008). For example, a study on 'Tifton 85' bermudagrass demonstrated that the addition of K fertilizer in soil improved the yield response related to an increase in N uptake (Haby et al. 2008). Increased levels of K- nutrition have found to enhance the root growth, dry matter content, and N accumulation in the shoot tissues of Maize seedlings (Xu et al. 2008). Because K can improve the plant health and disease resistance in turfgrass, the University of Georgia recommends the application of K, twice the rate of N in the fall season.

Research has shown that plant's ability to resist or tolerate insect-pest attack or disease depends significantly on the soil's physical, chemical, and biological properties. Thus, farming practices that cause nutrient imbalance can lower the plant's resistance against insect-pests (Altieri and Nicholls 2003). Generally, K application tends to diminish the incidence of fungal, bacterial, and insect-pests in crops. However, the opposite can be true as well. K nutrition affects the synthesis and distribution of primary metabolites in the plant tissue, which alters the plant's susceptibility to insect-herbivores and pathogens as well as their subsequent growth and development on/in the plant (Altieri and Nicholls 2003). The traits like resistance and tolerance in plants are mostly genetically driven. However, resistance can also be influenced by environmental factors. Hence, nutrition has always played a vital role in controlling pests and diseases (Huber and Wilhelm 1988, Amtmann et al. 2008). Research in this field would help to design sustainable agriculture programs by emphasizing the balanced nutritional status of the plants while exploiting the defensive benefits against pests and diseases (Amtmann et al. 2008).

# Potassium and fall armyworm

Soil's fertility status is linked to the plant's resistance against insect pests, and this could be used for pest management (Tingey 1981). The FAW is a major pest of Family, Gramineae in the central and southeastern U.S. (Luginbill 1928). The plant nutrient levels may influence grass's ability to resist FAW feeding. The FAW's larval weight and pupal weight were reduced when fed on tall fescue with low fertilizer than with high fertilizer treatments (Bultman and Conard 1998). In the same study, a higher number of FAW larvae (93% survival) survived when fed on plants treated with high fertilizer doses than those treated with low fertilizer.

During the FAW epidemic of 1977, losses to FAW infestation exceeded \$59 million USD for forage and hay in Georgia (Todd and Suber 1980). Damage to the coastal bermudagrass significantly increased in fertilized pastures. FAW larval survival and development rates were greater on fertilized than on the unfertilized bermudagrass. This increased survival and developmental rates are in direct response to increased N or protein within the grass tissue (Lynch 1984). There is often a positive correlation between pest incidence and N application in plants. In contrast, an increase in K application reduces pest infestation (Amtmann et al. 2008). In a study conducted on the rice plants, it was found that the food intake, growth, adult longevity, fecundity and population of white-backed planthoppers, *Sogatella furcifera* (Horváth) increased with increase in N application whereas, increase in K application was detrimental to *S. furcifera* (Salim and Saxena 1991). Foliar spray of 14 inorganic compounds, including K on 'coastal' bermudagrass clippings, showed that K has repellent properties against FAW larvae (Leuck et al. 1974). However, under field conditions, K is rarely applied as a lone nutrient. It is generally combined with other plant nutrients such as N. Clearly, there is a knowledge gap if

higher K fertilizer regimes can improve tolerance to insect herbivory, especially (FAW), and reduce insecticide use.

# Host plant resistance in turfgrass

Host Plant Resistance (HPR) is a tactic as part of the Integrated Pest Management (IPM) program. Among the major benefits of HPR include a reduction in insecticides, delay of resistance development against specific insecticides, reduced insecticide residues in water, and increased activity of beneficial arthropods under field conditions (Sharma and Ortiz 2002). Usually, the focus of the turfgrass breeding programs is on aesthetics and other abiotic traits. However, the development of insecticide resistance, impact on non-target organisms, and suspension of insecticides due to legislative issues create a need for alternative control options resistant turfgrass (Reinert and Engelke 2010).

Resistant cultivars are developed either through conventional breeding, where resistant traits are selected and incorporated into the turfgrass lines over multiple years or through genetic engineering, where resistant traits are artificially incorporated into the turfgrass lines (Bonos et al. 2006). In 1951, Painter defined three types of resistance mechanisms: Non-preference, antibiosis, and tolerance. Non-preference includes the resistance based on morphological characters of the plant, like color, the thickness of the cell wall, surface wax, spines, trichomes, etc. that render make the host plant unfavorable for feeding, reproduction, and oviposition. In antibiosis, the resistant plant produces secondary metabolites that are either fatal or retard the development of the insect pest. Most researchers prefer investigating antibiosis because of the clear visual effects on the insect, such as mortality, weight loss, and reduced development (Wiseman and Davis 1979). However, antibiosis can result in the development of specific biotypes in nature (Wiseman and Davis 1979).

# Fall armyworm and resistant turfgrass

Leuck et al. (1968) screened 441 clones of bermudagrass, for resistance against first instar FAW. Six days after infestation, around 1% (3 lines) were rated as resistant, 11% (48 lines) as intermediate, and 88% (390 lines) as susceptible. Three days later, when the same 441 clones were rated for FAW resistance, about 0.5% (2 lines) were resistant, 2% (9 lines) were intermediate, and 97% (429 lines) were found to be susceptible. Those two resistant lines were 'Tifton 292' and 'Tifton 296'. Lynch et al. (1983) used a host suitability index to evaluate resistance in nine bermudagrass clones against FAW, and 'Tifton 292' was rated resistant. The resistance was mainly antibiosis rather than non-preference. Most of the FAW larvae fed on 'Tifton 292' died within the first ten days of infestation, and none of them survived till pupation. 'Tifton 292' was, however, never commercially released, but it was used as one of the parents and was instrumental for forage bermudagrass cultivar 'Tifton 85' (Burton 2001). Previously, ten zoysiagrasses, 18 paspalums, 34 bermudagrasses, tall fescue, creeping red fescue, and perennial ryegrasses with and without endophytes were evaluated for resistance against FAW (Braman et al. 2002). Among endophyte-infected (E+) and endophyte-free (E-) cool-season grasses, the resistance to FAW was observed in the following order 'Dawson' E+> 'APR 1234' > 'Dawson' E- > 'Rosalin' E+ > 'Lp 5425', 'Rosalin' E-, 'ATF 480' > 'Tulsa' or E+ slender creeping red fescue > E+ turf-type perennial ryegrass > E- slender creeping red fescue > E+ forage-type perennial ryegrass > E- forage-type perennial ryegrasses, and E+ tall fescue > E- turf-type tall fescue. Reduction in weight gain was found in all the larvae fed on zoysiagrasses. Also, reduced larval weight was shown on bermudagrasses 'Tifsport', 'Tifgreen' and bermudagrass lines '97-4', '97–14', '97–22', '97–28', '97–39', '97–40', '97–54', '98–15', '98–30', and '98–45' when compared to the larvae fed on 'Tulsa' tall fescue or the diet control (Braman et al. 2002). Hostplant resistance is an environmentally and economically sustainable measure of pest management (Togola et al. 2017). Researchers have also tried tissue culture techniques such as somaclonal variation to enhance insect resistance in the host plants. Off seven bermudagrass lines regenerated through the tissue culture technique, 'Brazos-R3' and 'OSU LCB W26-R2' were resistance to FAW (Croughan and Quisenberry 1989). In another study, nine cultivars and three genotypes of zoysiagrass were tested for resistance against neonate and 4 d old FAW larvae. 'Cavalier', 'Emerald', and 'Belair' zoysiagrass were resistant as only5% of neonates survived after 4 d of feeding. For the 4 d, old larvae, which were previously fed on the susceptible host and later moved to the resistant zoysiagrass, showed that > 40% of FAW survived after 3-d of feeding on all the genotypes except for 'Cavalier' (Reinert and Engelke 2010). Both the antibiosis and non-preference mechanisms of resistance were reported FAW fed on common centipedegrass, *Eremochola ophiuroides* (Munro) Hack (Wiseman et al. 1982).

# Factors affecting resistance against FAW in bermudagrass

In nature, two genetically different strains of FAW exist, "corn" and "rice" strain; one predominantly feeds on the corn (*Zea mays* L.) and another on rice (*Oryza sativa* L.) and bermudagrass respectively (Pashley 1986). Pashley (1986) also reported that the genetic difference between the two strains is not due to their feeding on different host plants. These two FAW strains responded differently to the resistant bermudagrass in a previous study where 'Tifton 292' was resistant to corn strain of FAW but exhibited no resistance to the rice strain of FAW (Pashley et al. 1987). Along with the host strain, the diet used to rear laboratory colonies should also be taken into consideration while evaluating bermudagrass for FAW resistance (Quisenberry and Whitford 1988). The two strains responded discretely to different artificial diets (modified pinto beans, pinto beans, velvet bean caterpillar, southwestern corn borer) and

subsequently altered their development when they are later fed on turfgrasses (Quisenberry and Whitford 1988).

Moreover, the field-grown bermudagrass was showed to be of lower quality than the same grass grown in the greenhouse due to the higher neutral detergent fiber, and lower crude protein and in-vitro digestible dry matter content in the field-grown grass and that made it less favorable for FAW development. However, 'Tifton 292', which was found to be resistant to FAW under greenhouse conditions, showed no significant difference than the susceptible line 'Grazer' when grown under field conditions (Jamjanya et al. 1990). Similarly, several bermudagrass lines have been produced through somaclonal variation that showed resistance to FAW infestation under controlled conditions, but no resistance was observed under field conditions (Pitman et al. 2002). In another study, feeding suitability and preference of the 6th instar FAW larvae reared on seven bermudagrass lines were determined. The results showed that crude protein and in-vitro digestible dry matter (IVDDM) content in bermudagrass played a critical role in larval weight gain. Thus, larval growth and development were affected when FAW larvae were reared on 'Alicia', and 'OSU 6-7' and certain bermudagrass lines with low N and IVDDM levels than five other lines (Quisenberry and Wilson 1985). Thus, there are factors other than the quality that determine the susceptibility or resistance of bermudagrass lines toward FAW development (Jamjanya et al. 1990).

Quisenberry et al. (1988) showed the influence of toxic allelochemicals on FAW larval development when fed on a diet containing petroleum ether and dichloromethane extracts of 'Grazer,' 'Coastal', OSU 71 × 6-7, and 'Tifton 292'. The development of FAW larvae significantly reduced when fed on the diet containing extracts of 'Tifton 292' because of the higher concentration of toxic non-polar compounds in the diet, whereas the diet containing the

extracts of 'Grazer' was less toxic to the FAW because the concentration of non-polar toxic compounds was less than half the amount presents in the extracts derived from other lines (Quisenberry et al. 1988). Thus, screening for insect resistance under greenhouse conditions should be confirmed by the field trials. FAW larval growth is influenced by the neutral lipid profile of the bermudagrass genotypes (Mohamed et al. 1987). These lipid profiles vary significantly among different bermudagrass genotypes. In the same study, when FAW larvae were fed on a medic diet supplemented with a neutral lipid profile, the larval weight gain varied from 28-54% compared to the control. Moreover, an antifeedant, soxhlet extract was isolated from a bermudagrass line (OSU 71 × 6-7), which caused an 80% reduction in larval weight and a 70% increase in the time to reach pupae (Mohamed et al. 1987). The two strains of FAW, the one specialized on larger grasses (corn strain), and the one specialized on smaller grasses (rice strain) were reared on corn and bermudagrass to determine the larval development and reproductive traits in adults. It was found that the rice strain was more affected by the larval host than the corn strain and performed better on the preferred host plant bermudagrass (Pashley et al. 1995).

# Research objectives

The research objectives are to determine:

The effects of turfgrass parameters such as genotype, height and density, and thatch
thickness on the incidence and abundance of arthropods in the central Georgia sod farms.

The underlying hypothesis is that the occurrence of various arthropod communities will
be influenced by the grass genotype, and changes in the turfgrass height, density, and
thatch thickness.

- 2. The effects of potassium and nitrogen on survival and development of fall armyworm (Lepidoptera: Noctuidae). The underlying hypothesis is that the potassium and nitrogen will not support the survival and development of fall armyworm larvae under controlled conditions.
- 3. The levels of resistance and susceptibility of fall armyworm to 14 newly developed bermudagrass genotypes and compare with bermudagrass genotype 'TifTuf' and zoysiagrass genotype 'Zeon'. The hypothesis is that the resistance of these 14 genotypes will either be comparable or greater than that of 'TifTuf'.

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# **CHAPTER 2**

EFFECTS OF GRASS PARAMETERS AND THATCH THICKNESS ON INCIDENCE AND ABUNDANCE OF ARTHROPODS IN THE CENTRAL GEORGIA SOD FARMS<sup>1</sup>

<sup>1</sup>G. Singh and S. V. Joseph

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#### Abstract

The occurrence and abundance of arthropods have rarely been studied at commercial sod farms. The objective of the current study was to quantify the influence of turfgrass taxa [common bermudagrass, Cynodon dactylon (L.); centipedegrass, Eremochloa ophiuroides Munro Hack; and zoysiagrass, Zoysia spp.], height, density, and thatch thickness on the abundance of common turfgrass predators, parasitic hymenopterans, herbivores, and detritivores. Sweep net, vacuum, and pitfall traps were used for sampling at 20 sod field sites in the central-Georgia. Each site was sampled three times in the last week of May, June, and July 2019. The pitfall traps captured 89% and 96% of all the predators and parasitic Hymenoptera sampled during the study. The common turfgrass predators such as Anthocoridae, Formicidae, and Phlaeothripidae were significantly influenced by the grass taxa, whereas Carabidae, Staphylinidae, Geocoridae, and Araneae were equally abundant among turfgrass genotypes. The beneficial arthropods such as Anthocoridae, Araneae, and parasitic Hymenoptera were significantly greater in May samples than in June and July. The sampling date did not affect the captures of other predators such as predacious mirids, carabids, staphylinids, geocorids, and formicids. Herbivores such as the seed bugs (Rhyparochromidae) and spittlebugs (Cercopidae) significantly increased with the grass height. In contrast, the predacious mirids [Spanogonicus albofasciatus (Reuters)] and carabids did not change with grass height. The number of predatory arthropods did not vary with changes in thatch thickness, except formicids, were their numbers decreased with an increase in thatch thickness. Also, Curculionidae and Lepidoptera were significantly more abundant in the denser grasses. There was a positive correlation between the abundance of predatory arthropods such as Araneae, Carabidae, and Geocoridae increase in Delphacidae, Curculionidae, and Cicadellidae,

respectively. The results indicate that host-plant microenvironment characteristics play a role in shaping the pest and beneficial arthropod communities in the sod farms.

**Keywords:** Turfgrass, sod-farms, arthropod abundance, predators

Turfgrass is a multibillion-dollar industry in the U.S., (Haydu et al. 2006). Georgia is one of the major sod producing states in the U.S., and the sod production sector generated \$118.3 million USD to Georgia's economy in 2018 (Georgia Farm Gate Value Report 2018). Turfgrass in sod farms is a low-cut, evenly moved monoculture of the same grass species, expanding over a large area of land that supports a large number of insect pests (Layton and Held 2005). Currently, pest control in sod farms is mostly insecticide driven because of very low tolerance for the insect damage (Layton and Held 2005), and aesthetics of turfgrass is the prime objective for the managers (Potter 2005, Potter and Braman 1991, Held and Potter 2012). Turfgrass planted in golf courses, private properties, athletic fields, and public landscapes is produced in sod farms. Sod farm managers emphasize keeping the grass look attractive using an aggressive fertilizer regime, which favors the build-up of plant-feeding arthropods (Busey and Snyder 1993, Davidson and Potter 1995) and reduces alternate hosts for the natural enemies (Braman et al. 2002, Frank and Shrewsbury 2004). There is a knowledge gap in our understanding of the diversity and ecology of arthropods existing in commercial sod farms. Predicting the pest infestation is the real challenge in reducing the dependency on preventive insecticides (Held and Potter 2012).

The turfgrass ecosystem provides food and shelter to diverse arthropod communities, including herbivores, predators, and decomposers (Streu 1973, Cockfield and Potter 1984a, 1985, Potter et al. 1985). Many of these organisms play a pivotal role in controlling the insect

pests (Reinert 1978, Cockfield and Potter 1984b), improving soil physical properties (Turgeon et al. 1975), and aid in the thatch decomposition (Randell et al. 1972, Potter et al. 1985). Due to increased litigation and rising insurance costs, loss of registrations, and local ordinances restricting pesticide use, there is a constant need to develop alternative pest control options for the sod producers. The major constraint for the development of alternative options is the poor understanding of arthropod biology and ecology in commercial settings (Potter and Braman 1991). More research is required documenting faunal composition and seasonality of arthropods in turfgrass to develop effective pest management strategies. Several biotic and abiotic factors can influence the incidence and abundance of arthropods in turfgrass. Biotic factors such as grass height and density can alter micro-environment and favor specific arthropod communities. For example, taller and denser grasses have better shading, cooling, and moisture retention properties, which can either benefit or hinder the population build-up of certain types of arthropod species (Hull et al. 1994). Moreover, studies have shown that limiting irrigation before the beetle flight period, increasing mowing height, and applying aluminum sulfate in the spring reduces white grub infestations (Potter et al. 1996).

The application of insecticides has been shown to impact predaceous arthropods in turfgrass (Cockfield and Potter 1983; Cockfield and Potter 1984b). The predators such as spiders and rove beetles increased in numbers in the high-mowed tall fescue lawn grass (Dobbs and Potter 2014). In the same study, overall predation rates remained similar in both the low-mowed and high-mowed grasses because the ants, which were also the dominant predators, were equally distributed regardless of the mowing height. Joseph and Braman (2009) showed that several plant parameters, such as grass genotype, affected the abundance of arthropods in residential turfgrass. The predatory heteropterans, including anthocorids, lasiochilids, geocorids, and mirids

were most abundant in St. Augustinegrass and zoysiagrass, and taller grass supported high numbers of blissids, delphacids, cicadellids, and cercopids. The abundance of predatory heteropterans was not affected by the change in grass density (Joseph and Braman 2009). However, little is known about how plant parameters such as grass type, height density, and thatch thickness affect the incidence and abundance of arthropods in commercial production settings such as sod farms as commercial settings are intensively managed using pesticides and fertilizer applications than residential turfgrass. Thus, the current study's major objective is to determine if plant parameters such as grass type, density and height, thatch thickness, and abiotic factors such as soil temperature and relative humidity significantly affect the incidence and abundance of arthropods in sod farms. The null hypothesis was that these factors would have no effect.

#### Materials and methods

## Sampling sites

The survey was conducted in the sod farms of Marshallville, Fort Valley, and the Whitesburg area in central Georgia. A total of 20 sod farm fields were selected, which included five bermudagrass [ $Cynodon\ dactylon\ (L.)$ ], two centipedegrass ( $Eremochloa\ ophiuroides$ ) and thirteen zoysiagrass ( $Zoysia\ spp.$ ) fields. A  $4.3\times4.3$  m area was identified in each field, which was further subdivided into  $2.1\times2.1$  m quadrants. Arthropods were sampled from these quadrants. Each of the 20 sites was visited three times in the last week of May, June, and July.

### Sample collection and evaluation

Three sampling methods were employed: 1) sweep netting, 2) the vacuum suction, and 3) pitfall trap. All the samples were collected between 10:00 and 16:00 h. A total of forty sweeps were collected from each field using a 0.92 m long, 0.03 m<sup>2</sup> frame area sweep net (Ward's

Rochester, NY). Twenty sweeps were taken along the two parallel sides of the square area (ten along each side), ten sweeps along the transect passing through the center (parallel to the two opposite sides of the square), and ten along the diagonal. All these individual sweep samples were combined from a field site. For vacuum sampling, twenty 5 s suctions were sampled using a 'Vortis' vacuum sampler (Burkhard manufacturing Co., Ltd, Herefordshire, England) from each of the quadrant. An area of 0.2 m<sup>2</sup> was covered per suction of the vacuum sampler, making a total of 4 m<sup>2</sup> per quadrant with the air throughput of 10.5 m<sup>3</sup> per minute. Samples from all the four quadrants were combined from a site.

Four pitfall traps were deployed in each field and one trap per quadrant. To deploy the pitfall trap, a ~10 cm deep hole below ground level was dug using a 10 cm diameter cup-cutter and a 7 cm deep, 11 cm diameter plastic container was deployed into the hole. Ethyl glycol was added to the container to preserve the trapped arthropods. The pitfall trap was partially covered using a disposable plate to prevent the rainwater entering into the traps. The pitfall traps were exposed for 7 d during each sampling period. The samples from all the quadrants were combined from a site. All the sweep and vacuum samples were transported to the laboratory and temporarily stored at  $4\Box$ . The grass debris was removed from the samples and the arthropod collected in the samples were preserved in 70% ethanol. The preserved arthropods were quantified and identified to the order and family.

### Various parameters measured in the study

The grass density was recorded at the time of field visit, using a rectangular cardboard strip (8.5 cm in length, 6.5 cm in width, 2 mm in thickness) having a 2.5 cm<sup>2</sup> circular window in the center as described in Joseph and Braman (2009). The cardboard strip was randomly dropped four times, once in each quadrant in a field. The four density readings were averaged to get a

grass density value for that particular field per sampling date. The soil profiles collected for the pitfall traps using cup-cutter were transported to the laboratory. On each date, there were four soil profile samples for each field site. Grass height and thatch thickness were recorded using a ruler (mm) in the laboratory. The four grass height and thatch thickness measurements were averaged for each field site. Soil temperature and relative humidity were recorded in the field during sampling visits.

# Statistical analysis

All the analysis was conducted using SAS software (SAS Institute 2012). To determine the effect of grass height and density, thatch thickness, soil temperature, and relative humidity on the arthropod, the data was subjected to PROC COUNTREG procedure in SAS. The influence of sampling method, sampling date, and turfgrass taxa was analyzed using the PROC MIXED procedure in SAS after log link function. Grass height, grass density, thatch thickness, soil temperature, and relative humidity were the continuous effects, whereas turfgrass taxa and sampling date were the fixed effects. The sampling sites were treated as a random effect. Least square means were calculated using PROC PLM procedure in SAS. Pearson's correlation analysis was performed between plant parameters and arthropods as well as predators and herbivores using the PROC CORR procedure in SAS.

#### Results

## Influence of sampling method on arthropod abundance

Of the 227,811 arthropods collected in the study, 3,873, 89,497, and 134,441 were collected in the sweep, vacuum, and pitfall traps, respectively (Table 1.1). Arthropods in the pitfall samples represented 59% of all the arthropods collected in the study, whereas arthropods in the sweep samples were only 1.7% of the total arthropods sampled. The major predators

collected includes big-eved bugs (Geocoridae), assassin bugs (Reduviidae), plant bugs (Miridae), minute pirate bugs (Anthocoridae), damsel bugs (Nabidae), ground beetles (Carabidae), rove beetles (Staphylinidae), ants (Formicidae), earwigs (Dermaptera), spiders (Araneae), and Phlaeothripidae (Thysanoptera) (Table 1.1). Around 89% of all predators were collected in the pitfall traps (Table 1.1). Also, 96% of all the parasitic hymenopterans were collected in pitfall traps (Table 1.1). The numbers of total predatory Heteroptera (including Geocoridae, Nabidae, Reduviidae, Anthocoridae, and Miridae), total herbivorous Hemiptera (including Rhyparochromidae, Cercopidae, Cicadellidae, Delphacidae, Blissidae, Lygaeidae, and Aphididae), total predatory Coleoptera (including Carabidae, Staphylinidae, and Curculionidae), parasitic Hymenoptera, Formicidae, and Araneae were significantly greater in the pitfall traps than in sweep and vacuum samples (Table 1.3). A significantly greater number of Phlaeothripidae was collected in the pitfall traps than in the sweep and vacuum, whereas the number of Thripidae was significantly greater in the vacuum than in the sweep and vacuum samples (Table 1.3). The number of cercopids was not significantly different between the sweep and pitfall trap. The number of Acari was significantly greater in the sweep samples than in the vacuum or pitfall traps (Table 1.3).

Pitfall traps captured most arthropods among all the three sampling methods (Table 1.1). When the interaction between the sampling method and grass genotype was examined, numbers of Formicidae (Table 1.2; Fig. 1.2A), and Phlaeothripidae (Table 1.2; Fig. 1.2B) were significantly greater in the pitfall traps than in the sweep and vacuum samples, irrespective of the grass genotype. Curculionidae (Table 1.2; Fig. 1.2C), and parasitic Hymenoptera (Table 1.2; Fig. 1.2E) were captured in significantly greater numbers in the pitfall trap samples than in the sweep and vacuum samples in the zoysiagrass and bermudagrass, but not in the centipedegrass. Also,

the number of geocorids was significantly greater in the pitfall trap samples than in the sweep and vacuum samples in bermudagrass, but in the centipedegrass and zoysiagrass their captures did not vary significantly with the sampling method (Table 1.2; Fig. 1.2F).

## Influence of turfgrass taxa and date of sampling on arthropod abundance

Most of the arthropod families were not significantly influenced by turfgrass taxa (Table 1.2). Among the hemipterans, numbers of delphacids (Table 1.2; Fig. 1.1A), anthocorids (Table 1.2; Fig. 1.1B), and cicadellids (Table 1.2; Fig. 1.1C) were significantly greater in the centipedegrass than in the bermudagrass and zoysiagrass. The numbers of phlaeothripids (Table 1.2; Fig. 1.1D) and thripids (Table 1.2; Fig. 1.1F) were significantly greater in the centipedegrass than in the bermudagrass and zoysiagrass. Also, the number of formicids was significantly greater in the centipedegrass than in the zoysiagrass and bermudagrass (Table 1.2 and Fig. 1.1E).

The abundance of only a few arthropod families was affected by the sampling date. Numbers of anthocorids, delphacids, and nabids were significantly greater in May than in June and July samples (Table 1.2). The number of dermapterans was significantly greater in June and July than in May samples (Table 1.2). A significantly greater number of parasitic hymenopterans were collected in May compared to June and July samples (Table 1.2). The numbers of thripids and Araneae were significantly greater in May than in June and July samples (Table 1.2).

# Role of parameters such as height, density, and thatch thickness in determining arthropod abundance

The numbers of total herbivorous hemipterans (including Rhyparochromidae, Cercopidae, Cicadellidae, Delphacidae, Blissidae, Lygaeidae, and Aphididae) significantly increased with increase in grass height (Table 1.4; Fig. 1.3A). Numbers of Rhyparochromidae (Table 1.4; Fig. 1.3B) and Cercopidae (Table 1.4; Fig. 1.3F) significantly increased with an increase in grass

height. In contrast, the number of predatory heteropterans (including Geocoridae, Nabidae, Reduviidae, Anthocoridae, and Miridae) significantly decreased with increase in grass height (Table 1.4; Fig. 1.3C). The numbers of predatory mirids, *Spanogonicus albofasciatus* (Reuter) (Table 1.4; Fig. 1.3D) and the ground beetles (Carabidae) (Table 1.4; Fig. 1.3E) significantly decreased in the taller grasses.

With grass density numbers of phlaeothripids (Table 1.4; Fig. 1.5A), rhyparochromids (Table 1.4; Fig. 1.5B), and formicids (Table 1.4; Fig. 1.5E) significantly decreased with increase in grass density. Numbers of curculionids (Table 1.4; Fig. 1.5C), mirids (Table 1.4; Fig. 1.5D), and lepidopterans (Table 1.4; Fig. 1.5F) significantly increased with denser grass.

With thatch thickness, the numbers of hemipteran herbivores such as cercopids (Table 1.4; Fig. 1.4B) and aphidids (Table 1.4; Fig. 1.4C) significantly increased, whereas the number of coleopteran herbivores such as elaterids (Table 1.4; Fig. 1.4A) and curculionids (mostly, *Sphenophorus* species) (Table 1.4; Fig. 1.4D) significantly decreased with increase in thatch thickness. Also, the number of formicids (Table 1.4; Fig. 1.4E) significantly reduced with thicker thatch, whereas the numbers of Acari significantly increased with an increase in thatch thickness (Table 1.4 and Fig. 1.4F).

# Correlation among different arthropods and also between arthropods and plant parameters

Pearson's correlation coefficient shows a significant association between the predatory and herbivorous arthropod groups (Table 1.5). The number of Araneae collected displayed a positive correlation with the number of delphacids. The number of carabids was positively associated with the number of curculionids (Table 1.5). Similarly, the number of geocorids was positively associated with numbers of cicadellids and delphacids (Table 1.5(a)). Numbers of

parasitic wasps were positively correlated to numbers of hemipteran herbivores such as cercopids and aphidids (Table 1.5).

Arthropods were also significantly associated with various grass parameters. The number of formicids was negatively correlated with an increase in grass density and thatch thickness (Table 1.5). Numbers of carabids and rhyparochromids were negatively and positively associated with an increase in grass height, respectively (Table 1.5). The number of parasitic hymenopterans was negatively correlated to an increase in thatch thickness, whereas the number of aphids and cercopids was positively associated with an increase in thatch thickness (Table 1.5). Numbers of predatory mirids and herbivorous lepidopterans were positively correlated to an increase in grass density (Table 1.5). The number of Curculionidae was positively associated with an increase in grass density, whereas curculionids were negatively associated with an increase in thatch thickness (Table 1.5).

## **Discussion**

Results showed that the abundance of at least a few turfgrass arthropods in the sod farms was influenced by the plant parameters such as grass genotype, height and density, and thatch thickness. Among the predators, minute pirate bugs, Phlaeothripidae, and ants were more abundant in centipedegrass compared with bermudagrass and zoysiagrass. Most of the predators, such as big-eyed bugs, mirids, ground beetles, rove beetles, and spiders, were not influenced by the turfgrass genotype. However, in a previous study, predators such as geocorids, mirids, staphylinids, and spiders were more abundant in the zoysiagrass than in the bermudagrass and centipedegrass (Joseph and Braman 2009). The difference in results might be attributed to where the studies were conducted. Joseph and Braman (2009) studied the abundance of arthropods in

the residential lawns with varied levels of weed infestations and minimal pesticide use. Sod fields are intensively managed with regular pesticide applications.

The abundance of certain arthropod groups is affected by both turfgrass genotype and method used to sample arthropods. Anthocorids and geocorids were most often collected in the centipedegrass than the zoysiagrass and bermudagrass in vacuum samples. However, the data from the pitfall traps showed that an abundance of anthocorids and geocorids was greater in the bermudagrass than in the zoysiagrass and centipedegrass. Previously, the interaction between the grass genotype and sampling method has also been reported, where the carabids, staphylinids, and spiders were abundant in the zoysiagrass than the bermudagrass in vacuum samples. In contrast, the pitfall samples indicated that the carabids were commonly captured in the bermudagrass than the zoysiagrass, and staphylinids and spiders were equally abundant in all turfgrasses tested (Braman et al. 2003). These studies and our data provide guidance on selecting a sampling method for a specific turfgrass genotype for a better estimation of predaceous groups.

Data showed that the numbers of predatory mirids and carabids were influenced by grass height as these predators increased with an increase in grass height, whereas the other predators such as geocorids, staphylinids, formicids, and spiders were not affected by changes in the grass height. These results were not entirely consistent with previous studies. The abundance of ground-dwelling predatory beetles, ants, and spiders was affected by the grass height, with more individuals were collected in tall grasses (Smitley et al. 1998, Joseph and Braman 2009). Our data show that the herbivorous heteropterans such as cicadellids, and delphacids were unaffected by the height of the turfgrass. In contrast, more cercopids were collected from taller than low mowed grasses. Joseph and Braman (2009) showed that greater numbers of cicadellids and delphacids were captured from taller than shorter grasses. The variation in the previous and our

results could be caused by variation in the frequency and mowing height maintained in the sod farms and residential lawns. In the sod farms, turfgrass is grown for commercial purposes, and the emphasis is usually on growing the grass as quickly as possible. Thus, the grass is mowed frequently than residential lawns to promote the lateral spreading and thickness of the grass.

In the current study, the numbers of predatory mirids increased with an increase in the grass density, whereas the numbers of blissids, delphacids, and spiders were not affected. In contrast, Joseph and Braman (2009) showed that the predatory heteropterans were not affected, whereas the blissids, delphacids, and spiders were collected in greater numbers from denser than sparsely dense turfgrasses. Again, the variation in ours and previous results could be attributed to differences in the levels of management practices in commercial turfgrass and residential lawns. Moreover, the numbers of billbugs (*Sphenophorus* spp.) and lepidopterans, which are the major pest groups in the turfgrass, increased with an increase in the grass density in our study. This result was consistent with a previous study on Kentucky bluegrass sod field in Nebraska, where the bluegrass billbugs (*Sphenophorus parvulus* Gyllenhal) adults were abundant in dense grass (Kindler and Spomer 1986). These results suggest that the denser grasses might be favoring certain pests by providing a better micro-environment.

Our results show that the billbugs were negatively correlated to the thatch thickness in the sod field. This result is inconsistent with the previous study where the numbers of bluegrass billbugs adults were greater in denser grass regions with thicker thatch layer. Perhaps, thick thatch might have prevented desiccation of billbug larvae by providing shade, moisture, and protection from predators (Kindler and Spomer 1986). Further research is required to clearly understand the importance of thatch thickness in determining the abundance of billbug larvae in the sod farms growing warm-season grasses.

Cicadellids and delphacids were positively correlated to the abundance of geocorids in the current study. These results were consistent with previous studies where the predatory Heteroptera were positively associated with the delphacids (Braman et al. 2003, Joseph and Braman 2009) and blissids (Joseph and Braman 2009). Also, spiders were positively correlated to the delphacids, and the parasitic Hymenoptera were positively correlated to the cercopids and aphids in the current study. These results indicate some potential biological control activity in the sod farms.

Similarly, a positive correlation between the abundance of ground beetles and billbugs was documented. Dupuy and Ramirez (2019) showed that hunting billbug activity and mating were reduced by 56% and 28%, respectively, when exposure to the predators, although predaceous activity was 6% on adult billbugs. These experiments were conducted in the golf courses, where the carabids (60%) and spiders (28%) were mostly collected. These results are from golf courses that can be comparable to sod farms as both systems are intensely maintained, and generalist predators such as carabids and spiders are also abundant.

In summary, our study showed that the plant parameters such as grass genotype, height, and density, and the thatch thickness influenced the incidence and abundance of certain arthropods in the sod farms. Pitfall traps captured a diverse group of arthropods in the sod farms. Results also showed a positive association between predatory arthropods and an abundance of herbivorous arthropods. Based on the results from the current study, further research is needed to understand how host plant-insect interactions shape the communities of pest and predatory arthropods in the sod farms, which will help to refine the integrated pest management strategies for the major insect pests in sod farms.

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 $Table \ 1.1. \ The \ total \ arthropod \ sampled \ by \ various \ sampling \ methods \ in \ the \ sod \ field \ from \ May, \ June, \ and \ July \ in \ 2019.$ 

Arthropod Group	Taxon	Sweep	Vacuum	Pit-fall	Arthropod count
Predatory Heteroptera	Geocoridae	3	13	28	44
	Reduviidae	0	0	21	21
	Nabidae	0	0	4	4
	Anthocoridae	0	5	7	12
	Miridae	7	4	299	310
<b>Predatory Coleoptera</b>	Carabidae	0	44	528	572
	Staphylinidae	5	80	733	818
<b>Other Predators</b>	Formicidae	2	204	2,099	2,305
	Dermaptera	0	4	142	146
	Araneae	80	462	4,078	4,620
	Phlaeothripidae	1	14	94	109
Herbivorous Hemiptera	Lygaeidae	0	2	3	5
	Rhyparochromidae	0	42	53	95
	Blissidae	0	0	2	2
	Delphacidae	39	10	27	76
	Cicadellidae	128	38	282	448
	Cercopidae	86	21	25	132
	Aphididae	8	5	239	252
Herbivorous Coleoptera	Elateridae	0	1	230	231
	Chrysomelidae	14	5	33	52
	Lampyridae	0	0	41	41
	Mordelidae	0	0	16	16
	Curculionidae	0	1	1,210	1,211
<b>Other Herbivores</b>	Lepidoptera	1	0	82	83
	Diptera	211	170	4,463	4,844
	Thripidae	73	403	149	625
	Psocoptera	0	1	136	137
Hymenoptera	Parasitic	22	481	11,757	12,260
	Non-parasitic	1	3	44	48
Others	Collembola	656	39,554	85,462	1,25,672
	Acari	2,520	47,795	14,066	64,381
	Coleoptera	7	133	8,048	8,188
	Heteroptera	9	2	40	51
Total arthropods		3,873	89,497	1,34,441	2,27,811

Table 1.2. Influence of sampling method, turfgrass taxa, sampling date, and interactions between them on the abundance of different arthropod groups. The numbers in the table show F-values and P-values

Taxa	Method <sup>¥</sup>	<b>Genotype</b> <sup>¥</sup>	Date <sup>¥</sup>	Method × Genotype <sup>£</sup>	Method × Date <sup>£</sup>	Date × Genotype <sup>£</sup>
Predatory Heteroptera <sup>a</sup>	20.3***	-	-	-	-	2.99*
Anthocoridae	5.4**	4.0*	4.0*	4.8**	-	3.4*
Geocoridae	5.6***	-	-	3.1*	-	-
Nabidae	7.6***	-	6.2**	2.4*	-	3.4*
Reduviidae	16.3***	-	-	-	-	-
Miridae	9.3***	-	-	-	-	-
Herbivorous Hemiptera <sup>b</sup>	38.8***	-	4.7*	3.6**	3.1*	-
Rhyparochromidaeg	-	-	-	-	-	-
Blissidae	-	-	-	-	-	-
Lygaeidae	-	-	-	-	-	-
Cercopidae	-	-	-	-	-	-
Cicadellidae	28.3***	4.6*	-	7.3***	6.0***	7.2***
Delphacidae	-	4.3*	19.0***	-	-	7.2***
Aphididae	21.3***	-	-	-	-	-
Other Heteropterac	8.2***	-	-	-	-	-
Predatory Coleopterad	144.7***	-	-	-	3.5**	-
Carabidae	107.7***	-	-	-	4.2**	-
Staphylinidae	54.8***	-	-	-	-	-
Herbivorous Coleopterae	114.2***	3.5*	-	5.0***	-	-
Chrysomelidae	4.8**	-	-	-	-	-
Lampyridae	-	-	-	-	-	-
Mordellidae	-	-	-	-	4.2**	-
Curculionidae	90.5***	-	-	4.6**	-	-
Elateridae	59.0***	-	-	3.9**	3.9**	-
Other Coleoptera <sup>f</sup>	118.8***	-	-	-	-	-
Parasitic Hymenoptera	338.2***	-	5.7**	6.2***	4.4**	-
Non-parasitic Hymenoptera	13.5***	-	-	-	-	-
Formicidae	78.8***	3.4*	-	3.6**	2.8*	-
Diptera	183.1***	-	-	-	5.2***	-
Lepidoptera	19.6***	-	-	-	5.9***	-
Dermaptera	31.2***	-	5.0**	4.2**	2.9*	-
Phlaeothripidae	18.7***	5.5**	-	2.6*	-	-
Thripidae	19.9***	7.7***	49.3***	3.0*	6.5***	-
Collembola	102.4***	-	-	-	5.5***	-
Araneaeg	50.2***	-	7.8***	-	3.8**	-
Acari	67.0***	-	-	-	2.8*	-

"-" = not significant; "\*" = P < 0.05; "\*\*" = P < 0.01; "\*\*\*" = P < 0.001

<sup>a</sup>Predatory Heteroptera includes Anthocoridae, Geocoridae, Nabidae, Reduviidae, and Rhyparochromidae

<sup>b</sup>Herbivorous Hemiptera includes Miridae, Blissidae, Lygaeidae, Cercopidae, Cicadellidae, Delphacidae, and Aphididae

Other Heteroptera includes insects from families Saldidae, Pentatomidae, and other unidentified nymphal stages

<sup>d</sup>Predatory Coleoptera includes Carabidae and Staphylinidae

<sup>e</sup>Herbivorous Coleoptera includes Curculionidae, Elateridae, and Mordellidae

<sup>f</sup>Other Coleoptera includes Scarabaeidae, Silvanidae, and other unidentified families

ganalyzed through proc glm model because they were not converged in proc mixed model

<sup>§</sup>all arthropod groups have degree of freedom (2, 143) with a few exceptions. Predatory Heteroptera, Curculionidae, Collembola, Diptera, Acari and Other Coleoptera have degrees of freedom (2, 144). Rhyparochromidae and Araneae have degrees of freedom (2, 160).

<sup>£</sup>all arthropod groups have degrees of freedom (4, 143) with a few exceptions. Predatory Heteroptera, Curculionidae, Collembola, Diptera, Acari and Other Coleoptera have degrees of freedom (4, 144). Rhyparochromidae and Araneae have degrees of freedom (4, 160).

Table 1.3. Mean ( $\pm$  SE) no. arthropods sampled from 20 sod fields using sweep, vacuum, and pitfall during May, June, and July 2019 in central GA.

Arthropods	Sweep	Vacuum	Pitfall	df	f	P
Predatory Heteroptera <sup>a</sup>	$0.50 \pm 0.2c$	$1.10 \pm 0.5b$	$17.95 \pm 6.2a$	2	13.1	< 0.001
Anthocoridae	$0.00 \pm 0.0b$	$0.25 \pm 0.3a$	$0.35 \pm 0.2a$	2	3.8	0.032
Geocoridae	$0.15 \pm 0.1b$	$0.65 \pm 0.3a$	$1.40\pm0.4a$	2	6.7	0.004
Nabidae	$0.00 \pm 0.0b$	$0.00 \pm 0.0b$	$0.20\pm0.1a$	2	8.8	0.001
Reduviidae	$0.00 \pm 0.0b$	$0.00 \pm 0.0b$	$1.05 \pm 0.3a$	2	14.1	< 0.001
Herbivorous Hemipterab	$13.05 \pm 6.2b$	$5.9 \pm 3.2c$	$31.55 \pm 5.3a$	2	23.5	< 0.001
Rhyparochromidae	$0.00 \pm 0.0b$	$2.10 \pm 1.9ab$	$2.60 \pm 1.1a$	2	3.1	0.060
Miridae	$0.35 \pm 0.2b$	$0.20 \pm 0.1b$	$14.90 \pm 6.2a$	2	5.9	0.006
Blissidae	$0.00 \pm 0.0a$	$0.00 \pm 0.0a$	$0.10 \pm 0.1a$	2	0.1	0.869
Lygaeidae	$0.00 \pm 0.0a$	$0.10 \pm 0.1a$	$0.15 \pm 0.1a$	2	0.7	0.495
Cercopidae	$4.30 \pm 3.7a$	$1.05 \pm 1.1b$	$1.25\pm0.9a$	2	8.9	0.001
Cicadellidae	$6.40 \pm 3.9b$	$1.90 \pm 0.7c$	$14.10 \pm 3.2a$	2	14.1	< 0.001
Delphacidae	$1.90 \pm 1.1a$	$0.50 \pm 0.5a$	$1.30 \pm 0.5a$	2	1.4	0.271
Aphididae	$0.40 \pm 0.2b$	$0.25\pm0.2b$	$11.90 \pm 2.5a$	2	36.9	< 0.001
Other Heteropterac	$0.45 \pm 0.4b$	$0.10 \pm 0.1b$	$2.00\pm0.6a$	2	6.8	0.003
Predatory Coleopterad	$0.25 \pm 0.2c$	$6.20 \pm 1.3b$	$63.05 \pm 12.7a$	2	108.5	< 0.001
Carabidae	$0.00 \pm 0.0c$	$2.20\pm0.5b$	$26.40 \pm 3.8a$	2	86.5	< 0.001
Staphylinidae	$0.25 \pm 0.2c$	$4.00 \pm 1.1b$	$36.60 \pm 10.6a$	2	55.7	< 0.001
Herbivorous Coleoptera <sup>e</sup>	$0.70 \pm 0.4b$	$0.30 \pm 0.2b$	$65.00 \pm 13.1a$	2	84.4	< 0.001
Curculionidae	$0.00 \pm 0.0b$	$0.05 \pm 0.1b$	$60.50 \pm 13.0a$	2	87.2	< 0.001
Elateridae	$0.00 \pm 0.0b$	$0.05 \pm 0.1b$	$11.50 \pm 2.4a$	2	61.8	< 0.001
Mordellidae	$0.00\pm0.0a$	$0.00\pm0.0a$	$0.80 \pm 0.4a$	2	1.4	0.258
Other Coleoptera <sup>f</sup>	$0.35 \pm 0.2c$	$6.60 \pm 0.9b$	$402.40 \pm 199.3a$	2	76.6	< 0.001
Parasitic Hymenoptera	$1.10 \pm 0.5c$	$24.00 \pm 5.2b$	$587.80 \pm 86.5a$	2	255.9	< 0.001
Non-parasitic Hymenoptera	$0.05 \pm 0.1b$	$0.15 \pm 0.1b$	$2.20\pm0.4a$	2	16.6	< 0.001
Formicidae	$0.10 \pm 0.1c$	$10.20 \pm 2.7b$	$104.90 \pm 38.7a$	2	57.5	< 0.001
Diptera	$10.50\pm2.9b$	$8.50 \pm 1.9b$	$223.10 \pm 46.3a$	2	91.8	< 0.001
Lepidoptera	$0.05 \pm 0.1b$	$0.00 \pm 0.0b$	$4.10\pm0.9a$	2	18.8	< 0.001
Dermaptera	$0.00 \pm 0.0b$	$0.20 \pm 0.2b$	$7.10 \pm 2.9a$	2	20.3	< 0.001
Phlaeothripidae	$0.05 \pm 0.1b$	$0.70 \pm 0.3b$	$4.70\pm1.5a$	2	15.1	< 0.001
Thripidae	$3.60\pm0.8c$	$20.10 \pm 4.3a$	$7.50 \pm 1.5b$	2	14.6	< 0.001
Collembola	$32.80 \pm 15.9c$	$1977.70 \pm 613.5b$	4273.10 ± 505.1a	2	133.0	< 0.001
Araneae	$4.00 \pm 1.5c$	$23.10 \pm 4.9b$	$203.90 \pm 25.6a$	2	89.4	< 0.001
Acari	$126.00 \pm 68.2c$	$2389.70 \pm 636.0a$	$703.30 \pm 142.8b$	2	52.2	< 0.001

Means followed by the same letter are not significantly different (P > 0.05)

<sup>a</sup>Predatory Heteroptera includes Anthocoridae, Geocoridae, Nabidae, Reduviidae, and Rhyparochromidae

<sup>b</sup>Herbivorous Hemiptera includes Miridae, Blissidae, Lygaeidae, Cercopidae, Cicadellidae, Delphacidae, and Aphididae

Other Heteroptera includes insects from families Saldidae, Pentatomidae, and other unidentified nymphal stages

<sup>d</sup>Predatory Coleoptera includes Carabidae and Staphylinidae

<sup>e</sup>Herbivorous Coleoptera includes Curculionidae, Elateridae, and Mordellidae

<sup>f</sup>Other Coleoptera includes Scarabaeidae, Silvanidae, and other unidentified families

Table 1.4. Influence of turf attributes, relative humidity, and soil temperature on incidence and abundance of arthropod taxa. All three sampling methods were combined, but not the dates. The values in the table are t-values with their respective levels of significance

Insects	Grass height	Grass density	Thatch thickness	Relative humidity	Soil temperature
Predatory Heteroptera <sup>a</sup>	-2.1*	2.89**	-	4.3***	-
Anthocoridae	_	-	-	-	-
Geocoridae	-	-	-	-	-
Nabidae	-	-	-	-	-
Reduviidae	_	-	-	-	-
Herbivorous	3.1**	-	2.5*	-	-
Hemiptera <sup>b</sup> Rhyparochromidae	4.7***	-2.5*	-	-	-
Miridae	-2.7**	3.4***	-	4.1***	_
Blissidae	-	-	-	_	-41.0***
Lygaeidae	-	-	-	-	_
Cercopidae	3.7***	-	2.5*	2.5*	-2.2*
Cicadellidae	-	-	-	_	-
Delphacidae	-	-	-	1.9*	-
Aphididae	-	-	3.0**	3.2**	-2.68**
Other Heteroptera <sup>c</sup>	-	-	-	-	-
Predatory Coleopterad	-	-	-	-	-
Carabidae	-2.9**	-	-	-	-
Staphylinidae	_	-	-	-	_
Herbivorous	-2.4*	3.2**	-3.1**	-2.5*	_
Coleoptera <sup>e</sup>					
Chrysomelidae	-	-	-	-	-
Lampyridae	-	- -	-	-	-2.4*
Mordellidae	-	2.3*	- 0.54	-	-
Curculionidae	-	2.8**	-2.5*	-2.1*	- 
Elateridae	-2.4*	- -	-2.8**	-2.0*	2.5*
Other Coleoptera <sup>f</sup>	-	-2.8**	-	-4.8***	4.5***
Parasitic Hymenoptera	-	-	-	-	-
Non-parasitic Hymenoptera	-	-	-	-	-
Formicidae	-	-3.1**	-4.1***	2.1*	-
Diptera	-	-	-	3.9***	-2.9**
Lepidoptera	-	2.4*	-	_	-
Dermaptera	-	-	-	_	-
Phlaeothripidae	-	-3.1**	-	-	-
Thripidae	-	-	-	-	-
Collembola	-	2.5*	-	-	-
Araneae	_	-	-	-	-
Acari	2.1*	-	4.5***	_	-

<sup>a</sup>Predatory Heteroptera includes Anthocoridae, Geocoridae, Nabidae, Reduviidae, and Rhyparochromidae

<sup>b</sup>Herbivorous Hemiptera includes Miridae, Blissidae, Lygaeidae, Cercopidae, Cicadellidae, Delphacidae, and Aphididae

<sup>c</sup>Other Heteroptera includes insects from families Saldidae, Pentatomidae, and other unidentified nymphal stages

<sup>d</sup>Predatory Coleoptera includes Carabidae and Staphylinidae

<sup>e</sup>Herbivorous Coleoptera includes Curculionidae, Elateridae, and Mordellidae

<sup>f</sup>Other Coleoptera includes Scarabaeidae, Silvanidae, and other unidentified families

Table 1.5(a). Pearson's correlation between predatory arthropods, herbivorous arthropods, and parasitic Hymenoptera. All three sampling methods were combined, but the three sampling dates were kept separate. The values in the table are f-values with their respective levels of significance

Arthropod groups	Cercopidae	Cicadellidae	Delphacidae	Aphididae	Curculionidae
Araneae	-	-	0.3*	-	-
Formicidae	-	-	-	-	-
Carabidae	-	-	-	-	0.3*
Staphylinidae	-	-	-	-	-
Geocoridae	-	0.4**	0.3*	-	-
Miridae	-	-	-	-	-
Parasitic Hymenoptera	0.4**	-	-	0.4***	-

<sup>&</sup>quot;-" = not significant; "\*" = P < 0.05; "\*\*" = P < 0.01; "\*\*\*" = P < 0.001

Table 1.5(b). Pearson's correlation between arthropods and plant parameters. All three sampling methods were combined, but the three sampling dates were kept separate. The values in the table are f-values with their respective levels of significance

Arthropod groups	Grass Height	<b>Grass Density</b>	Thatch Thickness
Araneae	-	-	-
Formicidae	-	-0.3*	-0.3*
Carabidae	-0.3*	-	-
Staphylinidae	-	-	-
Geocoridae	-	-	-
Rhyparochromidae	0.5***	-	-
Parasitic Hymenoptera	-	-	-0.3*
Cercopidae	-	-	0.4**
Cicadellidae	-	-	-
Delphacidae	-	-	-
Aphididae	-	-	0.4***
Miridae	-	0.3*	-
Lepidoptera	-	0.3*	-
Curculionidae	-	0.3*	-0.3*

<sup>&</sup>quot;-" = not significant; "\*" = P < 0.05; "\*\*" = P < 0.01; "\*\*\*" = P < 0.001

Figure 1.1. Mean (± SE) no. arthropods, A. Delphacidae, B. Anthocoridae, C. Cicadellidae, D. Phlaeothripidae, E. Formicidae, and F. Thripidae) sampled from various sod fields.

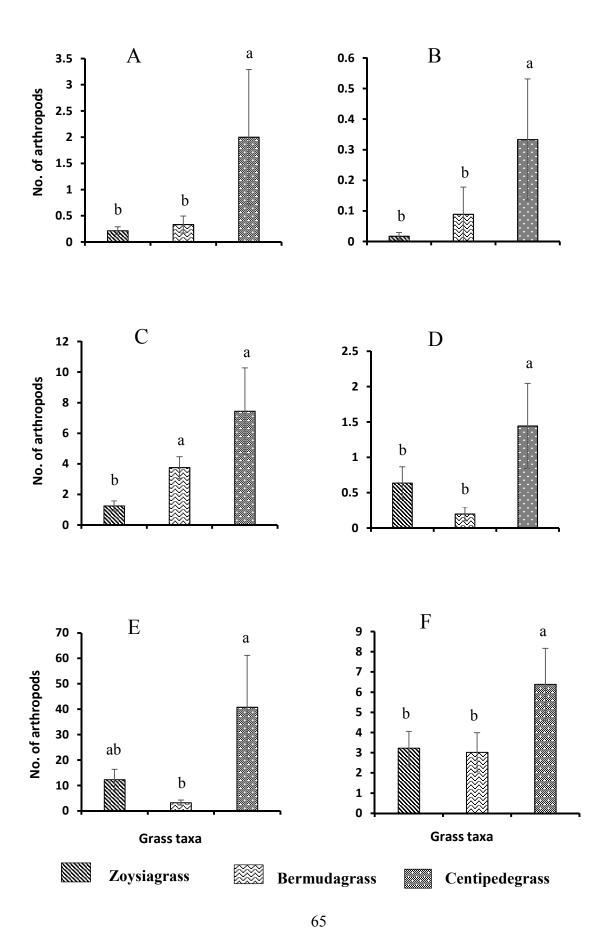
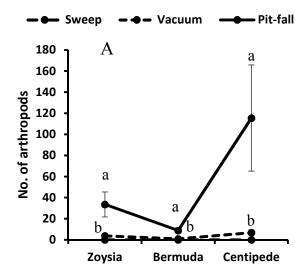
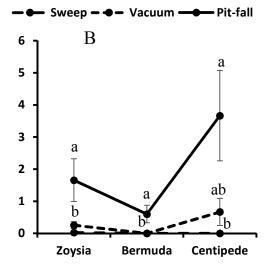
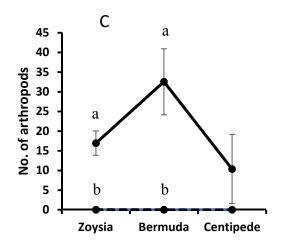
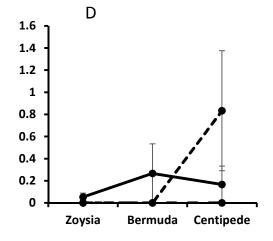


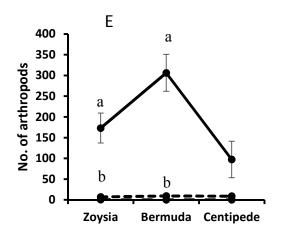
Figure 1.2. Mean  $(\pm$  SE) no. arthropods, A. Formicidae, B. Phlaeothripidae, C. Curculionidae, D. Anthocoridae, E. Parasitic Hymenoptera, F. Geocoridae sampled by sweep, vacuum and pitfall traps from various sod fields.











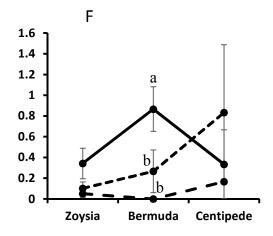
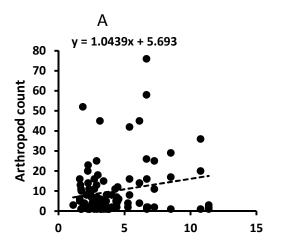
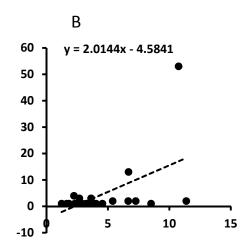
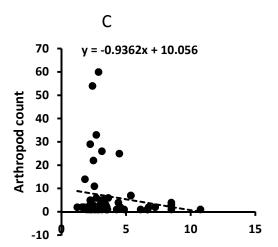
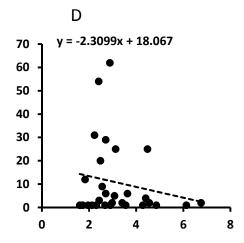


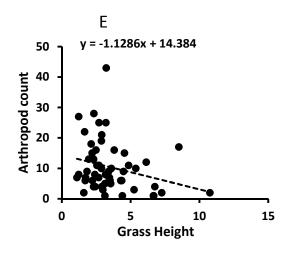
Figure 1.3. The relationship between grass height and arthropods, A. Herbivorous Hemiptera, B. Rhyparochromidae, C. Predatory Heteroptera, D. Miridae, E. Carabidae, and F. Cercopidae.











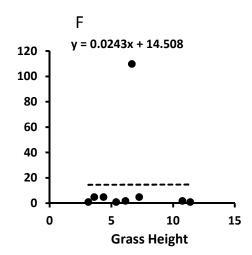
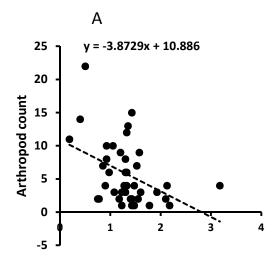
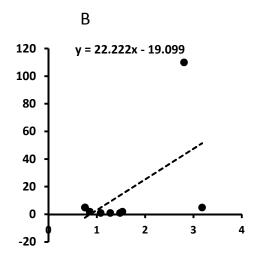
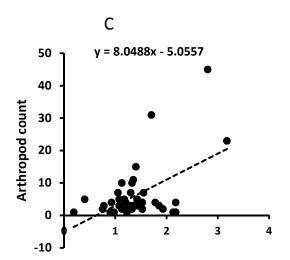
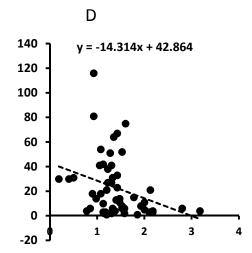


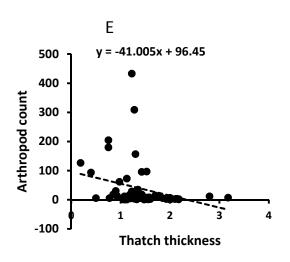
Figure 1.4. The relationship between thatch thickness and arthropods, A. Elateridae, B. Cercopidae, C. Aphididae, D. Curculionidae, E. Formicidae, and F. Acari.











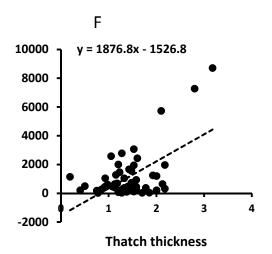
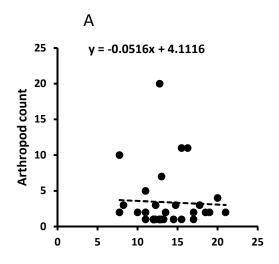
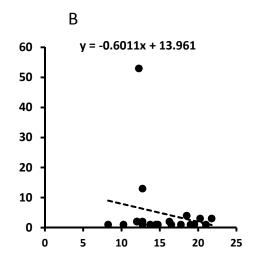
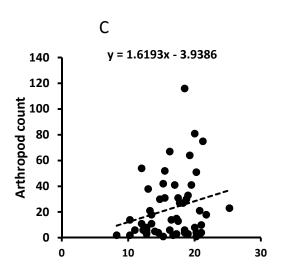
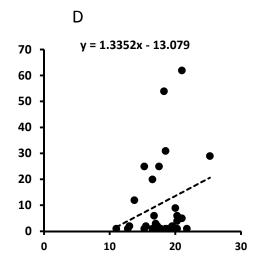


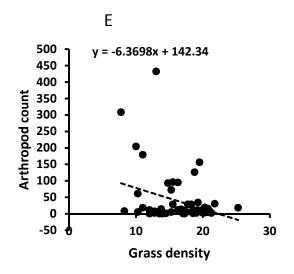
Figure 1.5. The relationship between grass density and arthropods, A. Phlaeothripidae, B. Rhyparochromidae, C. Curculionidae, D. Miridae, E. Formicidae, and F. Lepidoptera

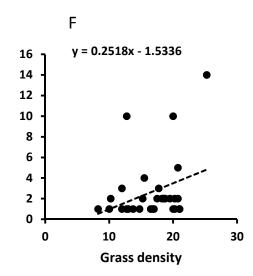












# **CHAPTER 3**

# EFFECTS OF POTASSIUM AND NITROGEN ON SURVIVAL AND DEVELOPMENT OF FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE)¹

<sup>1</sup>Gurjit Singh, Shimat V. Joseph and Clint Waltz

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#### **Abstract**

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a sporadic pest of turfgrass. Turfgrass is intensely managed using fertilizers, mostly driven by nitrogen. Although K is known to reduce the infestation of insect pests in other agro-ecosystems, little is known about the effects of K on S. frugiperda in turfgrass. Thus, the objective of this study was to determine the effects of N and K on the survival and development of S. frugiperda larvae. In 2018 and 2019, an experiment was conducted on 'TifEagle' bermudagrass. The treatments were various ratios of N: K and they were: 1) 0:0, 2) 1:0, 3) 2:1, 4) 1:1, 5) 0:1, and 6) 1:2 where 1 represents 113.4 g N or K per 92.3 m<sup>2</sup>. Each treatment was replicated ten times in 2018 and 14-15 times in 2019. The treatments were foliar applied from May to October at the biweekly interval. Survival and development of S. frugiperda were evaluated after introducing neonates of S. frugiperda in the laboratory conditions. In 2018, survival was significantly lower in 0:1, 1:2, 1:0, and 0:0 than in 2:1 and 1:1 treatment at 10 d and 24 d post-introduction, whereas in 2019, survival was significantly lower in 0:0 than in the rest of the treatments at 10 d and 24 d post introduction. The development of S. frugiperda larvae was significantly greater on 2:1 treatment as compared to 1:1 in both years. In 2018, the foliar K was similar in both the grasses fertilized with 2:1 and 1:1 treatment, whereas the K content was higher in 1:1 than in 2:1 treatment in 2019. Moreover, other micro-nutrients such as S, Na, and Zn were significantly greater in 2:1 than 1:1 treatment in 2018. However, in 2019 amounts of the same nutrients were found greater in 1:1 treatment than 2:1. Our data has shown that N favors, while K discourages the growth and development of *S. frugiperda* larvae on bermudagrass.

**Keywords:** Spodoptera frugiperda, turfgrass, integrated pest management

The fall armyworm, Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuiidae), is an important pest of bermudagrass (Cynodon spp., Family: Gramineae) in the central and southeastern U.S. (Luginbill 1928). Bermudagrass is a major pasture and turfgrass species in the southeastern U.S. (Burton 1991). The turfgrass has been ranked 23rd in 2018 Georgia Agricultural Commodity Rankings. Georgia is one of the major sod producing states in the U.S., and the turfgrass industry generates \$118.3 million USD to Georgia's economy (Georgia Farm Gate Value Report 2018). In Georgia, S. frugiperda does not diapause instead overwinters in tropical regions of Florida and Texas and disperses throughout the eastern and central U.S. during spring and summer every year. Younger larvae consume only the green tissue, causing skeletonization of the leaves. The older larvae (fourth to sixth instars) consume the entire leaf blade and stolon. Currently, the management of S. frugiperda is mostly accomplished using pyrethroid insecticides, although most of the insecticides are only effective against younger larvae. Pyrethroid insecticides are known for its hazardous effects against non-target organisms, especially beneficial insects such as predators and parasitoids (Sarto et al. 2014, Regan et al. 2017, Cheng et al. 2018). As part of the integrated pest management program, non-chemical options are available but rarely utilized for S. frugiperda management in commercial production and maintenance

Potassium (K) is known to increase fiber strength and quality in plants (Read et al. 2006). As an enzyme activator, K plays a vital role in various plant physiological processes such as photosynthesis, respiration, carbohydrate metabolism, translocation, and protein synthesis (Pettigrew 2008). Inadequately available K can not only contribute to nutrient imbalance (Miller 1999) but also increases susceptibility to arthropod infestation. Perrenoud (1990) reviewed more than 2000 studies and concluded that K fertilizer application tends to reduce the incidence of

diseases and insect pests in many agro-ecosystems. The K fertilizer application in rice has been shown to improve its tolerance to various abiotic and biotic stresses, including insect pests (Tiwari 2002). Similarly, resistance against insect pests and diseases is directly related to plant physiology and factors influencing plant's physiology may affect its resistance or tolerance levels (Altieri and Nicholls 2003). Studies also demonstrated that the K fertilizer inputs influence the effectiveness of applied nitrogen (N) especially, the yield response related to increasing in N uptake in 'Tifton 85' bermudagrass (Haby et al. 2007).

In Georgia, available soil K is highly prone to leaching and quickly become deficient (Mikkelsen 2007). Bermudagrass is highly responsive to N fertilizers but also requires a considerable amount of K fertilizer for root and shoot growth. Bermudagrass consumes considerable amounts of nutrients from the soil. For the production of every six tons of bermudagrass hay, around 136 Kg of N and 114 Kg of K nutrients are removed from the soil (Lee et al. 2017). Similarly, it was reported that K depletes from 160 to 50 ppm in three years under bermudagrass production (Nelson et al. 1983). Supplementing the soil with K fertilizer is critical as it improves plant health with a better root system and helps to withstand stress induced by disease and high salt content (Wang et al. 2013). Because K does not directly yield plant growth benefits relative to N, turfgrass managers are reluctant to regularly supplement K (Miller and Dickens 1996). Thus, the University of Georgia recommends the application of K, twice the rate of N in the fall season, to combat disease problems. However, it is not clear if high K levels in plant tissue can help reduce S. frugiperda pest incidence. Little is known on how plant nutrient levels influence the ability of turfgrass to resist *S. frugiperda* feeding in bermudagrass. Previously, Leuck et al. (1974) showed that foliar application of K could repel S. frugiperda larvae; however, the effects on survival and development were not well documented.

Thus, the objective of the current study was to determine if K as a stand-alone fertilizer and when combined with N significantly affects the survival and development of *S. frugiperda* on bermudagrass.

#### **Materials and Methods**

#### Plant material and insect

In 2018 and 2019, experiments were conducted in greenhouse and environmentally controlled chambers at the University of Georgia Griffin campus. The plugs of 'TifEagle' bermudagrass were obtained from a turfgrass field in Griffin and grown in 10.2 × 10.2 cm plastic pots in the greenhouse. The plugs were washed to remove soil and were planted in sand media. The grass pots were irrigated every day and fertilized at the weekly interval with 20:20:20 NPK at 5.9 g per L water (100 mL per pot). The plants were maintained at 25°C and ~60% relative humidity in the greenhouse. The *S. frugiperda* used in the experiments was purchased from Benzon Research Inc. (Carlisle, PA). After receiving larvae from the rearing facility, the whole shipment box was placed in the laboratory at room temperature (21°C and ~40% relative humidity) and was used for the experiment within a couple of hours.

### **Experiment design**

In 2018 and 2019, the N (Urea) and K (Muriate of potash) nutrients were applied at a biweekly interval from May to October. The treatments were ratios of N: K, 1) 0:0, 2) 1:0, 3) 2:1, 4) 1:1, 5) 0:1, and 6) 1:2 where, 1 represents 113.4 g N or K per 92.3 m<sup>2</sup>, and replicated 10 times in 2018 and 14 times in 2019 in randomized complete block design. The treatments were foliar sprayed on grass pots using CO<sub>2</sub> powered sprayer inside a spray chamber. After five months, ~ 4-10 g of grass clippings were removed and transferred into the 29.6 mL clear plastic container (Frontier Agricultural Sciences, Newark, Delaware, USA, product#9051). The

containers with fresh grass clippings were temporarily maintained in the refrigerator at 4°C before the introduction of *S. frugiperda* to prevent desiccation.

Within 3 h, three neonates of *S. frugiperda* larvae were introduced to the container with grass clippings. After the introduction, the container was sealed using a lid and a parafilm strip around the lid to reduce desiccation. Fresh grass clippings were added to the containers at 2 d intervals. The uneaten grass clippings and frass were removed before adding fresh grass clippings to the containers. The assay was maintained in the environmentally controlled chamber at 28°C and ~40% relative humidity and 16: 8 h (light: dark).

#### **Evaluation**

Survival and development of larvae were recorded at 2 or 3 d intervals until pupation.

The dead larvae shriveled up and turned dark in color. To determine development, the larval length, the width of the head capsule, and weight were recorded (in 2018, only the length and width of the larvae were recorded). Larval length and width of the head capsule were measured using a Vernier caliper. Larval weight was measured using a digital weighing balance. When there was more than one larva in the container, the measurements were averaged and recorded. Also, the pupal length, width around the thorax region, and weight were recorded for the surviving larvae, until the adult emergence (in 2018 only length and width were recorded). Foliage samples were collected for nutrient analysis when the adults emerged from all treatments. For the foliar nutrient analysis, leaf samples from two pots (two replications) for each treatment were combined; thus, there were five samples per treatment in both years.

The grass clippings were oven—dried (at 115°C for 48 h) in Griffin campus and transported to the Plant and Soil Testing Laboratory (University of Georgia, Athens, GA) for foliar nutrient analysis. 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) and the combustion method was used to quantify the total percent N (Colombo and Giazzi 1982).

#### Statistical analysis

All the analysis was conducted using SAS software (SAS Institute 2012). The survival data from 2018 and 2019 were log-transformed ( $\ln [x + 1]$ ) then subjected to a one-way analysis of variance (ANOVA) using the PROC GLM procedure in SAS. The length of larvae, the width of the head capsule, and weight data were analyzed only for treatments that had at least three replications with live *S. frugiperda* larvae. In 2018, the data were analyzed by student *t-test* using PROC TTEST procedure in SAS as only two treatments had live larvae whereas, in 2019, ANOVA using PROC GLM procedure in SAS was used after log-transformation ( $\ln [x + 1]$ ) as there were more than two treatments with one or more live larvae. The percentage foliar nutrients data were arcsine square-root transformed before one-way ANOVA was performed whereas, foliar nutrients data in ppm (parts per million) were subjected to one-way ANOVA using PROC GLM procedure in SAS after log-transformation ( $\ln [x + 1]$ ). Means were separated using the LSD method ( $\alpha = 0.05$ ). Means and standard error for the variables were calculated using the PROC MEANS procedure in SAS.

#### Results

#### Larval survival

In 2018, none of the larvae survived beyond 6 d after introduction in 0:0 treatment where N and K were not applied (Fig. 1A). A significantly lower number of larvae survived in 0:1, 1:2, 1:0 and 0:0 than in 2:1 and 1:1 treatment at 10 d ( $F_{5,45} = 5.3$ ; P < 0.001; Fig. 1A) and 24 d ( $F_{5,45} = 5.2$ ; P < 0.001; Fig. 1A) post-introduction. In 2019, survival of larvae in the various treatments

followed similar pattern as in 2018. None of the larvae survived beyond 6 d in 0:0 treatment (Fig. 1B). At 2 d post-introduction, survival of larvae was not significantly different between treatments (P < 0.05; Fig. 1B).

#### Larval development

In 2018, only two treatments had more than three replications with live *S. frugiperda* and so only these two treatments were compared. The larval length was greater in 2:1 than in 1:1 treatment at 6 d ( $t_9$  = 4.4; P = 0.002; Fig. 2A), 8 d ( $t_8$  = 2.66; P = 0.028; Fig. 2C) and 10 d ( $t_8$  = 3.1; P = 0.015; Fig. 2E) post-introduction. However, the width of larval head capsule was similar in 2:1 and 1:1 treatment at 6 d ( $t_8$  = 1.38; P = 0.2049; Fig. 2B), 8 d ( $t_8$  = 1.38; P = 0.2049; Fig. 2D), and 10 d ( $t_8$  = 1.38; P = 0.2049; Fig. 2F) post-introduction.

In 2019, larval length was not significantly different between treatments at 8 d ( $F_{4,7}$ = 2.4; P = 0.146; Fig. 3A), 10 d ( $F_{3,8}$ = 2.9; P = 0.105; Fig. 3D), 12 d ( $F_{2,5}$ = 3.25; P = 0.125; Fig. 3G) and 14 d ( $F_{2,5}$ = 3.7; P = 0.103; Fig. 3J) post-introduction. On 16 d, larval length was significantly lower in 1:1 than in 2:1 and 1:2 treatments ( $F_{2,5}$ = 21.7; P = 0.003; Fig. 3M). Similarly, the width of larval head capsule was found similar between treatments at 8 d ( $F_{4,7}$ = 1.5; P = 0.294; Fig. 3B), 10 d ( $F_{3,8}$ = 2.2; P = 0.163; Fig. 3E), 12 d ( $F_{2,5}$ = 2.9; P = 0.146; Fig. 3H), 14 d ( $F_{2,5}$ = 2.4; P = 0.183; Fig. 3K), and 16 d ( $F_{2,5}$ = 3.2; P = 0.127; Fig. 3N) post-introduction. The mean weight of surviving larvae was not significantly different between treatments at 8 d ( $F_{4,7}$ = 2.8; P = 0.108; Fig. 3C), 10 d ( $F_{3,8}$ = 2.6; P = 0.122; Fig. 3F), and 12 d ( $F_{2,5}$ = 3.5; P = 0.111; Fig. 3I) post introduction. Larval weight was significantly lower in 1:1 than in 1:2 treatments at 14 d post-introduction ( $F_{2,5}$ = 6.5; P = 0.041; Fig. 3L) whereas at 16 d post-introduction, the larval weight was significantly lower in 1:1 than in 2:1 and 1:2 treatments ( $F_{2,5}$ = 10.3; P = 0.0169; Fig. 3O).

#### **Nutrient analysis**

The percentage K in 2018 was lower in the grass fertilized with 0:0 and 1:0 treatments than in the rest of the treatments. However, it was similar in 2:1, 1:1, 0:1 and 1:2 treatments ( $F_{5}$ ,  $_{20}$  = 15.2; P < 0.001; Fig. 4A). The percentage N was greater in 2:1 treatment than in 1:1 and 1:2 treatments followed by 1:0 and 0:1 treatment. It was lowest in 0:0 treatment ( $F_{5,20}$  = 8.5; P < 0.001; Fig. 4B). In 2019, percentage K was significantly greater 1:1 than in the rest of the treatments ( $F_{5,20}$  = 3.1; P = 0.033; Fig 4C). However, percentage K was not significantly different between 1:1 and 2:1 as well as 2:1, 0:1 and 1:2 treatments. The grass fertilized with treatment 1:0 and 2:1 had greater percentage N than 1:1 and 1:2 treatments, followed by 0:0 and 0:1 treatment ( $F_{5,20}$  = 40.8; P < 0.001; Fig 4D).

In 2018, the percentage Ca was significantly greater in 1:0 than in 1:2 treatments (Table 1). Similarly, percentage of Mg was significantly greater in 1:0 than in 2:1, 0:1 and 1:2 treatments. The percentage of P was significantly greater in 0:0 and 0:1 treatment than in 1:2 treatment. The concentrations of Al and Zn were significantly greater in the treatment 1:0 than in 0:0 treatment. However, the S, B, Cr, Cu, and Mn nutrients were present in a similar quantity in all the treatments (Table 1). The concentration of Na was significantly greater for 1:0 followed by 0:0 treatment than for 1:1, 0:1 and 1:2 treatments.

In 2019, the foliar nutrient analysis showed that the percentage Ca was not significantly different between treatments (Table 1). Percentage Mg was significantly greater in 1:0 than in 1:2 treatment. The percentage of P was significantly greater in 2:1, than in 1:2 and 1:0 treatments. The percentage S was greater in 1:1 than in 0:1 and 0:0 treatment. The concentrations of nutrients such as Al, B, and Mn were not significantly different between treatments. The Fe content was significantly greater in 2:1 and 1:2 treatments than in 0:0 treatment. Similarly, the

Cu was in higher concentrations in 1:0 and 2:1 treatment than in 0:0 treatment. The concentration of Na was significantly greater in 1:0 treatment followed by 2:1, and 1:1 than in 0:0, 0:1, and 1:2 treatments. The levels of Zn were significantly greater in 1:0, 2:1, 1:1, 0:1 treatment than in 0:0 treatment (Table 1).

#### Discussion

Research in the past has demonstrated the effects of crop nutrition on plant's inherent resistance mechanisms and also on the biological development of insect pests feeding on them (Leuck et al. 1974, Amtmann et al. 2008, Rashid et al. 2016). The majority of the studies have shown that N supports (Scriber 1984, Altieri and Nicholls 2003), but K prevents the herbivory of insect pests (Amtmann et al. 2008). Results of the current study showed that N supported the development of S. frugiperda larvae whereas, K discouraged the development. In 2018, both the 2:1 and 1:1 treatment had a similar percentage of K in the plant tissues, but percentage N was greater in 2:1 treatment (Fig. 2A, C and E) and the S. frugiperda larval development was faster in 2:1 than in 1:1 treatment. Similarly, in 2019, a greater percentage of K and lower N levels in the plant tissues was observed in 1:1 than in 2:1 treatment (Figs. 3L, O, 4C, and D) and the development of S. frugiperda larvae was reduced in 1:1 than in 2:1 treatment. The evidence from 2018 and 2019 data suggests that K is not favoring the survival and development of S. frugiperda larvae, and maintaining a higher level of K in plant tissues may help to reduce feeding damage from S. frugiperda larvae in bermudagrass. Previously, Wiseman et al. (1973) found that the neonates of S. frugiperda larvae preferred corn foliage treated only with N fertilizer over the foliage treated with P, K and combination of P and K. Also, the larval mortality increased and gain in weight was reduced when S. frugiperda larvae were fed on foliage treated with K.

The data showed that the survival of S. frugiperda larvae was reduced to less than one after 6 d post-introduction. Although it is not exactly clear why the bermudagrass in the current study elicited high S. frugiperda larval mortality, this could be related to some possible reasons. In the current study, only two nutrients were applied to bermudagrass in varied ratios. Previously, increased survival of S. frugiperda larvae was observed on 'TifEagle' bermudagrass when all the macronutrients were applied at a regular interval (Braman et al. 2000, 2003). In Braman et al. (2000), bermudagrass was fertilized with 250 mg/L of 20:20:20 NPK in laboratory studies whereas 460.5 Kg/ha of 13:13:13 NPK was applied in a field study (Braman et al. 2003). In another study, the survival and development of S. frugiperda were enhanced on millet plants treated with all three macronutrients N, P, and K than the application of one or two nutrients (Leuck 1972). These studies suggest that the lower survival of S. frugiperda larvae observed in the current study could be related to the absence of all three macronutrients and low levels of N fertilizer application on bermudagrass. Another reason that could be related to the sand medium used in the current study, which can minimize the availability of other essential nutrients in the tissue. A principal constituent of sand is silica (Si), which can alter the plant's resistance response to herbivorous insects through a myriad of direct and indirect pathways (Reynolds et al. 2009). Elevated levels of Si in the plant tissues increase the hardness and abrasiveness of epidermal cells leading to reduced digestibility of insect feeding on them (Panda and Kush 1995). In other studies, reduced consumption was noted on the plant tissues with high levels of Si, causing high mortality rates, reduced larval growth, and reduced adult fecundity (Djamin and Pathak 1967, Horng and Chu 1990, Chu and Horng 1991, Salim and Saxena 1992). Thus, high Si content in the grass tissues could be another factor causing high mortality of S. frugiperda larvae in the current study.

Results show that the micronutrient levels were varied in plant tissues that received various ratios of N and K treatments. In the previous studies, various soil micronutrients such as S, B, P, Mg, Mn, Al, and Fe were associated with development, and reproduction of spotted alfalfa aphid, *Therioaphis maculata* (Buckton) (Kindler and Staples 1970), whitebacked planthopper, *Sogatella furcifera* (Horváth) (Salim and Saxena 1992) and hemlock wooly aphid, *Adelges tsugae* (Annand) (Hemiptera: Adelgidae) (Joseph et al. 2011). In this study, the sand was used as a growing medium, and no other micronutrient was applied. All the micronutrients found in the plant tissue should have been previously present. It is not clear from the data if those varying levels of certain micronutrients influenced the survival or development of *S. frugiperda* larvae.

In summary, results show that high levels of K in the bermudagrass can affect the development of *S. frugiperda* in controlled conditions. However, the survival and development of *S. frugiperda* larvae tend to improve with high levels of N in the foliage. More research is warranted to establish the role of optimized K and N levels that favor grass growth but reduce susceptibility to *S. frugiperda* larval survival and development in the field conditions. Also, varying levels of essential micronutrients were observed in the grass tissues that received various N and K treatments, which suggest that future studies are needed to determine how micronutrients are influencing the availability of N and K and ultimately affect the survival and development of *S. frugiperda* larvae. The current study showed that optimized N and K applications could become an important tool as part of the integrated pest management program for *S. frugiperda*.

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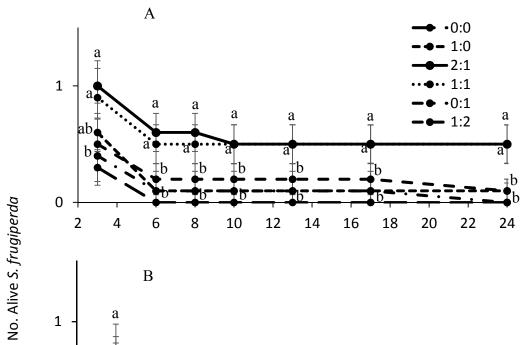
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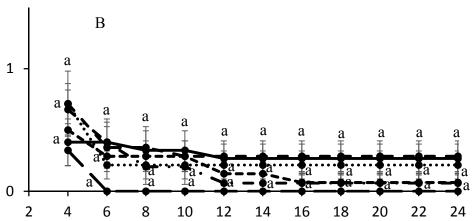
Table 1. Mean (±SE) percentage or parts per million (ppm) of foliar nutrients in 'TifEagle' bermudagrass after foliar spray of urea and Muriate of potash from May to October 2018 and 2019.

Elements	0:0	1:0	2:1	1:1	0:1	1:2	F	df1, df2	P
2018									
Ca	$0.613 \pm 0.022ab$	$0.702 \pm 0.039a$	$0.595 \pm 0.031ab$	$0.591 \pm 0.044$ ab	$0.591 \pm 0.062ab$	$0.463 \pm 0.028b$	3.3	5, 20	0.023
Mg	$0.184 \pm 0.006$ ab	$0.232 \pm 0.009a$	$0.172 \pm 0.008b$	$0.184 \pm 0.015ab$	$0.169 \pm 0.015b$	$0.138 \pm 0.006b$	7.5	5, 20	< 0.001
P	$0.219 \pm 0.011a$	$0.195 \pm 0.011ab$	$0.167 \pm 0.016ab$	$0.183 \pm 0.014ab$	$0.212 \pm 0.017a$	$0.150 \pm 0.009b$	4.8	5, 20	0.005
S	$0.387 \pm 0.015a$	$0.433 \pm 0.011a$	$0.431 \pm 0.017a$	$0.397 \pm 0.017a$	$0.360 \pm 0.028a$	$0.347 \pm 0.018a$	3.2	5, 20	0.029
Al	$13.9 \pm 1.4b$	$30.4 \pm 8.1a$	$17.8 \pm 1.9ab$	$21.9 \pm 1.9ab$	$21.2 \pm 2.2ab$	$20.7 \pm 1.4ab$	3.1	5, 20	0.029
В	$2.9 \pm 0.5a$	$4.1\pm0.2a$	$3.4 \pm 0.3a$	$3.9 \pm 0.8a$	$3.1\pm0.4a$	$2.5 \pm 0.1a$	1.9	5, 20	0.128
Cr	$1.29 \pm 0.09a$	$1.83 \pm 0.34a$	$1.37 \pm 0.14a$	$1.62 \pm 0.14a$	$1.37 \pm 0.17a$	$1.09 \pm 0.02a$	1.9	5, 20	0.128
Cu	$10.2 \pm 1.8a$	$8.7 \pm 0.7a$	$6.3 \pm 0.4a$	$8.0 \pm 0.6a$	$7.6 \pm 0.9a$	$6.7 \pm 0.5a$	2.4	5, 20	0.078
Mn	$65.0 \pm 7.4a$	$86.8 \pm 7.6a$	$80.7\pm10.2a$	$78.2 \pm 9.0a$	$89.9 \pm 7.8a$	$72.9 \pm 6.0a$	1.5	5, 20	0.226
Na	$1443.9 \pm 139.1b$	$2527.8 \pm 298.8a$	$1099.9 \pm 129.1$ bc	$893.3 \pm 111.3c$	$820.6 \pm 85.2c$	$706.9 \pm 24.3c$	21.5	5, 20	< 0.001
Zn	$39.4 \pm 1.6b$	$54.9 \pm 4.9a$	$47.2\pm1.5ab$	$43.7 \pm 2.3 ab$	$40.2 \pm 3.4b$	$38.3 \pm 2.7b$	4	5, 20	0.011
2019									
Ca	$0.398 \pm 0.044a$	$0.46\pm0.023a$	$0.366 \pm 0.027a$	$0.403 \pm 0.005a$	$0.408 \pm 0.023a$	$0.347 \pm 0.029a$	1.72	5, 20	0.175
Mg	$0.115 \pm 0.014ab$	$0.137 \pm 0.004a$	$0.113 \pm 0.006$ ab	$0.129 \pm 0.006$ ab	$0.114 \pm 0.004ab$	$0.096 \pm 0.009b$	2.79	5, 20	0.045
P	$0.157 \pm 0.011ab$	$0.111 \pm 0.004c$	$0.173 \pm 0.011a$	$0.162 \pm 0.009$ ab	$0.141 \pm 0.005$ abc	$0.127 \pm 0.011$ bc	5.31	5, 20	0.002
S	$0.215 \pm 0.015c$	$0.278 \pm 0.003$ ab	$0.266 \pm 0.008 abc$	$0.318 \pm 0.016a$	$0.25 \pm 0.011bc$	$0.263 \pm 0.021$ abc	5.56	5, 20	0.002
Al	$14.2 \pm 2.2a$	$30.2 \pm 10.5a$	$20.04 \pm 2.8a$	$23.2 \pm 4.6a$	$21.4 \pm 2.3a$	$17.8 \pm 1.7a$	1.37	5, 20	0.278
В	$3.8 \pm 0.6a$	$4.3 \pm 0.25a$	$4.3 \pm 0.25a$	$4.6 \pm 0.22a$	$3.7 \pm 3a$	$4.0\pm0.21a$	1.22	5, 20	0.335
Fe	$29 \pm 2.03b$	$57.8 \pm 8.52ab$	$104.1 \pm 48.05a$	$50.81 \pm 2.56ab$	$51.6 \pm 2.59ab$	$58.8 \pm 4.74a$	4.39	5, 20	0.007
Cu	$19.6 \pm 2.15c$	$38.1 \pm 4.38a$	$35.1 \pm 4.01a$	$20.8 \pm 0.88 bc$	$31.4 \pm 2.84ab$	$25.7 \pm 2.86 abc$	7.27	5, 20	0.0005
Mn	$37 \pm 3.61a$	$36.6 \pm 2.01a$	$49.6 \pm 2.46a$	$41.5 \pm 2.98a$	$35.1 \pm 2.95a$	$43.6 \pm 5.58a$	1.95	5, 20	0.13
Na	$379.5 \pm 37.5c$	$759.0 \pm 27.7a$	$557.2 \pm 43.1b$	$529.7 \pm 22b$	$364.2 \pm 17.1c$	$373.7 \pm 18.4c$	19.02	5, 20	< 0.001
Zn	$31.3 \pm 4.15b$	$50.9 \pm 4.65a$	$57.7 \pm 3.67a$	$51.8 \pm 3.65a$	$58.8 \pm 5.06a$	$44.5 \pm 3.06$ ab	6.11	5, 20	0.001

Means for Ca, Mg, P, and S are expressed as total percentage concentration (of dry weight), whereas Al, B, Na, Cu, Fe, Mn, and Zn are in ppm. Means in a row followed by different letters are significantly different (P < 0.05; LSD test). Analyses of variance were performed on the arcsine square root transformation for percentage data and log (x + 1) transformation for ppm data. Cr is only presented in 2018 whereas; Fe is presented only in 2019 data because below detectable levels. Other elements such as Ni, Mo, Pb, and Cd were below detectable levels, hence not presented.

Fig. 2.1. Mean ( $\pm$ SE) *S. frugiperda* larvae survived on various N and K ratio treatment (A) 2018 (B) 2019. Data points at each sample date with same letters are not significantly different (LSD test,  $\alpha = 0.05$ ).





Time after introduction (d)

Fig. 2.2. Mean ( $\pm$ SE) length of larvae (mm) and width of larval head capsule of *S*. frugiperda larvae survived on various N and K treatment at (A and B) 6 d, (C and D) 8 d, and (E and F) 10 d after introduction. Bars with the same letters are not significantly different (paired *t* test,  $\alpha = 0.05$ ). Bars without letters were not included in the analysis.

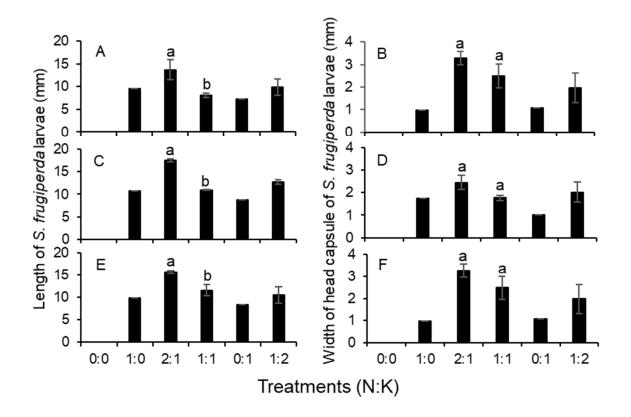


Fig. 2.3. Mean ( $\pm$ SE) length of larvae (mm), width of larval head capsule and larval weight (mg) of *S. frugiperda* larvae survived on various N and K treatment at (A, B and C, respectively) 8 d, (D, E and F) 10 d, (G, H and I) 12 d, (J, K and L) 14 d, and (K, M and N) 16 d after introduction. Bars with the same letters are not significantly different (LSD test,  $\alpha = 0.05$ ). Bars without letters were not included in the analysis.

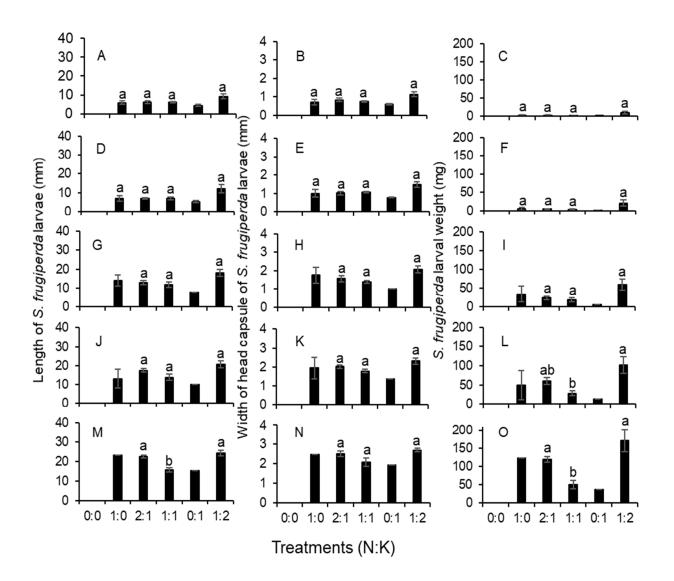
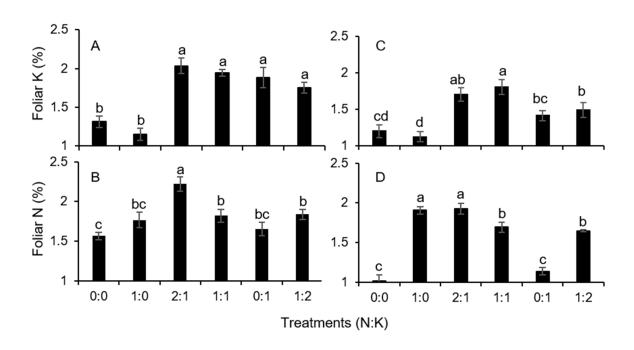


Fig. 2.4. Mean ( $\pm$ SE) percentage of (A) K and (B) N in 2018 and (C) K and (D) N in 2019 in the foliage when treated with various ratios of N and K after the conclusion of the experiment. Bars with the same letters are not significantly different (LSD test,  $\alpha = 0.05$ ).



# **CHAPTER 4**

# SCREENING FOR HOST PLANT RESISTANCE AGAINST FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) USING NEWLY DEVELOPED BERMUDAGRASS LINES<sup>1</sup>

<sup>1</sup>Gurjit Singh, Shimat V. Joseph and Brian Schwartz

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#### Abstract

The fall armyworm, Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae), is an important pest of warm season turfgrass species, including bermudagrass (*Cynodon* spp.). Bermudagrass continues to be a popular turfgrass genotype and is widely planted in golf courses, athletic grounds and ornamental landscapes across the country and throughout the world. Spodoptera frugiperda infestation is sporadic, but if it occurs, it can cause severe damage. Host plant resistance against S. frugiperda can be a valuable tool, as it can reduce or prevent the use of insecticides. Thus, the objective of this study was to quantify the level of resistance against S. frugiperda in selected elite bermudagrass lines. Fourteen bermudagrass lines plus two control cultivars, 'Zeon' zoysiagrass and 'TifTuf' bermudagrass, were evaluated against S. frugiperda to determine host plant resistance in the laboratory. The results show that the positive control, 'Zeon' zoysiagrass, was more resistant than the rest of the cultivars to S. frugiperda larvae. To understand the resistance of the lines relative to that of the controls, three indices were developed based on survival, development and overall resistance. According to the resistance index, '13-T-1032', 'T-822', '11-T-510', '12-T-192', '11-T-56', '09-T-31', '11-T-483', and '13-T-1067' were the top ranked bermudagrass lines. Among these, the resistances of '13-T-1032', 'T-822', '11-T-510', '11-T-56', '09-T-31' and '11-T-483' was comparable to that of 'TifTuf', and antibiosis was the underlying mechanism of resistance. Additionally, larval length, head capsule width and weight were negatively associated with the day of pupation and adult emergence as well as positively associated with pupal length, thorax width and weight.

**Keywords:** Spodoptera frugiperda, host plant resistance, turfgrass

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a sporadic but serious pest of various warm-season turfgrass species in the mid-southern and southeastern U.S. (Braman et al., 2000; Reinert and Engelke, 2010). In Georgia, the turfgrass industry is worth \$7.8 billion USD (Kane and Wolfe, 2012). This pest affects various sectors of the turfgrass industry. From July to late November, landscape maintenance companies and homeowners spend several thousands of dollars on insecticide sprays to protect residential and public lawns in urban and suburban areas. *Spodoptera frugiperda* also threatens golf courses and sod farms where insecticides are intensively used to maintain vast stretches of turfgrass. A typical sod farm spans more than 404,686 m², posing a considerable challenge in scouting operations. Currently, sod producers and golf course superintendents use pyrethroid insecticides, such as bifenthrin, throughout facilities starting in July.

The early larval stages of *S. frugiperda* usually go undetected, as they remain hidden within the turfgrass canopy during the daytime until the larvae reach the fourth or fifth instar. The young larvae feed on the grass blades, whereas the late instar larvae consume both the stems and grass blades. Severely affected turfgrass appears brown, as most of the grass blades are consumed. Compared to young instars, late instar larvae are tolerant to insecticides (Mink and Luttrell, 1989; Hardke et al., 2011).

S. frugiperda is a polyphagous pest and is known to damage more than 50 plant species, including corn, sorghum, cotton, rice and bermudagrass (Luginbill, 1928). Multiple overlapping generations of S. frugiperda occur within a year and infest several host species. Resistance to older insecticide formulations, such as carbamates, pyrethroids and organophosphates, has been reported in maize (Young, 1979; Carvalho et al., 2013; Diez-Rodriguez and Omoto, 2001; Okuma, 2015; Nascimento et al., 2016). Because most of the insecticides used in row crops, such

as corn, are also used in the turfgrass industry to control *S. frugiperda*, insecticide resistance by *S. frugiperda* in turfgrass is a real threat. This suggests that there is a dire need to develop alternate management options for *S. frugiperda* control in turfgrass.

The emphasis of turfgrass breeding programs has always been on improving horticultural attributes and tolerance to abiotic factors, such as drought and foot traffic (Patton et al., 2017; Baxter and Schwartz, 2018). Because insecticide resistance and nontarget effects of insecticides pose serious concern to the turfgrass industry, alternative control options, such as the development of S. frugiperda-resistant turfgrass, were recently incorporated into breeding programs (Reinert and Engelke, 2010). In pastures, S. frugiperda is a major pest of bermudagrass and critically affects grass quality and yield under severe infestations (Croughan and Quisenberry, 1989). Because extensive use of insecticides for pest management is still not an economically feasible option for pasture production, a major thrust was developing bermudagrass resistant to S. frugiperda. Previously, S. frugiperda-resistant 'Tifton 292' and 'Tifton 296' bermudagrass cultivars were developed for pasture production (Leuck et al., 1968), and antibiosis was identified as an underlying resistance mechanism (Lynch et al., 1983). In turfgrass, Braman et al. (2000) showed the zoysiagrass cultivars 'Cavalier', 'Emerald', 'DALZ8501', 'DALZ8508', 'Royal' and 'Palisades' exhibited high levels of antibiosis. Similarly, the zoysiagrass cultivars 'Cavalier', 'Emerald', and 'Belair' were found to be resistant to neonates of S. frugiperda (Reinert and Engelke, 2010). Clearly, there is a dearth of S. frugiperda-resistant bermudagrass cultivars available to the turfgrass industry. Thus, the objective of the current study was to test 14 promising bermudagrass lines for resistance against S. frugiperda and compare their performance to that of the standard bermudagrass 'TifTuf'.

These lines are considered "elite lines" because they underwent multiple years of field testing for horticultural attributes, such as drought tolerance and rapid growth, and resistance to foot traffic.

### **Materials and Methods**

## **Turfgrass and insects**

All the turfgrass lines were maintained in a greenhouse at the University of Georgia, Griffin Campus, Georgia, USA. The bermudagrass lines used in this study were '12-TG-101', '12-T-192', '13-T-1032', '11-T-483', 'KBUF-UF-1326', '97-45', '13-T-1067', 'T-822', 'B16-8', '11-T-510', 'T-789', 'FROST 1', '09-T-31', and '11-T-56'. These genotypes were used in multiple USDA grant experimental trials in the past. The line '12-TG-101' was previously evaluated in the golf courses as a putting-green surface. A bermudagrass cultivar ('TifTuf') and a zoysiagrass cultivar ('Zeon') were used as positive and negative controls, respectively. The turfgrasses were maintained at 25°C and 70% relative humidity in the greenhouse. There were 16 bermudagrass lines plus cultivars planted in  $7.8 \times 5.6 \times 5.9$  cm pots. The soil medium used was sand. The turfgrasses were irrigated every day and fertilized with 20:20:20 N:P:K at 5.9 g.L<sup>-1</sup> water (100 mL.pot<sup>-1</sup>) at weekly intervals. The grass was only mowed along the sides to prevent its growth in the nearby pots. The grass was never moved completely to ensure that there was enough material to feed the S. frugiperda larvae. The neonate larvae of S. frugiperda used in this study were purchased from Benzon Research Inc, (Carlisle, Pennsylvania, USA). Once the S. frugiperda larvae were received from the rearing facility, they were temporarily maintained at room temperature (21°C) and ~ 40% relative humidity in the laboratory. The S. frugiperda larvae were used in the experiment within 2 to 4 h after arrival.

## **Experimental procedure**

The 14 bermudagrass lines plus two standard cultivars were the treatments. The treatments were arranged in a completely randomized design. The experiment was conducted in 29.6 mL clear plastic containers (Frontier Agricultural Sciences, Newark, Delaware, USA, product #9051). One hour before introduction, grass clippings from mostly new grass blades were cut using a scissor from the treatment trays and transferred into clear plastic containers. The containers with grass clippings were placed inside a refrigerator for 2 to 3 h before introduction. Three 1<sup>st</sup> instar *S. frugiperda* larvae were introduced into each container with freshly cut grass clippings from the corresponding treatments. Every 2 d, 15 g of fresh turfgrass clippings was added to each container. The cups with larvae and grass clippings were maintained in environmentally controlled chambers at 28°C, ~ 40% relative humidity, and a 16:8 h (light: dark) light cycle.

This experiment was conducted in multiple groups. Ten replications from six to seven treatments were used at a time in each group, and these replications were repeated. The bermudagrass lines were evaluated 20 times, whereas the control cultivars were evaluated 60 times, because the control cultivars, 'TifTuf' and 'Zeon', were added to all the groups. The test of the first group was initiated on 2 Aug and repeated on 25 Sept 2019. The test of the second group was initiated on 28 Aug and repeated on 20 Oct 2019, and the test of the third group was initiated on 10 Dec 2019 and repeated on 8 Jan 2020. The same treatments were used in the tests of each group.

### **Evaluation**

Larval survival and development were recorded at 2-d intervals. To document larval development, larval length from the head to the tip of the abdomen, head capsule width, and larval weight were recorded. The larval length and head capsule width were measured using a

Vernier caliper. When there was more than one larva in a container, the measurements were averaged and recorded. Once those larvae were pupated, length, width (of the thoracic region), and weight were recorded at 2-d intervals. The pupation time and moth emergence were also noted when the evaluations were conducted at 2-d intervals.

To determine the performance of the bermudagrass lines relative to the controls, survival, development and overall resistance indices were developed. These indices were developed using the ranks of the means generated by the mean separation test (Tukey-Kramer test, P < 0.05). For the survival index, the means were ranked up to 14 d post-introduction, as most of the S. frugiperda were at the larval stage, whereas for the development index, the means generated by the mean separation test (Tukey-Kramer test, P < 0.05) for larval length, head capsule width and weight from 6 to 10 d were ranked. The means of the day of pupation and adult emergence were also incorporated into the development index. For the resistance index, the ranks based on the survival and development of the larvae as well as the day of pupation and adult emergence were pooled and analyzed. Further details of how the survival, developmental and resistance indices were developed are found in the statistical analysis subsection. A criterion was developed to compare and contrast the performance of the bermudagrass lines with the commercial standard, 'TifTuf'. The criterion was "High" if the line or cultivar was more resistant than 'TifTuf'; "Comparable" if either one of the survival or developmental ranks was  $\leq 5$ ; "Medium" if either one of the survival or developmental ranks was between 5 and 16; and "Low" if both the survival and developmental ranks were between 10 and 16. The assumption was that 'TifTuf' is ranked < 5 in all the indices.

## Statistical analysis

All the analyses were conducted using SAS software (SAS Institute 2012). To determine if the survival of S. frugiperda was affected when exposed to various bermudagrass lines, the number of live S. frugiperda larvae at 2, 6, 10 and 14 d after introduction was subjected to oneway analysis of variance (ANOVA) using the general linear model procedure (PROC GLM) in SAS after log-transformation (ln[x + 1]). After 14 d, S. frugiperda larvae started pupating in certain bermudagrass line treatments. The rate of larval development after consuming various bermudagrass lines was determined by calculating the change in larval length, head capsule width, and weight between 8 and 6 d, 10 and 8 d, and 12 and 10 d post-introduction. The data were log-transformed (ln[x + 1]), and one-way ANOVA was performed using the general linear model procedure (PROC GLM) in SAS. One-way ANOVA was also performed on larval length, head capsule width, and weight at 6 d to determine the effects of bermudagrass lines on S. frugiperda larval feeding. Similarly, the pupal and adult data, such as day of pupation and adult emergence, were subjected to one-way ANOVA using the general linear model procedure (PROC GLM) in SAS after log-transformation ( $\ln[x + 1]$ ). The means were separated using the Tukey-Kramer least square method ( $\alpha = 0.05$ ). The means and standard errors of the variables were calculated using the PROC MEANS procedure in SAS. Pearson's correlations between larval parameters, such as larval length, head capsule width and weight at 6, 8 and 10 d postintroduction, and pupal plus adult parameters, such as day of pupation, pupal length, thorax width and weight as well as day of adult emergence, were performed using the PROC CORR procedure in SAS. The mean larval length, head capsule width and weight as well as the day of pupation and adult emergence data were calculated for the correlation analysis. The mean pupal length, thorax width and weight were calculated after averaging the pupal parameters by treatment and replication across the observation dates.

Resistance to *S. frugiperda* could be quantified through mortality of young instars and delayed development of larvae when fed on various lines and control cultivars. To determine where the bermudagrass lines stand in relation to the positive control, "TifTuf', the survival and developmental parameters were ranked. To develop the survival index, the mean *S. frugiperda* larval survival data generated using the Tukey-Kramer test for the treatments (bermudagrass lines and controls) were ranked from 1 to 16, where 1 is the fewest survivors and 16 is the most survivors. The rank data were calculated at 2, 4, 6, 8, 10, 12 and 14 d post-introduction. The rank data were subjected to ANOVA using the general linear model procedure (PROC GLM) in SAS after log-transformation ( $\ln[x + 1]$ ), where the bermudagrass lines and controls and post-introduction dates were the treatment and replication, respectively. Using the mean ranks generated by the Tukey-Kramer test (survival index), the treatments were further ranked from 1 to 16, where 1 is the lowest mean rank and 16 is the highest mean rank.

A similar approach was adopted to determine the development and overall resistance indices. To determine the development index, the length, width and weight at 6, 8 and 10 d post-introduction as well as the days of pupation and adult emergence were individually ranked from 1 to 16, where 1 is the lowest value and 16 is the highest value. The rank data for each parameter was combined, log-transformed ( $\ln[x+1]$ ) and analyzed using the general linear model procedure (PROC GLM) in SAS. The treatments were further ordered by their rank means (separated by the Tukey-Kramer test) by assigning each a value from 1 to 16, where 1 is the lowest mean rank and 16 is the highest mean rank. To determine the overall resistance index, both survival and developmental rank data were combined and analyzed using the general linear model procedure (PROC GLM) in SAS after log-transformation ( $\ln[x+1]$ ). The rank means separated by the

Tukey-Kramer test were further ranked from 1 to 16, where 1 is the most resistant and 16 is the least resistant to *S. frugiperda* larvae.

### **Results**

### Larval survival

At 2 d post-introduction, the survival of *S. frugiperda* larvae in the 'Zeon' treatment was significantly lower than that in the '12-TG-101', '11-T-483, 'KBUF-UF-1326', 'T-789' and 'Frost 1' treatments ( $F_{15,325} = 2.7$ , P < 0.001; Fig. 3.1). At 6 d post-introduction, a significantly lower number of larvae survived in 'Zeon' than in the rest of the treatments except 'T-822' ( $F_{15,325} = 7.2$ , P < 0.001). At 10 d post-introduction, the number of larvae that survived in the 'Zeon' treatment was not significantly different from that in the 'T-822' and '11-T-483' treatments, although larval survival in 'Zeon' was significantly lower than that in the rest of the treatments ( $F_{15,325} = 6.2$ , P < 0.001; Fig. 3.1). At 14 d post-introduction, the number of larvae that survived in 'Zeon' was significantly lower than that in the rest of the treatments except 'T-822' and '11-T-483' ( $F_{15,325} = 8.1$ , P < 0.001).

## Larval development

At 6 d post-introduction, the length of the *S. frugiperda* larvae was significantly lower in 'Zeon' than in the rest of the treatments ( $F_{15,264} = 9.4$ , P < 0.001; Fig. 3.2A). The larval length was not significantly different between 'TifTuf' and the following treatments: '12-T-192', '13-T-1032', '11-T-483', '11-T-483', '97-45', '13-T-1067', '11-T-510', 'T-789', 'Frost 1', '09-T-31' and '11-T-56'. Similarly, there was no significant difference in the larval head capsule width between 'TifTuf' and the following treatments: '13-T-1032', 'Zeon', '11-T-510', 'T-789', 'Frost 1', and '11-T-56' ( $F_{15,264} = 11.4$ , P < 0.001; Fig. 3.2B). The larval weight was significantly lower in 'Zeon' than in the rest of the treatments except for '13-T-1032'; however, the larval

weight in 'TifTuf' was similar to that in '13-T-1032' and the rest of the treatments except for '12-TG-101' ( $F_{15,264} = 10.1$ , P < 0.001; Fig. 3.2C).

Hereafter, the rate of larval development was assessed at 2-d intervals, beginning at 6 d and for up to 10 d post-introduction, when the larvae started pupating in more than two treatments. From 6 to 8 d post-introduction, the change in larval length was not significantly different between the treatments ( $F_{15,263} = 1.5$ , P = 0.099; Fig. 3.3A). The change in head capsule width was significantly lower in the '12-T-192' treatment than in the '12-TG-101', '13-T-1032', 'KBUF-UF-1326', '97-45', '13-T-1067', 'Frost 1' and '11-T-56' treatments ( $F_{15,266} = 5.1, P < 10^{-5}$ 0.001; Fig. 3.3B). Compared to that in 'Zeon' and 'TifTuf', the head capsule width in the '12-T-192' treatment was not significantly different. The change in larval weight was significantly lower in 'Zeon' than in the rest of the treatments; however, there was no significant difference in larval weight between the 'TifTuf', '12-T-192', '13-T-1032', '11-T-483', 'T-822', '11-T-510', '09-T-31', and '11-T-56' treatments ( $F_{15,263} = 8.9$ , P < 0.001; Fig. 3.4A). Between 8 and 10 d post-introduction, a significantly lower change in larval length was observed between the '12-TG-101', '12-T-192', 'T-822', and 'Zeon' treatments than in the 'Frost 1' treatment, whereas other treatments ('13-T-1032', '11-T-483', 'KBUF-UF-1326', '97-45', '13-T-1067', 'B16-8', '11-T-510', 'T-789', '09-T-31' and '11-T-56) had similar changes in larval length as that in the 'TifTuf' treatment ( $F_{15,245} = 3.2$ , P < 0.001; Fig. 3.3C). For most of the treatments, the change in larval width was not significantly different from that in the 'TifTuf' treatment but was significantly higher than that in the '12-TG-101' treatment ( $F_{15, 233} = 3.2, P < 0.001$ ; Fig. 3.3D). The change in larval weight was significantly lower in the 'Zeon' treatment than in the 'KBUF-UF-1326', '97-45', 'B16-8', and 'Frost 1' treatments; however, none of the treatments was significantly different than 'TifTuf' ( $F_{15, 255} = 4.4$ , P < 0.001; Fig. 3.4B). Between 10 and 14 d

post-introduction, the change in larval length was significantly lower in the 'KBUF-UF-1326' treatment than in the '11-T-56' treatment ( $F_{15, 183} = 2.5$ , P = 0.002; Fig. 3.3E). There was no significant difference in the change in larval weight between 'TifTuf' and the rest of the treatments. The changes in both the larval head capsule width ( $F_{15, 231} = 0.9$ , P = 0.544; Fig. 3.3F) and weight ( $F_{15, 194} = 1.2$ , P = 0.244; Fig. 3.4C) were not significantly different from each other.

# **Pupal and adult parameters**

S. frugiperda larvae pupated significantly later in 'Zeon' than in the rest of the treatments  $(F_{15,253}=13.1, P<0.001; Fig. 3.5A)$ . The larvae in the '12-T-192', '13-T-1032', '11-T-510', '09-T-31', and '11-T-56' treatments took approximately similar times to pupate compared with the pupation time of the larvae in the 'TifTuf' treatment. Similarly, the emergence of adults was significantly more delayed in 'Zeon' than in the rest of the treatments  $(F_{15,228}=11.3, P<0.001; Fig. 3.5B)$ . The moth emergence time was not significantly different in the '12-T-192', '13-T-1032' and '11-T-510' treatments compared to that in the 'TifTuf' treatment.

Although there was no significant positive association between the larval length, width or weight and pupal length, width or weight at 6 d post-introduction, at 10 d post-introduction, both the larval length or weight and pupal length, width or weight had positive and significant associations (Table 3.1). At 12 d post-introduction, there were positive and significant associations between the larval and pupal widths and larval and pupal weights. At 6 and 8 d post-introduction, there were significant negative associations between the larval length, width or weight and days of pupation and adult emergence. At 10 d post-introduction, there was a significant negative association between the larval width or weight and days of pupation and adult emergence.

## **Resistance indices**

Based on the resistance index, the top ranked bermudagrass lines in this study were 13-T-1032', 'T-822', '11-T-510', '12-T-192', '11-T-56', '09-T-31', '11-T-483', and '13-T-1067' ( $F_{15}$ ,  $f_{255} = 33.5$ ,

### **Discussion**

The results show that the bermudagrass lines '13-T-1032', 'T-822', '11-T-510', '11-T-56', '09-T-31' and '11-T-483' were comparable to 'TifTuf' when resistance against *S. frugiperda* was evaluated in the laboratory. These lines were deemed comparable to 'TifTuf', eliciting resistance after carefully evaluating both the survival and development indices (Table 3.2). Currently, 'TifTuf' and 'Zeon' are standards and widely planted bermudagrass and zoysiagrass cultivars, respectively, across the U.S. (Schwartz, personal communication). Previous studies showed that zoysiagrass cultivars were resistant to young larvae of *S. frugiperda* (Braman et al. 2000, Reinert and Engelke, 2010). In the current study, 'Zeon' was the most resistant treatment, followed by 'TifTuf'. Because 'TifTuf' is the commercial standard, the emphasis of the current study was to determine where the bermudagrass lines stand relative to this cultivar. Based on the resistance index, which incorporated the ranks from both the survival and developmental data, the '13-T-1032', 'T-822', '11-T-510', '12-T-192', '11-T-56', '09-T-31', '11-T-483', and '13-T-1067' bermudagrass lines were among the top 10 treatments. When the most resistant lines (based on the resistance index) were compared with 'TifTuf', all the lines

elicited comparable resistance as that of 'TifTuf'. The bermudagrass lines tested in the current study were not bred specifically for *S. frugiperda* resistance but were previously tested for desirable horticultural traits. These *S. frugiperda* resistance results are important for the understanding of where these genetically novel lines stand relative to popular commercial cultivars and thus provide direction for future breeding research.

Based on the survival index, the bermudagrass lines 'T-822', '09-T-31', and '11-T-483' were within the top five treatments along with the 'TifTuf' and 'Zeon' cultivars. Because young larvae of the fall armyworm are vulnerable to food deprivation, they constantly consume grass tissue starting from the neonate stage. When fall armyworm moths invade a landscape, their egg masses are found on manmade structures adjacent to turfgrass so that emerging larvae have immediate and constant access to turfgrass tissues. In the current study, the survival of young larvae was affected when they were placed on certain bermudagrass lines, which suggests that these lines prevented access to the vital nutrients and moisture in the grass tissue. The physical characteristics of grass cells, such as thick cell walls, or biochemical compositions of the cells, such as secondary metabolites toxic to larvae, can interfere with the ability of young larvae to access these vital resources (Braman et al., 2000, 2002, Reinert and Engelke, 2010). Thus, the survival index can be an indicator of resistance or tolerance mechanisms, as evidenced by the survival of *S. frugiperda* exposed to certain bermudagrass lines.

The development of larval stages of *S. frugiperda* was affected when the larvae consumed certain bermudagrass lines. The results show that '13-T-1032', '11-T-56' and '11-T-510' were among the top five bermudagrass lines in addition to 'TifTuf' and 'Zeon' based on the development index (Table 3.2). The underlying mechanism for the delayed development is antibiosis, which has been reported previously for turfgrass genotypes and their cultivars

(Braman et al., 2000, 2002, Reinert and Engelke, 2010). In the current study, the *S. frugiperda* larvae developed more quickly on certain bermudagrass lines than on other resistant lines and cultivars (Figs. 3.3-3.5). This suggests that the biochemical composition of certain bermudagrass lines affects normal larval growth and development. The longer the *S. frugiperda* remains in the larval stages, the greater the chance of them succumbing to predation, pathogens or severe weather. Previous studies have shown that the days to pupation and days to adult emergence decrease with an increase in the susceptibility of turfgrass lines (Reinert and Engelke, 2010; Lynch et al., 1983).

The data show that the larval development parameters, such as length, head capsule width and weight, were negatively associated with the day of pupation or adult development. Additionally, the results show that the *S. frugiperda* larvae that fed on the resistant bermudagrass lines and cultivars spent more time pupating and emerging into adults than those that fed on the susceptible bermudagrass lines. This suggests that the *S. frugiperda* larvae that develop on relatively resistant bermudagrass lines tend to be smaller and lighter than the *S. frugiperda* larvae that develop on susceptible bermudagrass lines. Perhaps the fitness of the *S. frugiperda* adults developed on the resistant lines was compromised. More studies are warranted where parameters such as the longevity of adults, fecundity and viability of eggs are evaluated as secondary effects for *S. frugiperda* adults developed on resistant lines. The results also show that the larval length, head capsule width and weight are positively associated with the pupal length, thorax width and weight. This suggests that measurements of the length, thorax width and weight of *S. frugiperda* pupae can be used for evaluating resistance.

In summary, this study identified few resistant bermudagrass lines ('13-T-1032', 'T-822', '11-T-510', '11-T-56', '09-T-31' and '11-T-483') comparable to the bermudagrass 'TifTuf'.

Some lines resistant to neonates of *S. frugiperda*, whereas a few other lines lead to reduced development rates, potentially exposing the larvae to severe weather and predation. The results also show that resistance screening can be achieved by evaluating pupal parameters, which will be especially useful when breeders screen for *S. frugiperda* resistance on several lines at a time. Because the current study only focused on evaluating the resistance against *S. frugiperda* through antibiosis mechanisms, other mechanisms, such as antixenosis (nonpreference) and tolerance, were not evaluated and merit further investigation. Although this study identifies few promising lines, more studies are warranted to understand the consistency of their performance in field conditions, as host plant resistance continues to be a valuable tactic for managing *S. frugiperda* in turfgrass.

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Table 3.1. Pearson's correlation coefficients between larval and pupal or adult parameters of *S. frugiperda*.

Larval parameter	Pupal	Pupal	Pupal	Day of	Day of adult
	length	width	weight	pupation	emergence
6 d after introduction					
Larval length				-0.85***	-0.85***
Larval width				0.75***	-0.72**
Larval weight				-0.78***	-0.79***
10 d after					
introduction					
Larval length	0.50*	0.62*	0.51*	-0.94***	-0.92***
Larval width				-0.95***	-0.93***
Larval weight	0.60*	0.67**	0.58*	-0.92***	-0.91***
12 d after					
introduction					
Larval length					
Larval width		0.50*		-0.92***	-0.89***
Larval weight			$0.48^{a}$	-0.66**	-0.68**

The notations indicate significant correlations (P: a, 0.1; \*, 0.05; \*\*, 0.01; and \*\*\*, 0.001) between the larval and pupal or adult parameters

Table 3.2. Overall ranking based on the resistance index.

Overall rank <sup>a</sup>	Treatment <sup>b</sup>	Survival rank <sup>c</sup>	Development rank <sup>d</sup>	Resistance index <sup>e</sup>	Resistance status relative to 'TifTuf' f
1	'Zeon'	1	1	$1.0 \pm 0.0 \text{ g}$	High
2	'Tiftuf'	3	2	$3.2 \pm 0.4 \text{ f}$	-
3	'13-T-1032'	9	3	$5.4 \pm 1.0 \text{ ef}$	Comparable
4	'T-822'	2	9	$7.2 \pm 1.2 de$	Comparable
5	'11-T-510'	7	5	$6.6 \pm 0.5 \text{ c-e}$	Comparable
6	'12-T-192'	8	6	$6.9 \pm 0.6 \text{ c-e}$	Medium
7	'11-T-56'.	13	4	$7.5 \pm 0.9 \text{ c-e}$	Comparable
8	'09-T-31'	4	7	$7.7 \pm 0.8 \text{ b-e}$	Comparable
9	'11-T-483'	5	10	$8.3 \pm 0.7 \text{ b-d}$	Comparable
10	'13-T-1067'	6	12	$9.5 \pm 0.8 \text{ a-d}$	Medium
11	'T-789'	15	8	$10.1 \pm 0.7 \text{ abc}$	Medium
12	'Frost 1'	7	11	$10.0 \pm 0.6 \text{ abc}$	Medium
13	'97 <b>-</b> 45'	12	13	$11.3 \pm 0.5 \text{ ab}$	Low
14	'B16-8'	14	15	$13.0 \pm 0.5 a$	Low
15	'12-TG-101'	11	16	$13.6 \pm 0.9 a$	Low
16	'KBUF-UF-1326'	13	14	$14.1 \pm 0.4 a$	Low

<sup>&</sup>lt;sup>a</sup>Based on the mean value of the resistance index

<sup>&</sup>lt;sup>b</sup>Bermudagrass lines and control cultivars arranged by the overall rank

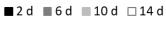
<sup>&</sup>lt;sup>c</sup>Ranks based on the survival index (mean survival ranks arranged in decreasing order)

<sup>&</sup>lt;sup>d</sup>Ranks based on the developmental index (mean developmental ranks arranged in decreasing order)

<sup>&</sup>lt;sup>e</sup>Ranks based on the resistance index (mean combined survival and developmental ranks arranged in decreasing order)

 $<sup>^{</sup>f}$ High = more resistant than 'TifTuf'; Comparable = either one of the survival or developmental rankings is  $\leq 5$ ; medium = either one of the survival or developmental rankings is between 5 and 16; and low = both the survival and developmental rankings are between 10 and 16

Fig. 3.1. Mean ( $\pm$ SE) number of *S. frugiperda* larvae that survived at 2, 6, 10 and 14 d after the introduction of the neonates at 28°C, ~40% relative humidity. Three neonates were initially released per cup. The treatment numbers on the x-axis refer to the following lines and cultivars: 1 = `12-TG-101'; 2 = `12-T-192'; 3 = `13-T-1032'; 4 = `11-T-483'; 5 = `KBUF-UF-1326'; 6 = `97-45'; 7 = `13-T-1067'; 8 = `T-822'; 9 = `Zeon'; 10 = `B16-8'; 11 = `TifTuf'; 12 = `11-T-510'; 13 = `T-789'; 14 = `Frost 1'; 15 = `09-T-31'; and 16 = `11-T-56'. The bars of the same shade with the same types of letters (i.e., upper or lower cases, in *italics* or not) are not significantly different (Tukey-Kramer test, P < 0.05).



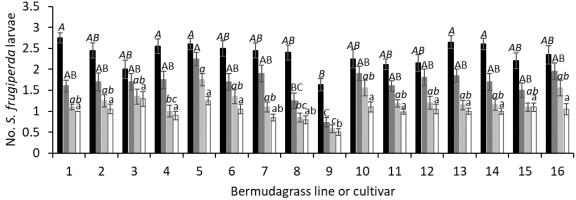


Fig. 3.2. Mean ( $\pm$ SE) *S. frugiperda* larval length (A), head capsule width (B), and weight (C) at 6 d. The treatment numbers on the x-axis refer to the following lines and cultivars: 1 = `12-TG-101'; 2 = `12-T-192'; 3 = `13-T-1032'; 4 = `11-T-483'; 5 = `KBUF-UF-1326'; 6 = `97-45'; 7 = `13-T-1067'; 8 = `T-822'; 9 = `Zeon'; 10 = `B16-8'; 11 = `TifTuf'; 12 = `11-T-510'; 13 = `T-789'; 14 = `Frost 1'; 15 = `09-T-31'; and 16 = `11-T-56'. The bars with the same letters are not significantly different (Tukey-Kramer test, P < 0.05).

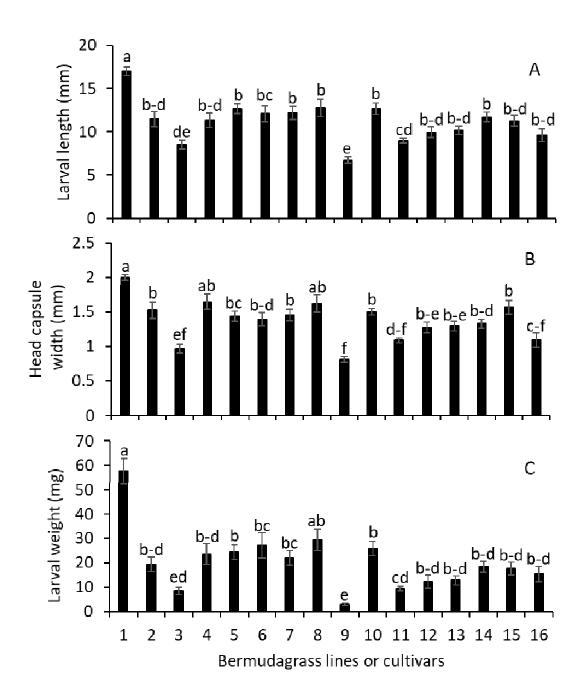
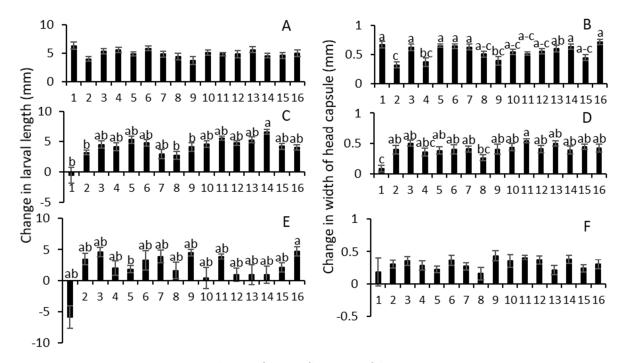


Fig. 3.3. Mean ( $\pm$ SE) change in *S. frugiperda* larval length and head capsule width between 8 and 6 d (A and B, respectively), 10 and 8 d (C and D, respectively), and 12 and 10 d (E and F, respectively). The treatment numbers on the x-axis refer to following lines and cultivars: 1 = `12-TG-101'; 2 = `12-T-192'; 3 = `13-T-1032'; 4 = `11-T-483'; 5 = `KBUF-UF-1326'; 6 = `97-45'; 7 = `13-T-1067'; 8 = `T-822'; 9 = `Zeon'; 10 = `B16-8'; 11 = `TifTuf'; 12 = `11-T-510'; 13 = `T-789'; 14 = `Frost 1'; 15 = `09-T-31'; and 16 = `11-T-56'. The bars with the same letters are not significantly different (Tukey-Kramer test, P < 0.05). Where no differences were observed, no letters are given.



Bermudagrass lines or cultivars

Fig. 3.4. Mean ( $\pm$ SE) change in *S. frugiperda* larval weight between 8 and 6 d (A), 10 and 8 d (B), and 12 and 10 d (E and F, respectively). The treatment numbers on the x-axis refer to the following lines and cultivars 1 = '12-TG-101'; 2 = '12-T-192'; 3 = '13-T-1032'; 4 = '11-T-483'; 5 = 'KBUF-UF-<math>1326'; 6 = '97-45'; 7 = '13-T-1067'; 8 = 'T-822'; 9 = 'Zeon'; 10 = 'B16-8'; 11 = 'TifTuf'; 12 = '11-T-510'; 13 = 'T-789'; 14 = 'Frost 1'; 15 = '09-T-31'; and 16 = '11-T-56'. The bars with the same letters are not significantly different (Tukey-Kramer test, P < 0.05). Where no differences were observed, no letters are given.

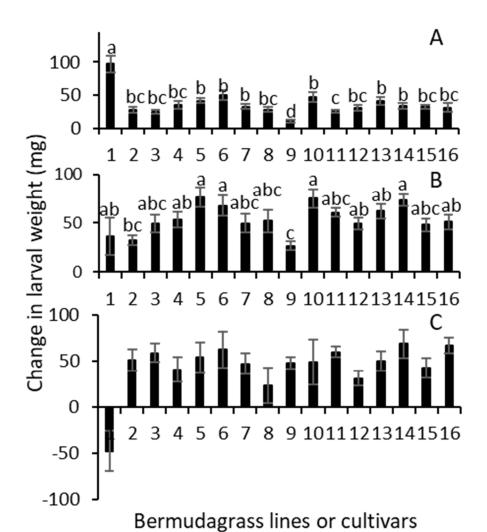
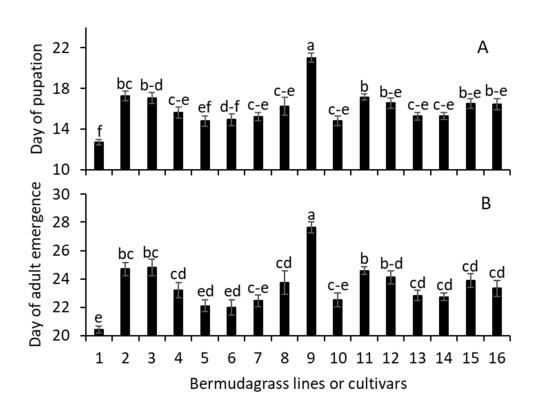


Fig. 3.5. Mean ( $\pm$ SE) *S. frugiperda* day of pupation (A) and day of adult emergence (B). The treatment numbers on the x-axis refer to the following lines and cultivars:  $1 = ^{1}2$ -TG-101';  $2 = ^{1}2$ -T-192';  $3 = ^{1}3$ -T-1032';  $4 = ^{1}1$ -T-483';  $5 = ^{1}$ KBUF-UF-1326';  $6 = ^{1}3$ -T-1067';  $8 = ^{1}3$ -T-822';  $9 = ^{1}3$ -T-1068';  $11 = ^{1}3$ -T-11067';  $12 = ^{1}3$ -T-789';  $14 = ^{1}3$ -T-781'; and  $16 = ^{1}3$ -T-786'. The bars with the same letters are not significantly different (Tukey-Kramer test, P < 0.05). Where no differences were observed, no letters are given.



### **CHAPTER 5**

## **SUMMARY**

The incidence and abundance of various arthropods were analyzed under the influence of turfgrass genotype, height, density, and thatch thickness at twenty sod field sites in central Georgia during May, June, and July 2019. Sweep nets, vacuums, and pitfalls were used for sampling. Results showed that pitfall traps captured 89% and 96% of all the predatory arthropods and parasitic Hymenoptera sampled during the study. The beneficial arthropods such as Anthocoridae, Araneae, and parasitic Hymenoptera were significantly greater in the May samples than in June and July. Herbivorous insects such as the seed bugs (Rhyparochromidae) and spittlebugs (Cercopidae) significantly increased with an increase in the grass height. The abundance of predatory arthropods did not vary with changes in thatch thickness, except formicids, where their numbers decreased with an increase in thatch thickness. Also, Curculionidae and Lepidoptera were significantly more abundant in the denser grasses. A positive correlation was found between the abundance of predatory arthropods such as Araneae, Carabidae, and Geocoridae and an increase in Delphacidae, Curculionidae, and Cicadellidae, respectively. These results suggest that beneficial arthropods are abundant in sod fields and their service can be incorporated to the IPM program for S. frugiperda in sod farms.

Effects of N and K were assessed for the survival and development of *S*.

frugiperda larvae on 'TifEagle' bermudagrass in the laboratory in 2018 and 2019. The fertilizers were applied in various ratios of N: K and the treatments were: 1) 0:0, 2) 1:0, 3) 2:1, 4) 1:1, 5) 0:1, and 6) 1:2 where 1 represents 113.4 g N or K per 92.3 m<sup>2</sup>. The grasses were treated with foliar applications of different treatments from May to October at the biweekly interval. After

five months of fertilization, survival and development of *S. frugiperda* were evaluated after introducing neonates of *S. frugiperda* in the laboratory conditions. In 2018, survival was significantly lower in 0:1, 1:2, 1:0, and 0:0 than in 2:1 and 1:1 treatment at 10 d and 24 d post-introduction, whereas in 2019, survival was significantly lower in 0:0 than in the rest of the treatments at 10 d and 24 d post introduction. The development of *S. frugiperda* larvae was significantly greater on 2:1 treatment as compared to 1:1 in both years. In 2018, the foliar K was similar in both the grasses fertilized with 2:1 and 1:1 treatment, whereas the K content was higher in 1:1 than in 2:1 treatment in 2019. Moreover, other micro-nutrients such as S, Na, and Zn were significantly greater in 2:1 than 1:1 treatment in 2018. However, in 2019 amounts of the same nutrients were found greater in 1:1 treatment than 2:1. These results suggest that higher levels of K in the bermudagrass can deter *S. frugiperda* activity. The tactic needs to be further evaluated in the field conditions.

Fourteen newly developed bermudagrass lines plus two control cultivars, 'Zeon' zoysiagrass and 'TifTuf' bermudagrass, were evaluated for resistance against *S. frugiperda* in the laboratory. The results showed that the positive control, 'Zeon' zoysiagrass, was more resistant than the rest of the cultivars to *S. frugiperda* larvae. Three indices were developed based on survival, development, and overall resistance to understand the resistance of the lines relative to that of the controls. Based on the larval survival and developmental rankings, the top lines compared with 'TifTuf' bermudagrass were '13-T-1032', 'T-822', '11-T-510', '11-T-56', '09-T-31' and '11-T-483'; the other line were categorized as medium and low. According to the resistance index, '13-T-1032', 'T-822', '11-T-510', '12-T-192', '11-T-56', '09-T-31', '11-T-483', and '13-T-1067' were the top-ranked bermudagrass lines. Among these, the resistances of '13-T-1032', 'T-822', '11-T-510', '11-T-56', '09-T-31', and '11-T-483' were comparable to that

of 'TifTuf', and antibiosis was the underlying mechanism of resistance. Promising lines further warrant field trials.

The results indicate that incidence and abundance of beneficial, herbivores, and decomposers arthropods in sod fields are influenced by factors associated with grass genotype, cultural practices, and environment. Our data suggest that N favors, but K discourages the growth and development of *S. frugiperda* larvae on bermudagrass. Optimized N and K applications could be developed as a tactic as part of the integrated pest management program for *S. frugiperda*. Also, results show that a few bermudagrass lines are resistant to *S. frugiperda* and can be developed as a valuable tool for *S. frugiperda* control.