## DEVELOPING INTEGRATED PEST MANAGEMENT STRATEGIES FOR HUNTING BILLBUG (COLEOPTERA: CURCULIONIDAE) IN SOD FARMS

by

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(Under the Direction of SHIMAT V. JOSEPH)

#### **ABSTRACT**

The billbugs, *Sphenophorus* spp. (Coleoptera: Curculionidae), are important pests attacking turfgrass. Evaluation of linear pitfall trap captures revealed *Sphenophorus venatus vestitus* as the major billbug species (> 98%) in the sod farms of central Georgia. Also, seasonal billbug captures were influenced by turfgrass phenology (e.g., early and late growth stage and fully grown turfgrass). The numbers of *Sphenophorus* spp. collected were significantly greater in the fully grown turfgrass than in the early- and late-growth stages. Adult billbugs were sampled from harvested and nonharvested areas of sod farms by using linear pitfall traps. A significantly greater number of billbug adults were captured from the nonharvested than from the harvested sod. *Sphenophorus* spp. adults actively emerged from the harvested and nonharvested sod areas. A series of laboratory, semi-field, and field assays were conducted to determine the influence of abiotic factors on the walking behavior of adult *S. venatus vestitus*. *S. venatus vestitus* males and females moved further when the temperature increased from 15 to 28 °C under laboratory and semi-field assays. The increase in temperature and relative humidity did not affect the distance moved by adults in the outdoor assay, but the increase in wind speed reduced the distance moved.

The spatial distribution patterns of *S. venatus vestitus* larvae and adults were analyzed at four sod farm sites in central Georgia using SADIE and variograms. Analyses revealed a significant aggregation pattern for adults, whereas aggregated distributions were detected for larvae with variogram analyses. The average ranges of spatial dependence for larval and adult samples were 3.9 m and 5.4 m, respectively. A survey was conducted to determine the major pests and current management practices in the commercial turfgrass industry. Most golf courses, and sod farms respondents identified fall armyworm, white grubs, and mole crickets as major pests than billbugs, chinch bugs, and others. Also, respondents use insecticides multiple times a year and indicated that management of the major pests is driven mainly by insecticide use.

INDEX WORDS: *Sphenophorus* spp., surface movement, temperature, spatial distribution, SADIE, variogram, survey, fall armyworm.

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#### **CHAPTER 1**

#### INTRODUCTION AND LITERATURE AND REVIEW

Billbugs, *Sphenophorus* spp. are weevils native to the U.S. (Dupuy and Ramirez 2016). They are serious pests of turfgrass, especially in sod farms in Georgia. Although over 60 native billbug species occur in the U.S., hunting billbug, Sphenophorus venatus vestitus Chittenden (Coleoptera: Curculionidae) is the most damaging and abundant species in warm-season turfgrass (Huang and Buss 2009). In Florida and the Carolinas, the hunting billbug is the dominant species (Huang and Buss 2009, Doskocil and Brandenburg 2012) with peak adult activity during late spring and from late summer to early fall (Doskocil and Brandenburg 2012, Chong 2015). This information on billbug species composition and distribution reported from the Carolinas and Florida was based on trap captures from golf courses with bermudagrass. The biology and diversity of billbug species that colonize the grass differ among turfgrass species (Huang 2008, Chong 2015). Therefore, the information obtained on billbug biology and damage from golf courses cannot be entirely applied to sod farm ecosystems. To develop an effective IPM strategy for billbug problems in Georgia sod farms, a clear understanding of the billbug species composition, phenology and seasonal abundance, spatial distribution, dispersal capabilities, and movement behavior are essential.

#### **Turfgrass system**

Turfgrass is defined as a regular stand of grass of different varieties managed at a low height and utilized mainly for recreational and functional purposes or to enhance human surroundings (Beard 1973, Potter and Braman 1991). The species of turfgrass grown in the U.S. are classified based on their ability to adapt to different climatic conditions (Beard 1973). The cool-season grasses, which include bluegrasses (*Poa* spp.), fescues (*Festuca* spp.), ryegrasses (*Lolium* spp.), and bentgrasses (*Agrostis* spp.), are the main species grown in the northern part of the US. Whereas Warm-season grasses, including bermudagrasses (*Cynodon dactylon* (L.) Pers.), zoysiagrass (*Zoysia* spp.), St. Augustinegrass (*Stenotaphrum secondatum* (Walter) Kuntze), bahiagrass (*Paspalum notatum* Flugge), and centipedegrass (*Eremochloa ophiuroides* (Munro) Hack) are grown in the southeast and the warm, semiarid zones of the South and southwest (Beard 1973, Potter and Braman 1991 and Hanna et al. 2013).

Turfgrass is an essential component in golf courses and general landscapes, such as residential and public lawns worldwide and especially in the eastern region of the U.S. Turfgrass production in the U.S. is valued at \$40-60 billion USD annually and covers approximately 20 million ha (Morris 2003). In Georgia, sod is produced on approximately 10,785 ha across 64 counties and is valued at \$118.3 million USD (Wolfe and Stubbs 2019).

The six essential components of turfgrass quality are uniformity, density, texture, growth habit, smoothness, and color. Turfgrass is grown primarily for its utility and appearance, and any discoloration is unacceptable in settings such as golf courses and sod farms (Dupuy and Ramirez 2016).

#### **Common arthropod pests of turfgrass**

Arthropod pests of turfgrass attack in multiple ways. Some feed upon roots or damage turfgrass through their burrowing activity, whereas others consume leaves and stems. Certain pests suck the plant juice leading to abnormal growth (Potter and Braman 1991). Common arthropod pests that feed on warm-season turf include mole crickets, *Neoscapteriscus vicinus* Scudder and *Scapteriscus borellii* Giglio—Tos (Potter and Braman 1991 and Vittum 2020), white grubs such as Japanese beetles, *Popillia japonica* Newman (Potter and Braman 1991), and hunting billbug (Gireesh and Joseph 2020), black cutworm, *Agrotis ipsilon* (Hufnagel) (Held and Potter 2012), fall armyworm, *Spodoptera frugiperda* JE Smith, several species of sod webworm (Lepidoptera: Pyralidae), southern chinch bug, *Blissus insularis* Barber, bermudagrass mite, *Eriophyes cynodoniensis* (Sayed) (Huang 2008), and rhodesgrass mealybug, *Antonina graminis* (Maskell) (Joseph and Hudson 2019).

In recent years, billbugs have increased in importance in turfgrass systems throughout the U.S., causing significant losses to sod farms, golf courses, and the landscape care industry maintaining residential, commercial, and public lawns (Dupuy and Ramirez 2016). Although over 60 native billbug species occur in the U.S. (Niemczyk and Shetlar 2000), about 10 species threaten the sustainability of turfgrass (Potter and Braman 1991, Vittum et al. 1999, Dupuy and Ramirez 2016). Of these, the bluegrass billbug, *Sphenophorus parvulus* Gyllenhal and the hunting billbug are most widely identified as serious pests of turfgrass. The bluegrass billbug primarily feeds on Kentucky bluegrass (*Poa pratensis* L.) and other cool season turfgrass varieties (Fry and Cloyd 2011). Hunting billbug is the most destructive billbug species in warm-season grasses (Potter and Braman 1991, Huang and Buss 2009). Billbug larvae cause

damage by feeding within the stems, roots, and the crowns of the turfgrass. This feeding causes severe discoloration of the turfgrass, resulting in eventual grass mortality (Potter and Braman 1991).

The other common arthropod pests of turfgrass that consume leaves and stems include cutworms, fall armyworms, sod webworms, and skippers (Lepidoptera: Hesperiidae) (Potter and Braman 1991). These pests feed mainly on grass blades, causing an uneven and patchy appearance to the turfgrass (Buss and Turner 2004). Feeding by the bermudagrass mite results in shortened stems and stolons, yellow and curly blades, and tufts of grass plants (Short and Buss 2005). Southern chinch bug is an important pest of turfgrass that feed by sucking plant juices. The aggregations of nymphs and adults suck sap from stems and crowns resulting in localized injury that may combine into expanded patches of dead and dying turf (Potter and Braman 1991).

#### **Billbugs**

Billbugs are both stem boring and root-feeding turfgrass insects (Potter 1998). These are a complex of weevils that feeds primarily on grass and reduces the aesthetic and functional qualities of turfgrass. The *Sphenophorus* genus comprises 71 species, with 50 species found in the U.S. sand Canada (Dupuy 2016).

The adult billbug is sturdy, with sclerotized forewing covering the thorax and abdominal segments. It has a long beak-like snout with chewing mouthparts at the tip of the snout (Dupuy and Ramirez 2016). They are commonly known as billbugs due to this long beak-like snout. The snout is at least half the length of the pronotum. The antennae are clubbed and

elbowed (geniculate) with a long scape inserted at the proximal end of the snout. Adults are black or dull red or brown (Reynolds 2013) and often appear in lighter color shades (Niemczyk and Shetlar 2000). Billbug species can be differentiated from one another with the help of patterns on the pronotum and markings on the elytra, color, and relative size (Shetlar et al. 2012). Billbug species are distinguished from other related genera by the shape of the antennal club, the relative separation of the coxae, the shape of the mesoepimeron, metaepimeron, and intercoxal processes, the number of segments on the claw, and the quantity and arrangement of setae on the underside of the third tarsal segment (Vaurie 1951). Duffy et al. (2018) utilized molecular tools to identify billbug larval species. However, no morphological keys are available to distinguish billbug larvae easily. The billbug species is typically determined using morphological characters of adults (Johnson-Cicalese et al. 1990).

#### Distribution and host range

Billbugs are distributed throughout the continental U.S. and in Hawaii. Earlier it was thought that the bluegrass billbug and hunting billbug were the only species that damage turfgrass, but later it was observed that different species of billbugs dominate other parts of the country (Johnson Cicalese et al. 1990, Dupuy and Ramirez 2016). While hunting billbug dominates the southeastern U.S., bluegrass billbug dominates in the northern half of the country. Phoenix billbug, *S. phoeniciensis* Chittenden and the rocky mountain billbug, *S. cicatristriatus* Fabraeus dominate the southwestern U.S. and rocky mountain region. Overall, 10 species of billbugs attack turfgrass in the U.S., but detailed biological observations were limited only to bluegrass billbug and hunting billbug (Held and potter 2012, Dupuy and Ramirez 2016).

The normal host range of bluegrass billbug include certain cool-season grasses, especially Kentucky bluegrass (*Poa pratensis* L.) but may also feed on certain warm-season grasses like zoysiagrass and nonturf, such as orchardgrass (*Dactylis glomerata* L.), corn (*Zea mays* L.), wheat (*Triticum* spp.) (Dupuy and Ramirez 2016, Vittum 2020). Hunting billbug primarily feeds on warm-season grasses, especially zoysiagrass and bermudagrass. Additional hosts include certain cool-season turfgrass and grassy weeds. However, rocky mountain billbug, uneven billbug, *S. inaequalis* Say, and the lesser billbug, *S. minimus* Hart feed mainly on cool-season turfgrasses, such as tall fescue, perennial ryegrass, and Kentucky bluegrass. The host range of phoenix billbug includes mainly warm-season turfgrasses such as bermudagrass, zoysiagrass, and certain nonturfgrass hosts, including Johnsongrass [*Sorghum halepense* (L.)] and oats (*Avena sativa* L.).

#### **Hunting billbug**

The hunting billbug, also known as the "zoysia billbug," is a weevil native to the southeastern U.S. (Kuhn et al. 2019). The common name comes from the translation of *venatus*, meaning "the chase or hunting" (Vaurie 1951). In the U.S., the hunting billbug ranges from Washington, DC., to Florida and as far west as New Mexico and southeastern Kansas (Dupuy and Ramirez 2016). The hunting billbug was first reported as a serious pest of zoysiagrass sod in Florida (Doskocil 2012). Gireesh and Joseph (2020) reported hunting billbug as an important turfgrass pest in Georgia sod farms. Although hunting billbugs attack all major turfgrass genotypes, damage on zoysiagrass cultivars can be particularly serious. The nonturf host range of hunting billbug includes timothy hay (*Phleum pratense* L.), wheat, yellow nutsedge (*Cyperus esculentus* L.), corn, and sugarcane (*Saccharum officinarum* L.) (Satterthwait 1931, Satterthwait 1932,

Woodruff 1966, Kuhn et al. 2019). Hunting billbug infestations in turfgrass are not easily detected until the first signs of feeding damage, such as discoloration or irregular dead patchesscattered across the turfgrass, are found.

#### **Biology**

Because hunting billbug has emerged as a serious pest of warm-season turfgrass in recent years, understanding its biology and ecology has become a very critical need in turfgrass systems.

(Doskocil and Brandenberg 2012). Hunting billbugs are usually observed on actively growing turfgrasses. Adult hunting billbugs mostly move around by crawling rather than flying. The adults are nocturnal (Dupuy and Ramirez 2016). Adults emerge from overwintering sites on warmer days in the spring. The females prefer turfgrasses with thick stems for oviposition or actively growing grasses (Dupuy and Ramirez 2016). The females lay creamy-white, beanshaped eggs inside holes they have chewed into the base of the grass stem (Doskocil 2010). Eggs are inserted singly into the feeding holes on the stem or stolon. The eggs hatch between 3 and 10 days, and the larvae undergo five larval instars. The first larval stage is about 1.33 mm in length, whereas the later instars are 6-10 mm long. The first instar larvae feed within stems, but as their size increases (2<sup>nd</sup> instar) they no longer fit inside the stem and drop into the soil (Huang and Buss 2009). The older instars (3<sup>rd</sup> to 5<sup>th</sup> instars) feed on roots and pupate in chambers about 2 to 5 cm deep (Brandenburg and Villani 1995). The hunting billbug larval stages generally take 21-35 days to become pupae. Pupae are initially cream-colored, but they gradually change into reddish-brown with time. For hunting billbug, the time required to develop from egg to adult varies in bermudagrass and zoysiagrass (Huang and Buss 2009). On

bermudagrass, it takes nine weeks at ~25 °C whereas it takes about one week less in zoysiagrass at ~27 °C (Huang and Buss 2009). Billbugs mostly overwinter as adults in protected areas, although larvae are also found during winter months (Dupuy and Ramirez 2016). Larval feeding causes economic damage, whereas adult feeding causes superficial damage to grass blades. However, reports from North Carolina showed that adult hunting billbug causes severe damage to warm-season turfgrass (Doskocil and Brandenburg 2012). Two overlapping generations of hunting billbug have been reported from the Carolinas, whereas up to six generations are reported from Florida (Doskocil and Brandenburg 2012).

#### **Damage**

Billbug feeding damage is often misdiagnosed as drought or disease problems (Huang and Buss 2009). On zoysiagrass, billbug damage is noticed in the spring as the turfgrass struggles to grow out of winter dormancy. On established turfgrass (e.g., golf courses), the feeding damage appears as a brown or discolored patch. Because smaller larval instars feed within the stem, the infested stems are filled with yellow sawdust-like frass near the root zone. The second and older instars feed on the roots and stolons of the turfgrass. The adult and larval feeding injury on roots and oviposition injury cause economic damage (Doskocil and Brandenburg 2012). Larval feeding injury initially appears as yellowing of turfgrass; then later, the yellowing expands to a larger area, gradually turning into a distinct brown patch causing grass death. The dead turfgrass patch comes off easily with a gentle pull (Huang and Buss 2009).

In sod farms, machine-harvest of billbug-infested sod is challenging because of sustained feeding injury to the roots and rhizomes. The harvested sod slab does not hold

together or falls apart while harvesting. This inability to harvest is a severe problem causing by billbugs in sod production in Georgia. The domestic and international demand for zoysiagrass [Zoysia matrella (L.) Merrill and Zoysia japonica Steudel] has increased dramatically (Patton 2009). As much as 6593.5 ha were planted with zoysiagrass on golf courses in the U.S. in 2006 (Lyman et al. 2007), and the demand is consistently increasing in the southeastern and south-central U.S. as more golf courses and residential lawns have been established with or converted to this species. In sod farms, the damage potential of billbugs on zoysiagrass is magnified because zoysiagrass is a slow-growing species that does not recover well from billbug damage. The damage to turfgrass by billbugs differs between golf turf and sod fields. Moreover, the biology and diversity of billbugs differ among turfgrass species (Huang 2008, Chong 2015). As a result, billbug biology and damage to golf courses cannot be directly applied to sod farms.

Although the hunting billbug attacks bermudagrass and zoysiagrass alike, the damage is more serious in zoysiagrass. The damage to bermudagrass is observed, especially when the grass is under abiotic or disease stress. Usually, bermudagrass can tolerate billbug damage as it rapidly grows and repairs the injury (Young 2002). In North Carolina, the most severe damage to warm-season turfgrasses often occurs during late spring and early fall in the same areas every year.

#### **Dispersal**

The movement of an organism is an essential component of ecological and evolutionary processes that determine pest dispersal and establishment (Bykova and Blatt 2018). Long-distance movement is usually linked to the pest colonization rate. Movement within a field or

between small-scale habitat patches is related to pest reproductive success and the ability to consume resources (Dingle 1996) effectively. Short-distance movement is defined by four mechanical elements for most organisms: internal state, motion capacity, navigation, and external factors. The external factors involve biotic interactions and environmental variables, landscape characteristics, and soil properties (Dingle 1996). The physical and structural components of the environment play an important role in modifying insect movement, and also affect population dynamics, evolution, and distribution (Grez and Villagran 2000). Bykova and Blatt (2018) examined the effect of soil type on carrot weevil, *Listronotus oregonensis* (LeConte), and showed that soil type influences carrot weevil burrowing activity and movement. They discovered that carrot weevil is more inclined to burrow into mineral and organic soils than pure sand. Corneil and Wilson (2017) examined how light and temperature affect adult pales weevil behavior, and they observed that the weevils remain at the base of their host trees during the day and move onto the trees after dark. The distribution of billbugs in sod farms is poorly understood, even though spatial distribution has been studied on golf courses for annual bluegrass weevil, Listronotus maculicollis Kirby, where adults and larvae tend to aggregate along the edge of fairways (McGraw and Koppenhöfer 2010). Insects that primarily establish in certain areas can remain aggregated or spread to a broader area from initial colonization. This type of information on hunting billbug is not available but will be valuable to develop effective IPM tactics. Ultimately, science-based action thresholds and reliable sampling plans to guide decision-making for billbug management should be prepared for southeastern sod farms. Many insect sampling plans were developed based on the models using the mean-variance relationship (Taylor 1984, Young and Young 1990, Kuno 1991) without considering the actual spatial distribution pattern of the pest population. Sampling

plans that fail to account for the spatial distribution patterns of pest populations in the field can produce unreliable infestation assessments and lead to incorrect treatment decisions. Besides, non-spatial techniques cannot quantify and develop the distribution-based density maps that provide a visual representation of the infestation and can be useful in site-specific pest management.

#### Management

Billbugs are particularly difficult to manage due to the differences in susceptibility of life stages to management options (Dupuy and Ramirez 2016). Managing billbug populations is challenging as the larvae are hidden within leaf sheaths and stems of the host plant (Potter 1998). Also, larval stages display varying levels of susceptibility to insecticides or management approaches. Tashiro and Personius (1970) set a 15 to 25 billbug adults threshold, counted on paved surfaces in one minute by one person, for treatment. Pitfall traps have been used to estimate adult billbug densities (Johnson-Cicalese et al. 1990, Young 2002, Huang and Buss 2009). Monitoring traps several times a week and counting the collected adults can indicate when management strategies are needed (Potter 1998). For optimal management, turfgrass managers must first have a sound understanding of billbug seasonal activity and biology.

Limited research has been conducted looking at traditional cultural management tactics for billbugs such as irrigation, mowing height, and fertilization. One study reported severe injury on Kentucky bluegrass from billbugs when grass was maintained at high mowing height and under low nitrogen levels (Bishop et al. 1981). Resistant turfgrass varieties provide a non-chemical and economical method of long-term billbug management paired with other IPM

strategies (Dupuy and Ramirez 2016). Research showed that warm-season grasses *Z. matrello* 'Diamond', 'Zorro', 'Cavalier', and 'Royal' and bermudagrass 'TifEagle' were resistant to hunting billbug feeding damage (Huang and Buss 2013). Billbug damage is worst in turfgrass under stress, such as drought conditions or poor fertility (Shetlar et al. 2012). Light to a moderate infestation of turfgrass by billbugs can be managed by proper irrigation and fertilization (Watschke et al. 2013, Dupuy and Ramirez 2016).

#### **Biological control**

Even though diverse predatory arthropods have been described in turfgrass, the ability of these predators to suppress the billbug population has not been well documented (Dupuy and Ramirez 2016). A few parasitoids, nematodes, and fungal pathogens have been associated with various billbug species. The presence of *Neotyphodium* spp., an endophytic fungus in fescue and ryegrass, has been associated with resistance to many insects, including billbugs (Doskocil and Brandenburg 2012). These endophytic fungi live within the plant tissue and release toxins that attack insects and other herbivores feeding on the colonized plants (Johnson-Cicalese and White 1990).

Dupuy and Ramirez (2019) observed that the predator exposure reduced overall billbug activity by 56%, and for hunting billbugs, specifically, reduced mating by 28%. They found that the predatory arthropod community consisted mainly of carabids and spiders, representing 60% and 28% of all predators, respectively. This study sheds light on the need for more biological control strategies to effectively reduce the billbug population and other existing IPM techniques.

Another potential biological control for billbug larvae is entomopathogenic nematodes (Georgis et al. 2006, Dupuy and Ramirez 2016). Three species of nematodes that have been used against billbugs are *Steinernema carpocapsae* (Weiser) *S. feltae* (Filipjev), and *Heterorhabditis bacteriophora* Poinar (Niemczyk and Shetlar 2000). These authors suggested that treatments using nematodes were most effective when the larvae inhabited the crowns of the plants. Although nematodes are effective against billbug larvae and turfgrass is ideal for using nematodes, insecticides remain a cheaper option for management than nematodes, particularly on a large hectarage in sod farms or golf courses.

#### **Chemical control**

Demand for high-quality turfgrass has been accompanied by growing public concern about the negative impacts of insecticides, especially groundwater contamination and potential risks to human health (Potter and Braman 1985). Insecticides have always been the primary tool for reducing subterranean insect pests in turfgrass systems (Shetlar 2003). Even though cultural and biological control options exist, the use of insecticides remains an effective control option for billbugs. Most work assessing insecticide efficacy against billbugs has been tried in coolseason turfgrass. Reynolds and Bradenburg (2015) evaluated the effectiveness of conventional insecticides against hunting billbug larvae and adults in warm-season turfgrass. The authors observed that bifenthrin, clothianidin, cyantraniliprole, and a combination of bifenthrin and clothianidin had > 80% efficacy against adults. In contrast, imidacloprid was the most effective insecticide against larval stages, with approximately 33.6% mortality (Dupuy and Ramirez 2016). The low mortality of billbug larvae is attributed to insufficient exposure on larvae deep in the soil.

Accurate information on an insect's biology and seasonal activity is critical to maximizing the efficacy of insecticides (Doskocil and Brandenburg 2012). Insecticide applications are recommended in the spring as a preventative approach to control adults in areas with consistent billbug pressure (Shetlar 1995). Larval control can be difficult because they spend most of their time within turfgrass stems and are sheltered from contact insecticides, such as pyrethroids. The pyrethroid insecticides can target emerging adults from overwintering sites in spring. Systemic insecticides, such as imidacloprid and chlorantraniliprole, are effective against larval stages of billbug, even those developing inside the stems of turfgrass. If applied before the egg hatch, these preventative insecticides can affect the early stages of billbug larvae.

#### **Rationale**

As insecticides are primarily used for billbug control, insecticide resistance and the negative impact of insecticides on the environment, people, and other nontarget organisms are real concerns. There is limited information on the seasonal occurrence, abundance, species diversity, dispersal, and within-field distribution, associated with incidence and abundance of billbugs. This information on this pest is critical in developing an effective integrated pest management program. Moreover, most of the previous research on billbugs has been conducted only on golf courses. Billbug biology and management approaches adopted in the golf courses may not be the same in the sod farms. Therefore, the projects outlined in the dissertation are conducted to improve our understanding of abundant billbug species and their seasonal occurrence, dispersal behavior and movement capacity, and within-field distribution of billbugs in Georgia sod farms.

#### **Research Projects**

**Project 1**: Seasonal occurrence and abundance of billbugs in Georgia sod farms.

The objective of the study was to determine the occurrence and seasonal abundance of billbugs relative to the growth stages of turfgrass in sod farms. The anticipation is that the adult billbug populations be higher in the fully grown turfgrass than in younger growing stages.

**Project 2**: Surface movement of billbugs in harvested and nonharvested sod.

Gireesh and Joseph (2020) showed that adult billbugs in sod farms were more abundant in fully grown turfgrass than more juvenile growing stages. At the fully-grown stage, the billbug population size may be large enough to be effectively managed using insecticide sprays. This indicates that billbug management could be improved if the movement behavior of billbugs is understood when sod is harvested in the field and the turfgrass begins to grow. The first objective of this study was to determine the movement activity of billbugs in harvested and nonharvested areas of sod fields, and the second was to document if the billbugs adults still emerge from the harvested areas of the sod. This information can be utilized to develop an IPM program for billbugs as harvested areas are not considered a potential threat for billbug infestations in sod farms.

Project 3: Influence of abiotic factors on walking behavior of hunting billbugs

Adult hunting billbug is a nocturnal insect and is only reported to move by walking (Dupuy and Ramirez 2016). Gireesh and Joseph (2021) showed that adult hunting billbugs moved to and from harvested and nonharvested areas in a sod field. This indicates that adults emerging from

the nonharvested, fully grown areas of sod could reinfest the newly developing grass stems in harvested areas. However, the walking behavior of adult hunting billbug is still not well documented. Understanding the walking behavior of hunting billbugs and the factors that influence their movement will immensely improve IPM strategies by reducing insecticide use. Thus, the objective of the project was to determine the influence of abiotic factors such as temperature, relative humidity, and wind speed on the walking capacity of males and females of adult hunting billbug.

#### **Project 4**: Spatial distribution of hunting billbugs in sod farms

The hunting billbug is a major and damaging insect pest species of sod farms in Georgia.

Hunting billbugs are usually managed using insecticides. An improved sampling plan for larvae and adults is warranted to improve management decisions. Understanding how hunting billbug is spatially distributed within sod fields will help develop an effective sampling strategy.

Developing sampling plans using mean and variance-based models only utilize the frequency distributions of pest counts without considering the spatial locations of the pest population samples. Therefore, the objective of the project was to determine the spatial distributions of hunting billbug larvae and adults in sod farms using geospatial techniques such as variograms and SADIE.

**Project 5**: A survey on major insect pests and management practices adopted by the commercial turfgrass industry in Georgia

Many herbivorous arthropod pests can invade turfgrass. However, it is not clear if all the pests invade all the turfgrass systems alike. Moreover, the current management practices adopted for

major pest species problems can vary by turfgrass type and facility. Most of the arthropod pests are managed using insecticides, and some insecticides, if used inconsistent with the label, can affect nontargets such as predators, parasitoids, and pollinators. The major objective of this survey was to determine the major pests and management approaches adopted by various turfgrass systems in Georgia. As there is an increased demand for quality turfgrass, the demand for an improved IPM program is at an all-time high. The information generated on major pests and current management practices in various turfgrass systems will shape the focus and allocation of resources.

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### **CHAPTER 2**

# SEASONAL OCCURRENCE AND ABUNDANCE OF BILLBUGS (COLEOPTERA: CURCULIONIDAE) IN GEORGIA SOD FARMS

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**ABSTRACT** The billbug, *Sphenophorus* spp. (Coleoptera: Curculionidae), is an important pest complex in sod farms in Georgia. Larval feeding within stolons and on roots affects spring recovery of slow-growing zoysiagrass and poses a serious challenge to machine harvesting, as the damaged turfgrass rarely holds together. Little is known about major billbug species and their seasonal occurrence and abundance in Georgia sod farms, as most previous research was conducted in golf courses in the region. In 2018 and 2019, adult billbugs were sampled from five zoysiagrass sod field sites in central Georgia. Four linear pitfall traps were used per site from February to December each year, and the traps were checked at weekly intervals. The data show that > 98% of the sampled billbugs were the hunting billbug, Sphenophorus venatus vestitus Chittenden, whereas the nutgrass billbug, S. cariosus Olivier; uneven billbug, S. inaequalis Say; and vegetable weevil, Listroderes difficilis Germain were the minor species. Seasonal billbug capture was influenced by turfgrass phenology (e.g., early-growth-stage, late-growth-stage or fully grown turfgrass). The numbers of *Sphenophorus* spp. collected were significantly greater in the fully grown turfgrass than in the early- or late-growth-stage turfgrasses. Significantly greater densities of billbug were found in Zoysia matrella (L.) Merrill ('Zeon') and the Z. matrella × Z. pacifica (Goudswaard) M. Hotta & S. Kuroki hybrid ('Emerald') than in the Z. japonica (Steudel) cultivars 'El Toro' and 'Zenith'. Similar numbers of male and female billbugs were collected from the sod field sites.

Keywords Sphenophorus spp., turfgrass, growth stages, zoysiagrass

Turfgrass is an important component in golf courses and general landscapes such as residential and public lawns worldwide and especially in the eastern region of the U.S. In Georgia, the turfgrass industry is estimated to be worth \$7.8 billion USD (Kane and Wolfe 2012). Sod farms in Georgia span ~ 11331.2 ha across 61 counties and are valued at \$1.12 billion USD (Farm Gate Value Report 2018). The billbug pest complex, *Sphenophorus* spp. (Coleoptera: Curculionidae), has increased in importance in turfgrass systems throughout the U.S. in recent years, causing significant losses to sod farms, golf courses, and the landscape care industry, maintaining residential, commercial and public lawns (Dupuy and Ramirez 2016). Although over 60 native billbug species occur in the U.S. (Niemczyk and Shetlar 2000), approximately 10 species threaten the sustainability of turfgrass (Potter and Braman 1991, Vittum et al. 1999, Dupuy and Ramirez 2016). In warm-season grasses, the hunting billbug, *Sphenophorus venatus vestitus* Chittenden, is the most destructive species (Potter and Braman 1991, Huang and Buss 2009).

Previous studies and reports from the Carolinas, Florida and elsewhere have allowed turf scientists and pest managers to develop a picture of the general biology of billbugs (Kelsheimer 1956, Niemczyk 1983, Vittum et al. 1999, Shetlar 2003, Huang 2008, Doskocil and Brandenburg 2012, Reynolds 2014). Adult female billbugs oviposit eggs inside the stolons (stem) of turfgrass. The emerging first instars feed within the internodal space of the stolons. When they molt into second instars, they break out of the stolons because of the inadequate space within the stolons and drop into the soil. Thereafter, they molt through four more instars before becoming pupae. The pupal stage occurs in the soil.

The domestic and international demands for zoysiagrass [Zoysia matrella (L.) Merrill and Zoysia japonica Steudel] have increased dramatically (Patton 2009). Zoysiagrass was planted on as much as 6593.5 ha on golf courses in the U.S. in 2006 (Lyman et al. 2007), and the demand is

consistently increasing in the southeastern and south-central U.S., as more golf courses and residential lawns have been established with or converted to this species (Patton et al. 2017). On sod farms, the damage potential of billbugs on zoysiagrass is magnified because zoysiagrass is slow-growing and does not recover well once adult and larval billbugs have caused damage. Additionally, the damage to turfgrass by billbugs differs between golf turf and sod fields. Whereas billbugs cause patches of dead grass on golf courses, sods infested by billbugs struggle to grow and hold together when machine-harvested, rendering the harvested but broken slabs unsalable. Because the generalized understanding of billbugs was built on information gathered from golf courses planted largely with bermudagrass, this information may not be wholly applicable to turf production, particularly zoysiagrass production. Moreover, the biology and diversity of billbugs clearly differ among turfgrass species (Huang 2008, Chong 2015), and as a result, the information on billbug biology and damage gathered from golf courses cannot be entirely applied to sod farms.

Billbugs can spread to other non-infested regions or around the country through the movement of infested sods (Tashiro 1987). The domestic market is critical for sod producers in Georgia. Additionally, some Georgia growers export sod to other countries. All sods undergo rigorous phytosanitary inspection before shipment to European, Middle Eastern, Asian and South American countries and Australia, which have zero-tolerance policies on billbug larvae-infested sods (J. Arrington, GA Dept. of Agriculture, personal communication). Because hunting billbug eggs are deposited inside stolons (stems), the emerging first instars feed within the stolons and remain undetected until the larvae molt into the second instar and break out of the stolons. The sod leaves the U.S. with no detectable billbug larvae, but the larvae become visible at the destination, which leads to the rejection of the sod. Rejection has become more common and

seriously affects some sod producers in Georgia. Currently, growers sprig their sod and repeatedly treat it with insecticides to control young billbug larvae. This process provides reasonable control but not enough to meet the export standards. Thus, an effective and timely field-level management program is necessary.

Knowledge of seasonal occurrence, abundance and species diversity is critical to developing IPM strategies, such as sampling plans or management tactics, for billbugs in sod farms. To date, management approaches have been adopted from previous research on golf courses in North and South Carolina and Florida. Hunting billbugs undergo two generations per year in North Carolina (Doskocil and Brandenburg 2012), whereas there are up to six generations per year in Florida (Huang and Buss 2009). The variability in billbug voltinism underscores the need to understand regional billbug phenology in turfgrass, because the number of generations will dictate the number of insecticide applications needed. Most previous billbug research has been conducted on golf courses. Billbug management approaches on golf courses may not directly apply to sod farms because management options and goals, such as fertilization, irrigation and plant protection practices, of the two systems vary distinctly. Turfgrass on golf courses is not harvested and sold; it is fully grown, and emphasis is placed on maintenance. Turfgrass in sod farms is harvested and sold, and then the grass is grown from sprigs or rhizomes from the same cut patch within a short time frame. Thus, the major objective of the current study was to determine the occurrence and seasonal abundance of billbugs relative to the growth stages of turfgrass in sod farms.

#### **Study site**

A survey of adult billbugs was conducted in sod farms in central Georgia in 2018 and 2019.

Each year, the sampling was initiated from February to December. The selected sod fields have a

history of billbug infestation. The details of the selected fields, such as their locations and grass cultivars, are listed in Table 1. In 2018, sampling was conducted in four sod fields within four distinct sod farms. For the fifth site, sampling was conducted in three fields at the same sod farm in Whitesburg, GA. In 2019, sampling at the Whitesburg site was discontinued, and a new site in Fort Valley, GA, was selected. The turfgrass genotype in all the sod fields was zoysiagrass (*Zoysia* spp.) but the cultivars varied. The zoysiagrass cultivars were 'El Toro', 'Emerald', 'Zenith', and 'Zeon'. The cultivar 'Zeon' is *Z. matrella*, and 'El Toro' and 'Zenith' are *Z. japonica*, while 'Emerald' [*Z. matrella* × *Z. pacifica* (Goudswaard) M. Hotta & S. Kuroki] is a hybrid. 'El Toro', and 'Zenith' zoysiagrass are wide-blade cultivars, whereas 'Emerald' and 'Zeon' zoysiagrass are narrow-blade cultivars. At the onset of sampling in 2018 and 2019, all the zoysiagrass in the field sites was fully grown and ready for harvesting.

In 2018, sampling was performed continuously at the five sites from mid-February to mid-December without any interruption (Table 1). In 2019, harvesting at site 1 began in January and was completed by late May, and the site was replanted with a new crop (soybean) instead of turfgrass. Thus, a new field adjacent to site 1, site 1a, was selected for further sampling (Table 1). The newly recruited zoysiagrass site used in 2019 was also fully grown at the onset of sampling. The sampling continued at site 1a from the first week of June to mid-December. In 2019, sampling at site 5 was discontinued because of logistical reasons and upon grower request; thus, a new site, site 6 in Fort Valley, GA, was selected for further sampling (Table 2.1). All the sites except site 2 received no fungicide or herbicide application. Site 3 was intensively managed by the grower for billbug control, as the maximum label rates of imidacloprid, bifenthrin and chlorantraniliprole were applied once each during May and June 2018. The rest of the sites received no insecticide applications.

# **Sampling**

Linear pitfall traps as described in Huang and Buss (2009) and Doskocil and Brandenburg (2012) were used to sample adult billbugs. This trap was constructed using white, 2.5 cm diameter, 0.6 cm thick, polyvinyl chloride (PVC) pipes by cutting a 1 cm wide linear slit. Four 152.4 cm long PVC pipes with slits were attached to a five-way PVC adaptor (Fig. 2.1A). Four PVC stopper caps were attached to the distal ends of the slited PVC pipes. This was done to prevent the movement of the trapped adult billbugs to the distal end of the PVC pipe. The fifth vent of the five-way adaptor was attached to the plastic dome lid of a 236.6 mL disposable Dixie PerfecTouch coffee cup (Fig. 2.1B). A 2.5 cm hole was drilled in the plastic dome lid to allow it to fit tightly on the fifth vent of the five-way adaptor. This coffee cup served as a collection device. Each linear pitfall trap was created by burying a 7.6 L plastic pail (24.13 deep × 24.8 cm diameter). Four 2.7 cm diameter holes were drilled along diagonal lines placed 0.5 cm below the top edge of each plastic pail. Ten 2.5 mm diameter holes were drilled along the bottoms of the pails to drain rainwater. The collection coffee cup inside the pail was supported by placing a 5 cm tall (5 cm length × 5 cm breadth) brick under the coffee cup. The PVC pipes were installed with the slits facing upward when the pail was deployed in the soil (Fig.2.1C). The traps were deployed at the mowing height of the grass. The gaps along the lengthwise edges of the slited PVC pipes were filled with soil so that the adult billbugs could walk over the pipe, fall into the slit and become trapped in the coffee cup. Because stoppers were placed at the distal ends of the PVC pipes and the other end emptied into a collection container, the billbugs were ultimately forced to move in one direction and become trapped in the collection device. The curved edges of the slits along the PVC pipe prevented the escape of the adult billbugs that fell into the trap.

Four linear pitfall traps were placed in each site. In site 5, however, two linear pitfall traps were deployed in one field, and one trap was placed in each of the other two fields.

One-third of the collection container was filled with ethylene glycol as a preservative agent. These traps were serviced at weekly intervals, and the billbugs were recovered by pouring the content through a copper strainer. Then, the strainer was emptied into plastic bags in the field. The billbugs were transported to the entomology laboratory, University of Georgia, Griffin, GA, for further identification. The billbugs were preserved in 70% ethyl alcohol.

### **Evaluation**

The adult billbugs were identified to species based on the morphological characters described by Vaurie (1951) and Johnson-Cicalese et al. (1990). The males and females were distinguished by the presence of a groove or depression on the metasternum and the first two abdominal sterna (Johnson-Cicalese et al. 1990). The growth stages of the turfgrass in the sod farms were recorded quarterly. The turfgrass was considered to be in an early growing stage when the turfgrass surrounding the trap had been harvested and < 50% of the soil was covered by grass. The grass was considered to be in the late growing stage when > 50% of the soil was under grass cover. The grass was classified as fully grown when it was at least ~5 cm tall (measured from the soil) and ready to harvest. The data were organized by turfgrass growth stage and season. The seasons were as follows: spring (February to May), summer (June to August) and fall (September to December).

### **Statistical analyses**

All the analyses were conducted using SAS software (SAS Institute 2012). To determine the incidence and abundance of billbugs from February to December 2018 and 2019, the number of billbugs captured in the four traps at each site were averaged by sampling date. The average

billbug data were subjected to one-way analysis of variance (ANOVA) using the general linear model procedure (PROC GLM) in SAS after log transformation (ln[x+1]), where the sample date and sites served as the treatment and replication, respectively. Because the growth stages of turfgrass were not uniform across the sampling dates, the billbug trap captures from all the sites were organized by turfgrass growth stage and season. Two-way ANOVA was performed on these data using the general linear model procedure (PROC GLM) in SAS after log transformation (ln[x+1]), where the effects of turfgrass growth stage and season were analyzed with interaction. The means and standard errors of the variables were calculated using the PROC MEANS procedure in SAS.

For each field, the billbug data were subjected to one-way ANOVA using the general linear model procedure (PROC GLIMMIX) in SAS with a log link function and a negative binomial distribution. The turfgrass growth stage had a fixed effect and replication (pitfall trap) had a random effect in the model. The least squares means were back transformed and separated for a pairwise t test (P < 0.05). The billbug data from sites 1, 1a, 5 and 6 were combined when the analysis was performed. For the combined data from sites 5 and 6, billbug data were obtained from only two traps in late-growth-stage turfgrass; thus, the data from the late growth stages were not included in the analysis. A one-way ANOVA was performed using the general linear model procedure (PROC GLM) after log transformation ( $\ln[x+1]$ ) to determine the effect of zoysiagrass genotypes on billbug captures. For this analysis, billbug capture data only from February to June 2018 were used, as all the turfgrass was fully grown during this period. The means were analyzed using the Tukey HSD method ( $\alpha = 0.05$ ). To determine the effect of gender, male and female billbug data for two years were combined by trap for each site, and a paired Student's t test was performed on the data using the PROC TTEST procedure in SAS after

log transformation (ln[x +1]). When reporting the statistics, the "pooled" data were used. The means and standard errors of the variables were calculated using the PROC MEANS procedure in SAS.

### **Results**

# **Billbug species**

From mid-February 2018 to mid-December 2019, 3,320 adult billbugs were collected from five different sod fields in central Georgia using linear pitfall traps. Four different species of billbugs were captured: the hunting billbug, *S. venatus vestitus*; nutgrass billbug, *S. cariosus* Olivier; uneven billbug, *S. inaequalis* Say; and vegetable weevil, *Listroderes difficilis* Germain.

Sphenophorus venatus vestitus accounted for 98.3% of the total adult captures for two years, whereas 1.3% of the total captures were *S. cariosis*. Similarly, *S. inaequalis* and *L. difficilis* accounted for 0.3% and 0.1% of the total adult captures, respectively.

# Overall seasonal abundance of billbugs and the influence of turfgrass phenology

A total of 2,695 adult billbugs were captured in 2018, whereas 625 adults were captured in 2019. In 2018, billbugs were significantly more abundant in most sampling dates in the early part of the year, from mid-February to late May, than in October and November (F = 6.4; df = 42, 168; P < 0.001; Fig. 2.2A). More than 86% of the total billbugs captured in 2018 were collected from mid-February to late June. However, the number of billbugs captured during the mid-season period (June to September) was not significantly different from the number of billbugs captured during the early season period (mid-February to late May). When the total number of billbugs captured in 2018 and 2019 were combined, only approximately 19% of the billbugs were captured in 2019. In 2019, the number of billbugs captured was significantly greater on a

few sampling dates, especially during late February, late March, late September and early October, than during the rest of the sampling period (F = 2.3; df = 45, 185; P < 0.001; Fig. 2.2B).

When the traps from all the sites were analyzed by turfgrass phenology and season, the number of billbugs captured was significantly greater in fully grown turfgrass than in early- or late-growth-stage turfgrass (F = 9.2; df = 2, 81; P < 0.001; Fig. 2.3). The number of billbugs captured was not significantly different by season, i.e., spring, summer and fall (F = 2.5; df = 2, 81; P = 0.092). Similarly, the interaction effects between turfgrass phenology and season were not significantly different (F = 0.1; df = 4, 81; P = 0.991).

# Site-by-site assessment of billbug captures relative to turfgrass phenology

To understand the seasonal incidence and abundance pattern of billbugs at the individual site level, billbug captures were assessed in relation to the turfgrass growth stages at each site. At site 1, only fully grown and late-growth-stage turfgrass was present during 2018 and 2019. The number of billbugs captured per trap was not significantly different between the fully grown and late-growth-stage grasses (F = 5.4; df = 1, 7; P = 0.053; Fig. 2.4A). In 2018, the number of billbugs captured in the traps gradually decreased throughout the season until late fall, when the turfgrass was ready to harvest (Fig.2.5A). In 2019, the number of billbugs captured was low during the spring when the turfgrass was in the harvesting phase (Fig. 2.5A). On the new, adjacent site with fully grown turfgrass, the number of billbugs captured increased in August and September. At site 2, there was no significant difference in the number of billbugs captured between the fully grown and early-growth-stage turfgrasses (F = 3.7; df = 1, 4; P = 0.128; Fig. 2.4B). The turfgrass was fully grown and ready to harvest, and it was not harvested until September 2019 (Fig.2.5B). Captures of billbugs spiked in spring 2018, and thereafter, the number of billbug captures remained low (Fig.2.5B). At site 3, there was not a significant

difference between the fully grown, early-growth-stage and late-growth-stage turfgrasses (F = 5.4; df = 2, 6; P = 0.654; Fig. 2.4C). The number of billbugs collected from this site was generally low, with some spikes in captures during June and October (Fig. 2.5C). At site 4, the number of billbugs captured was significantly greater in fully grown turfgrass than in early- or late-growth-stage turfgrass (F = 10.1; df = 2, 6; P = 0.012; Fig. 2.4D). Billbug captures were relatively high during spring and summer of 2018 and fall of 2019 (Fig. 2.5D). When sites 5 and 6 were combined, the number of billbugs collected was significantly greater in the fully grown turfgrass than in the early-growth-stage turfgrass (F = 22.4; df = 1, 9; P = 0.001; Fig.2.4E). The largest number of billbugs were captured on fully grown turfgrass in spring of 2018 (Fig. 2.5E).

# Effect of grass genotype and gender

A significantly higher number of billbugs was collected in the traps in *Z. matrella* ('Zeon') and the hybrid 'Emerald' than in the *Z. japonica* cultivars 'El Toro' and 'Zenith' (F = 32.7; df = 2, 6; P = 0.002; Fig. 2. 6). There was no significant difference between the number of male and female billbugs collected from any site (site 1: t = -0.4, df = 6, P = 0.672; site 2: t = 0.1, df = 6, P = 0.999; site 3: t = -0.1, df = 6, P = 0.999; site 4: t = -0.7, df = 6, P = 0.537; site 5: t = -0.3, df = 6, t = 0.804; Fig. 2.7).

### **Discussion**

The results show that the hunting billbug, *S. venatus vestitus*, is the most common billbug species collected from sod farms in Georgia. This result is consistent with billbug captures in the Carolinas (Johnson-Cicalese et al. 1990, Docksoil and Brandenburg 2012) and Florida (Huang and Buss 2009), where *S. venatus vestitus* was also the most abundant species in warm-season turfgrass. Other minor species captured in the current study include *S. inaequalis* and *S. cariosis*, which were also found on golf courses in Florida and South Carolina (Huang and Buss 2009,

Chong 2015), although *S. cariosis* was not reported from golf courses in North Carolina (Doskocil and Brandenburg 2012). However, other *Sphenophorus* spp. such as the bluegrass billbug, *S. parvulus* Gyllenhal, and *S. minimus* Hart found on golf courses in Florida and the Carolinas were not found on sod farms in Georgia (Huang and Buss 2009, Docksoil and Brandenburg 2012, Chong 2015). Billbugs such as *S. apicalis* (LeConte), *S. deficiens* Chittenden, *S. cubensis* Buchanan, *S. necydaloides* Dunedin, and *S. pontederiae* Chittenden found in Florida (Huang and Buss 2009); *S. rectus* Say and *S. callosus* Olivier found in North Carolina (Docksoil and Brandenburg 2012); and *S. coesifrons* Gyllenhal found in South Carolina (Chong 2015) were not found on sod farms in Georgia in the current study. Few vegetable weevils, *L. difficilis* were collected from sod farms in central Georgia; previous studies did not find this species on golf courses in neighboring states.

The data show that billbugs were abundant in sod farms when the turfgrass was fully grown or ready to harvest. Unlike in golf courses or residential or public lawns, turfgrass in sod farms is continuously grown, cut and sold throughout the year. Within a sod farm, sod producers maintain turfgrass at various stages in several fields so that they can meet the demand year round. Hunting billbugs undergo two generations per year in North Carolina (Doskocil and Brandenburg 2012) and up to six generations per year in Florida (Huang and Buss 2009). The number of hunting billbug generations in Georgia can vary between two and six, as sod is produced in approximately 61 counties across the state. This also suggests that the longer the turfgrass stays in the ground, the more vulnerable it is to the constant influx and colonization of billbugs, resulting in a high population buildup. Although billbugs are found on both zoysiagrass and bermudagrass, zoysiagrass is slow growing and takes approximately six more months to be ready for harvest in central Georgia. This longer time frame not only exposes zoysiagrass to

within-field accumulation and invasion of several overlapping generations of billbugs but also magnifies the damage potential of billbugs to zoysiagrass because this species does not recover well once damaged by adult and larval billbugs. This was evident in the data, as the number of billbugs captured was higher in the fully grown turfgrass than in grass in the early or late developmental stages.

Understanding synchronous billbug voltinism can help determine the incidence of the most vulnerable life stage. In turn, this helps determine the insecticide types, such as contact or systemic, and the number and timing of applications needed as part of integrated pest management methods. Degree-day models have been used to predict the synchronous emergence of adult billbugs (Dupuy et al. 2017, Duffy et al. 2018) for the best use of control measures, such as insecticides. Based on the data from the current study, it is not clear how useful degree-day models are for predicting the emergence of adult billbugs for management decisions, as billbug populations vary by turfgrass growth stage. Thus, this approach may not be generally useful for determining billbug emergence in sod farms unless fully grown sod is maintained for a long time period and grown through the winter months into the spring months in the following year or beyond. At a given time, turfgrass can be found at various stages in a sod field. The key strategy in any pest management approach is to tackle the pest before the population size grows beyond a certain threshold. If this holds true for billbug management in sod farms in Georgia, it is critical that management is administered when turfgrass is still growing. More studies are warranted to determine the best insecticide application timing, as the data from the current study suggest that the size of the billbug population could be influenced by the time of the previous sod harvest and indicate the length of time that exposes sods to multiple overlapping generations of billbugs.

We resorted to sampling adult billbug populations rather than billbug larvae, although developing larval stages are more destructive and cause more economic damage than adults (Doskocil and Brandenburg 2012). It is challenging to determine the phenology of billbugs by sampling larvae. There are potential overlapping generations during the growing seasons, and early larval stages develop inside stems, while the later stages develop in the soil while consuming roots (Huang and Buss 2009). Moreover, the distribution pattern of billbugs on zoysiagrass is still not known, although this information could help determine how many samples are required from an area as well as the optimal spatiotemporal sampling plan. Lack of specific biological data on larval behavior and infestation pattern in the soil, it is difficult to determine a reliable sampling plan to determine larval phenology. The current sampling method (using cup cutters) is very time consuming and laborious (Doskocil and Brandenburg 2012) and is less likely to yield reliable data for determining billbug phenology in sod farms.

Billbug density was greater on the *Z. matrella* cultivar 'Zeon' and hybrid 'Emerald' than on the *Z. japonica* cultivars 'El Toro' and 'Zenith'. Previous studies show that zoysiagrass cultivars such as 'Emerald', 'Royal', 'Zeon', and 'Zorro' are resistant to the hunting billbug, whereas 'Zenith' and 'El Toro' are susceptible to this pest (Reinert and Engelke 2001, Huang et al. 2014). The exact reason for this is not clear. Previous data also showed that *Z. matrella* cultivars were less susceptible to hunting billbug damage than are *Z. japonica* cultivars (Reinert and Engelke 2001, Reinert et al. 2011, Huang et al. 2014). In the current study, the sex ratio of male to female hunting billbugs from all five sod farm sites was approximately the same, which was consistent with a previous study (Huang and Buss 2009).

In summary, the results show that *S. venatus vestitus* is the dominant billbug species in Georgian sod farms. Other minor species of billbugs found in the traps included *S. inaequalis* 

and *S. cariosis*. The data showed that billbugs were abundant when the turfgrass was fully grown or ready to harvest. This suggests that the management of billbugs in sod farms should not be based on billbug phenology data described from golf courses in neighboring southern states (where sequential spikes of billbug abundance were observed), which are used to time insecticide application for specific life stages of billbugs. The data suggest that a field-by-field management strategy needs to be developed to reduce population surges in slow-growing zoysiagrass. More studies are necessary to understand the risk of incidence and abundance of billbugs in a given sod field; factors such as when turfgrass was previously harvested, the turfgrass growth stage and the temporal exposure windows to billbug populations within and adjacent to turfgrass fields in sod farms should be studied. Clearly, current integrated pest management plans, including the use of insecticides, timing of applications, stage of the turfgrass, and time of the harvest, need to be revisited to improve billbug control in sod farms.

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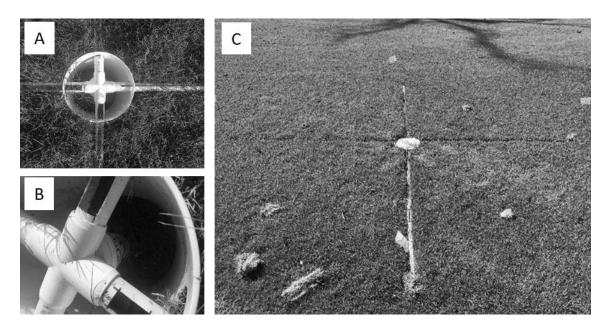
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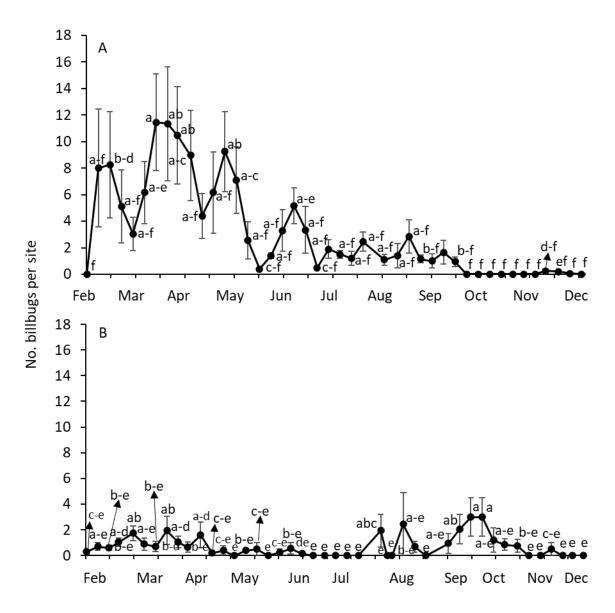
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Table 2.1. Details of the sod farm sites selected for weekly *Sphenophorus* spp. sampling in central Georgia in 2018 and 2019.

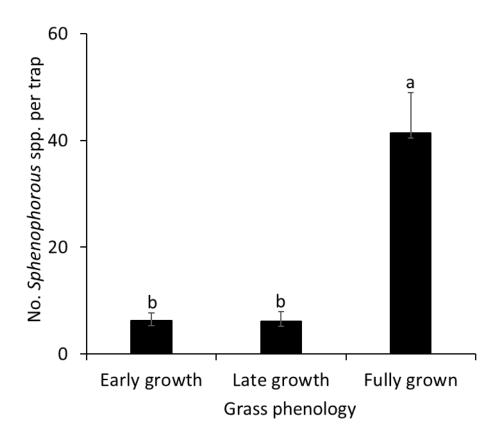
Site	Year	Location	Zoysiagrass cultivar	Field coordinates of trap location	Components of surrounding landscape	Field size (ha)
1	2018, 2019 (up to May 2019)	Marshallville, GA	'Emerald'	1) 32.4286, -84.0022 2) 32.4285, -84.0032 3) 32.4284, -84.0051 4) 32.4296, -85.0051	Wood line, open fields	14.02
1a	2019	Marshallville, GA	'Zenith'	1) 32.4287, -83.9943 2) 32.4287, -83.9940 3) 32.4292, -83.9945 4) 32.4316, -83.9952	Open fields	27.55
2	2018, 2019	Marshallville, GA	'Zenith'	1) 32.3864, -83.9920 2) 32.3843, -83.9918 3) 32.3814, -83.9934 4) 32.3800, -83.9974	Wood line, open fields	66.33
3	2018, 2019	Fort Valley, GA	'El Toro'	1) 32.5193, -83.9463 2) 32.5206, -83.9459 3) 32.5215, -83.9448 4) 32.5196, -83.9437	Pecan orchard, wood line, open fields	7.89
4	2018, 2019	Marshallville, GA	'Zeon'	1) 32.4241, -83.8880 2) 32.4237, -83.8902 3) 32.4276, -83.8928 4) 32.4293, -83.8927	Wood line, open fields	31.12
5	2018	Whitesburg, GA	'El Toro'	1) 33.4918, -84.8604 2) 33.4964, -84.8639 3) 33.4989, -84.8544 4) 33.5516, -84.8512	Wood line, creek, open fields	Traps 1&2: 4.66 Trap 3: 4.44 Trap 4: 1.82
6	2019	Fort Valley, GA	'El Toro'	1) 32.5075, -83.9412 2) 32.5059, -83.9406 3) 32.5070, -83.9393 4) 32.5804, -83.9398	Pecan orchard, woods	9.66



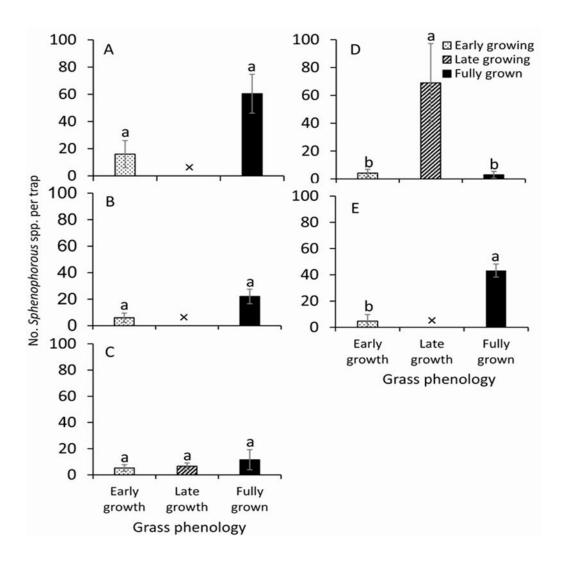
**Fig. 2.1.** A four-pipe linear pitfall trap with (A) four slited PVC pipes connected to a central fiveway adaptor; (B) the five-way adaptor empties into a coffee cup in a plastic pail; (C) shows the fully deployed linear pitfall trap on the turfgrass.



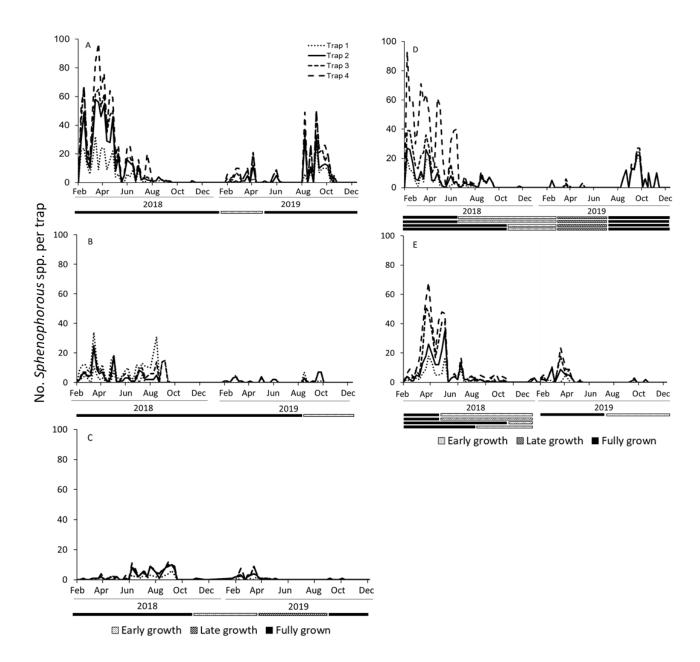
**Fig. 2.2.** Mean ( $\pm$ SE) (A) number of *Sphenophorus* spp. captured from February to December in the linear pitfall traps in (A) 2018 and (B) 2019. The average billbugs captured across the sampling dates with the same letters are not significantly different (Tukey's HSD test, P < 0.05).



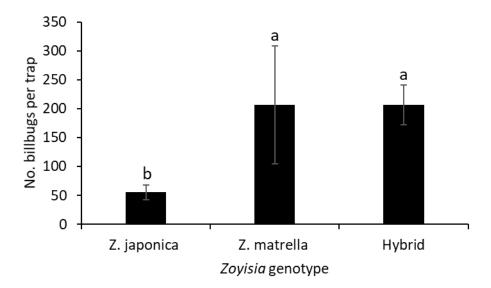
**Fig. 2.3.** Mean ( $\pm$ SE) number of *Sphenophorus* spp. captured per trap by turfgrass development stages when all the traps at all the sites were combined. The bars with the same letters are not significantly different (Tukey's HSD test, P < 0.05).



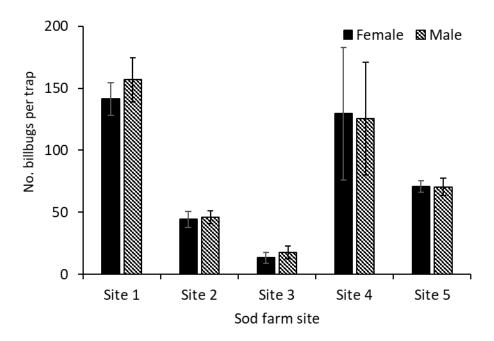
**Fig. 2.4** Mean ( $\pm$ SE) number of *Sphenophorus* spp. captured per trap by turfgrass development stage at (A) sites 1 and 1a, (B) site 2, (C) site 3, (D) site 4, and (E) sites 5 and 6. The symbol  $\times$  indicates that a specific turfgrass stage was not present. The bars with the same letters are not significantly different (pairwise t test, P < 0.05).



**Fig. 2.5.** The number of *Sphenophorus* spp. captured per trap stage from February to December in 2018 and 2019 at (A) sites 1 and 1a, (B) site 2, (C) site 3, (D) site 4, and (E) sites 5 and 6. The horizontal bars with various patterns represent the grass phenology status corresponding to the sampling dates. A single horizontal bar represents a single grass phenology status around all four traps, and four stacked horizontal bars represent the different grass phenology stages around four traps, from 1 to 4.



**Fig. 2.6.** Mean ( $\pm$ SE) number of *Sphenophorus* spp. captured by turfgrass genotype. The bars with the same letters are not significantly different (Tukey's HSD test, P < 0.05).



**Fig. 2.7.** Mean ( $\pm$ SE) numbers of male and female *Sphenophorus* spp. captured by site from February to June 2018. The bars (paired by site) without symbols are not significantly different at  $\alpha = 0.05$  (Student's t test).

# **CHAPTER 3**

# SURFACE MOVEMENT OF BILLBUGS (COLEOPTERA: CURCULIONIDAE) IN HARVESTED AND NONHARVESTED SOD.

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**ABSTRACT** The billbug, *Sphenophorus* spp. (Coleoptera: Curculionidae), is an important pest complex on the sod farms of Georgia. The feeding damage of larvae within stolons and on roots adversely delays the sod harvest and makes it difficult to conduct machine harvests. To develop an effective management strategy, the timing of insecticide applications is critical. The activity of billbugs, especially soon after sod harvest, has not been documented, as newly emerging adults could reinfest the harvested area or adjacent nonharvested sod fields. In 2019 and 2020, adult billbugs were sampled from harvested and nonharvested areas of sod farms by using linear pitfall traps. Although a significantly greater number of billbug adults were captured from the nonharvested sod, the data showed that adults were present in the harvested sod area. To understand the direction of billbug movement in both harvested and nonharvested sod, a square area was selected, and the sod inside the square was removed. Linear pitfall traps were deployed along the perimeter of square areas to collect adults from outside and inside the square. In 2020, a significantly greater number of billbug adults were collected in the traps from the nonharvested areas outside the square than from harvested area inside the square, whereas in 2019, adult captures were similar from both areas. The data documented the activity of billbugs in the areas where sod was harvested, posing a risk of infestation for both strips of nonharvested grass in the harvested area and the adjacent, nonharvested sod fields that were near harvest.

Keywords Sphenophorus spp., turfgrass, growth stages, zoysiagrass

The billbug pest complex, *Sphenophorus* spp. (Coleoptera: Curculionidae), is one of the serious pest problems in sod production in the eastern U.S. (Dupuy and Ramirez 2016, Gireesh and Joseph 2020). Among ten pest species of billbugs that occur in turfgrass (Potter and Braman 1991, Vittum et al. 1999, Dupuy and Ramirez 2016), the hunting billbug, *Sphenophorus venatus vestitus* Chittenden, is the most abundant pest of warm-season grasses (Potter and Braman 1991, Huang and Buss 2009) and is abundant on sod farms (Gireesh and Joseph 2020). Other billbug species, such as *S. inaequalis* and *S. cariosis*, have also been captured from sod farms (Gireesh and Joseph 2020). In the U.S., at least 20.2 million ha is covered by turfgrass (National Turfgrass Federation 2020). In 2017, sod production was valued at \$1.148 billion USD, and 1,465 farms produced 137,411 ha (USDA Ag Census 2017). Georgia's turfgrass industry is valued at \$7.8 billion USD (Kane and Wolfe 2012), with sod grown in 61 from the 159 counties in the state (Farm Gate Value Report 2018).

The females of *Sphenophorus* spp. insert eggs into internodal regions of the turfgrass stolons. First instar larvae feed within the stolons and remain in the internodal space (Dupuy and Ramirez 2016), which weakens the integrity of the stolon. As the larvae grow, the second instars emerge out of the stolon. After that, all subsequent instars feed on the root system (Vittum et al. 1999, Doskocil and Brandenburg 2012). The larval feeding damage to the stolon and roots pose a challenge to machine harvest, as the affected sod slab can rarely hold itself together.

Additionally, the stress imposed by this damage causes a delay in normal growth and development of the billbug-infested sod, especially when the sod breaks winter dormancy (Dupuy and Ramirez 2016). These two billbug-mediated problems cause economic constraints for sod producers by interrupting the timely supply of sod to their clientele.

In sod farms, *Sphenophorus* spp. attacks both zoysiagrass [*Zoysia matrella* (L.) Merrill and *Zoysia japonica* Steudel] and bermudagrass [*Cynodon dactylon* (L.)]. Zoysiagrass and bermudagrass have distinctly different growth habits (Huang et al. 2014). Because zoysiagrass grows and develops at a slower rate than bermudagrass, zoysiagrass is particularly vulnerable to *Sphenophorus* spp. feeding damage (Gireesh and Joseph 2020). In recent decades, the popularity of zoysiagrass has increased over the years in the southeastern and south-central U.S. (Patton et al. 2017). Because some of the zoysiagrass cultivars, such as 'Zeon' and 'El Toro', grow faster and become ready for harvest sooner, they are highly preferred by sod producers, and more sod producers are growing these two zoysiagrass cultivars (Patton 2009).

Sod farms present unique ecological conditions for billbugs. The turfgrass on sod farms is grown rapidly from sprigs, residual grass strips or ribbons of grass left over from the previous harvest (Gireesh and Joseph 2020). The fully grown sod is harvested as needed in strips, and the harvest is completed in several weeks. Sometimes, the fully grown sod is held in the inventory for a shorter period of time until market demand and value increase. Within a sod farm, sections of the field are typically harvested, while sod continues to grow in other areas of the same or adjacent fields (Gireesh and Joseph 2020). The sod harvest continues through the growing period and during the late fall and winter months. Previously, it has been shown that the numbers of adult billbugs in sod farms were considerably higher when the sod was fully grown than at early growing stages (Gireesh and Joseph 2020). Usually, sod development initiates when fully grown sod is harvested from the field. This process can occur at any time of the year, depending on when sod is ready and current market demand. Gireesh and Joseph (2020) showed that phenology of *Sphenophorus* spp. doesn't strictly follow a seasonal pattern in sod farms; instead, the sod growth stage at a given time and exposure to the number of overlapping generations of

Sphenophorus spp. play a critical role in phenology. If the recently harvested field is billbug infested, the larval and pupal stages can be found in the root. The immature billbugs in the soil develop feeding on the root system and molt into adults. Newly emerged adults pose a risk of reinfestation on the strips of leftover grass in the already harvested area of the field or in the adjacent fields where sod of various stages is growing. Knowledge of billbug movement activity, especially at the early growth stages of turfgrass in the harvested areas of the field, can be useful information for developing integrated pest management strategies, including a refined insecticide application timing.

The objectives of the current study were two-fold: 1) to determine the activity of billbug adults in the harvested and nonharvested areas of the sod fields and 2) to document whether the billbug adults emerge from the harvested area of the sod. This information can be integrated into the billbug management program because currently, harvested sod areas are not typically considered to pose a threat for billbug infestation on sod farms.

### Materials and methods

# **Study site**

Experiments were conducted in sod production farms belonging to two producers in Marshallville and Fort Valley, Georgia, in 2019 and 2020. The selected sod field sites for the experiments had a history of billbug infestation. The linear pitfall trap method common to both objectives is described below (pitfall trap section below). The sampling for both objectives was initiated in April 2019 and May 2020. Additional information on study sites for the first objective is indicated in Table 3.1. For the second objective, the experiment was conducted in Marshallville, Georgia.

### Billbug activity in harvested and nonharvested sods

This experiment was conducted on sod farms in Marshallville and Fort Valley, Georgia, in 2019 and 2020. The details of the sod field sites are presented in Table 1. Three zoysiagrass and three bermudagrass sod fields were selected to conduct this experiment. These sod fields were partially harvested in certain areas, and other areas were not harvested when the experiment was initiated. In most sites, harvest began in November, and it was completed at some sites within a month, while it lasted for a few months in others. At a few sites, the harvest was started in February and was continued during the experiment (Table 3.1). The nonharvest areas where the traps were deployed had no active harvest activities for the duration of the experiment. During the winter months (November to January), adult billbugs are typically not active, as they are mostly at immature stages. This experiment was initiated when the adults were active in the spring month on fully grown sod (Gireesh and Joseph 2020).

Two linear pitfall traps (see pitfall section below) were deployed in each harvested and nonharvested areas (Fig. 3.1). The harvested and nonharvested areas were at least 100 m apart, while traps deployed within each area were ~ 50m apart. The growers in the selected field harvested the entire section of the field, and the new grass shoots were developed mostly from the rhizomes. The experiments lasted for a month in both years. The linear pitfall traps were monitored at weekly intervals for four weeks on 3, 10, 17, and 24 April 2019; and 19 and 26 May and 2 and 9 June 2020. The details on the processing and evaluation of collected billbugs are described in the pitfall trap section common to both objectives. The experiment was arranged in a completely randomized design (CRD) with sites that served as replications. Six sod field sites each were sampled in both years. The adult billbugs captured from the two linear pitfall traps were combined for the harvested or nonharvested areas in each site.

### Emergence of billbugs from the harvested and nonharvested areas

This experiment was conducted in Marshallville, Georgia, in 2019 and 2020. In 2019, the selected experimental site was on 'Zeon' zoysiagrass, whereas in 2020, the site was planted with 'TifWay' bermudagrass. Each year, sod from six 3.05 × 3.05 m square areas was removed, and hereafter, the area devoid of sod was referred to as harvested. At each square area, four twopiped linear pitfall traps (see pitfall section below) were deployed along the sides of the square, with one pitfall trap per side (Fig. 3.2). Black plastic resin edging (Suncast Corporation, Batavia, IL) was placed in corners between tubes of the pitfall trap to restrict billbug movement and avoid the gap. To understand the billbug movement emerging from the inside harvested area, or outside nonharvested sod area, aluminum foil sheets were attached to the linear pitfall traps to block the movement from one direction. On the two-piped linear pitfall trap, aluminum foil was attached to the edge of the slits created on the two PVC pipes using binder clips. The 30.5 cm wide aluminum foil sheet was cut to the length of the PVC pipe on one side and folded in half lengthwise before being attached to the slits on the PVC pipe. In each square, aluminum foil was attached to the inner edges of the slits for two linear pitfall traps, whereas on the other two linear pitfall traps, aluminum foil was attached to the outer edges of the slits. This arrangement allowed the adult billbugs to walk and fall into slits from one side, and aluminum-foil blocked their entry from the other side. Preliminary laboratory studies showed that adult hunting billbugs failed to walk over the smooth surface, which was the reason behind using aluminum foils in the study to prevent billbug movement from the desired direction. The attached aluminum foil was flipped between the inner and outer edges of the PVC pipe of linear pitfall traps within the square, and they were attached to one side on each linear pitfall trap. The square side that received the aluminum foil attachment on the inner or outer side of the traps was randomly assigned. The

experiments were initiated on 22 April 2019 and 19 May 2020. The sod within the square areas was removed two weeks before the experiment was initiated in both years. The linear pitfall traps were monitored at weekly intervals for up to five and four weeks in 2019 and 2020, respectively. The billbugs collected from the same side were combined for each square. The experiment was arranged in RCBD with six replications.

# Pitfall traps

A modified version of the linear pitfall trap, as described in Gireesh and Joseph (2020), was used to sample adult billbugs. White, 152.4 cm long, 2.5 cm diameter, 0.6 cm thick polyvinyl chloride (PVC) pipes were used to build the trap, and only two pipes rather than four PVC pipes were attached to the centrally located adaptor, which was the only modification (Fig. 3.1A and B). The two pipes with a 1 cm wide lengthwise slits facing up were deployed at a 180° angle and were attached to a three-way, T-shaped PVC adaptor. This adaptor was pointed downward (Fig. 3.1C) into a 236.6 mL disposable Dixie PerfecTouch coffee cup (Dixie cup company, Easton, PA) hereafter referred to as the collection cup (Fig. 3.1D). The cup was secured to the adaptor after drilling a 2.5 cm hole on the lid. Two PVC stopper caps were attached to PVC pipes at opposite ends to direct adult billbugs toward the collection cup. The collection cup was housed in a 7.6 L plastic pail (24.1 deep × 24.8 cm diameter) buried in the soil. Two 2.7 cm diameter holes were drilled at the opposite ends along the diagonal line approximately 0.5 cm below the top edge of plastic pail, and two pipes passed through the two holes on either side of the pail. The upper margin of the slits flushed with the soil surface. At the bottom of the pail, approximately ten 2.5 mm diameter holes were drilled to drain rainwater. A  $5 \times 5 \times 5$  cm (length  $\times$  width  $\times$ height) brick was placed inside the bottom of the pail to support the collection coffee cup. The soil was used to fill the gaps along the outer margins of the PVC pipes to enable a continuous

surface for the adult billbugs to walk over the pipe, fall through the slit, walk toward the center, and become trapped in the coffee cup. Once a billbug falls through the slit on the PVC pipe, the curved edges prevent it from escaping. They would wander inside the pipe and ultimately fall into the coffee cup in the center.

When trapping was initiated, ~10 mL of ethylene glycol was added to the coffee cup as a preservative agent. These traps were serviced at weekly intervals, and the billbugs were recovered by pouring the content through a copper strainer. Then, the content of the strainer was emptied into plastic bags in the field and transported to the laboratory. The billbugs were sorted and preserved in 70% ethyl alcohol and identified to species at the entomology laboratory, University of Georgia, Griffin, GA. The morphological characteristics described by Vaurie (1951) and Johnson-Cicalese et al. (1990) were used to identify adult billbugs to species.

# **Statistical analyses**

All analyses were conducted using SAS software (SAS Institute 2012, Cary, NC). To determine the effect of the billbug activity on the harvested and nonharvested areas of the sod, the number of billbugs captured in the two traps at the harvested or nonharvested areas at each site were combined. The combined data with each sample date were subjected to a mixed model analysis with repeated measures as dates (PROC GLIMMIX) in SAS after log transformation (ln[x +0.25]). Homogeneity of variance (normality) was checked using the PROC UNIVARIATE procedure in SAS before the transformation. The sample date, harvest status, turfgrass genotype (bermudagrass and zoysiagrass), and their interactions served as the treatments, and the sod field sites were the replications. To further understand the sod harvest status effect, combined data for each sample date and overall data were subjected to two-way ANOVA using the general linear

model procedure (PROC GLM) in SAS after log transformation (ln[x +0.25]). The means were separated post-ANOVA, using the Tukey HSD method ( $\alpha = 0.05$ ).

To determine the effect of the number of billbugs captured from harvested (inside) and nonharvested (outside) areas of the square areas, the billbug capture data were combined by the direction of the linear pitfall trap (facing the harvested and nonharvested areas) for each square. In 2019, all captured billbugs were S. venatus vestitus, whereas in 2020, S. inaequalis was also captured. The combined data with each sample date were subjected to a mixed model analysis with repeated measures as dates (PROC GLIMMIX) in SAS after log transformation (ln[x +0.25]). The sample date, harvest status, and their interactions served as the treatments, and the squares were the replications. In 2020, repeated measures analysis for combined data where both species together and by species was conducted. To further understand the effect of sod harvest status, the data were analyzed by sampling date. Thus, the data were analyzed by sampling dates for these two species plus on combined data. Overall, the data were created for each billbug species, and the species were combined. Paired Student's t-tests were performed on the datasets using the PROC TTEST after log transformation (ln[x + 0.25]). When reporting the statistics, the "pooled" data were used ( $\alpha = 0.05$ ) since variances were homogeneous. The means and standard errors of the variables were calculated using the PROC MEANS procedure in SAS.

### **Results**

### Billbug activity in harvested and nonharvested sod

The repeated measures analysis show that the sample date was not significantly different in 2019 and 2020 (P > 0.05). The effects of sod harvest status were significant on adult S. venatus vestitus captures in 2019 (F = 28.0; df = 1, 26; P < 0.001) and in 2020 (F = 25.1; df = 1, 28; P < 0.001). The grass genotype did not affect the adult S. venatus vestitus captures in 2019 and 2020

(P > 0.05). The sod harvest status × grass genotype interaction was not significantly different in 2019 and 2020 (P > 0.05). Similarly, the sod harvest status × sample date interaction was not significantly different in 2019 and in 2020 (P > 0.05).

In 2019, adult *S. venatus vestitus* captures were significantly greater in nonharvested sods than in harvested sods on 18 April (F = 10.4; df = 1, 4; P = 0.032; Fig. 3.3A). On 10 and 25 April, similar numbers of adult *S. venatus vestitus* were collected from the nonharvested sod and the harvested sod (P > 0.05). When all the adult *S. venatus vestitus* captures were combined during the sampling period, significantly more individuals were collected from the nonharvested sod than from harvested sod (F = 8.5; df = 1, 4; P = 0.043; Fig. 3.3B). In 2020, more adult *S. venatus vestitus* were captured from the nonharvested sod than from the harvested area on 19 May (F = 9.2; df = 1, 4; P = 0.038) and 1 June (F = 25.5; df = 1, 4; P = 0.007; Fig. 3.3C). Overall, a significantly greater number of *S. venatus vestitus* were captured from the nonharvested sod than from harvested sod (F = 8.2; df = 1, 4; F = 0.045; Fig. 3.3D). No other factors or their interactions (grass genotype; sod harvest status × genotype) were significantly different (F > 0.05).

### Emergence of billbugs from harvested and nonharvested sods

The repeated measures analysis show that sample date was not significantly different (P > 0.05; for adult S. venatus vestitus) in 2019, but significantly different in 2020 (F = 13.2; df = 3, 20; P < 0.001; for adult S phenophorus spp.). In 2020, sample date was significantly different for adult S. venatus vestitus (F = 12.1; df = 3, 20; P < 0.001) and for adult S phenophorus in 2020; P = 0.041. The effects of sod harvest status were not significant on adult S phenophorus phenophorus spp. in 2020 (P > 0.05). In 2020, the sod harvest status did not affect the adult S phenophorus spp. in 2020 (P > 0.05) but on adult S.

inaequalis captures were affected (F = 8.3; df = 1, 20; P = 0.009). The sod harvest status × sample date interaction was not significantly different in 2019 (for adult *S. venatus vestitus*) and in 2020 (P > 0.05; for adult *Sphenophorus* spp.). In 2020, sod harvest status × sample date interaction was not significantly different for adult *S. venatus vestitus* captures (P > 0.05) but was significantly different for adult *S. inaequalis* captures (P = 8.1; df = 3, 20; P = 0.001).

In 2019, the number of adult *S. venatus vestitus* emerging from and moving to the square area was not significantly different among sampling dates and when the adults were combined (P > 0.05; Fig. 3.4A and B). In 2020, both adult *S. venatus vestitus* and *S. inaequalis* were captured in the traps. Similar to 2019, there was no significant difference in the number of adult *S. venatus vestitus* collected between movement from and to the harvested square area for any of the sample dates and when adult captures were combined (P > 0.05; Fig. 3.5A and B). The number of adult *S. inaequalis* collected in linear pitfall traps was greater from the nonharvested into the harvested square area on 21 May (t = 13.8; df = 10; P < 0.001; Fig. 3.5C), and this trend was maintained when all adult *S. inaequalis* captures were combined (t = 2.5; df = 10; t = 0.035; Fig. 3.5D). A similar effect was noticed when both the adult *S. venatus vestitus* and *S. inaequalis* captures were combined (t = 0.035; Fig. 3.5D). A significantly greater number of individuals were collected in traps that received adults from the nonharvested areas than from inside area of the squares on 21 May (t = 4.1; df = 10; t = 0.002; Fig. 3.5E) and when captures from all dates were combined (t = 2.5; df = 10; t = 0.035; Fig. 3.5F).

### **Discussion**

The results showed that *S. venatus vestitus* adults were active in both harvested and nonharvested areas of the field; their captures were greater in the nonharvested than in the harvested areas (Fig. 3.3). This suggests that *S. venatus vestitus* adults either emerged from the harvested areas as they

underwent the pupal stage in the soil or that the adults migrated from the nonharvested to harvested areas. Because S. venatus vestitus adults can walk to harvested areas from nonharvested areas, another follow-up study was conducted where the traps were deployed facing harvested and nonharvested areas to record the activity of adult emergence. The results showed that the *Sphenophorus* spp. adults actively emerged from the harvested area and moved toward nonharvested areas of sod (Figs. 3.4 and 3.5). This suggested that the billbugs collected from the harvested areas of the field also originated from the harvested area. However, the number of adults collected from nonharvested sod might be disproportionate as a large area of nonharvested sod surrounded the harvested square area. These are critical results that have not been reported previously, as sod producers rarely manage Sphenophorus spp. adults immediately after sod harvest, especially in the harvested areas of the field. Sphenophorus spp. adult density was greater at fully grown stages of the sod than at the developing stages (Gireesh and Joseph 2020). Typically, those sod growers who use insecticides for *Sphenophorus* spp. larval control often sprays systemic insecticides such as imidacloprid or chlorantraniliprole in March or April, and adults are not targeted.

The activity of *S. venatus vestitus* adults observed in the current study might be related to their unique biology in sod farms. The flight activity of *S. venatus vestitus* adults is still unknown, and they move around by walking (Johnson-Cicalese et al. 1990). Adult females oviposit by inserting their eggs into the stolon of turfgrass (Johnson-Cicalese et al. 1990, Gireesh and Joseph 2020). This suggests that the adults, after mating, must be looking for a suitable site for oviposition. If they emerge from the nonharvested area, grass tissue is readily available for egg laying. However, if they emerge from already harvested grass, they do not have immediate access to grass tissue for egg laying. The data on the direction of adult movement (Figs. 3.4 and

3.5) suggested that adults continuously emerged from the harvested areas. These adults are likely to infest the areas that are not harvested in the sod fields. Additionally, when the sod is harvested, most sod producers leave ribbons of turfgrass shoots, especially zoysiagrass, which promotes faster recovery, as grass rapidly grows and spreads from those ribbons (Gireesh and Joseph 2020). These ribbons could be a suitable substrate for oviposition for those adults emerging from the harvested areas, which increases the chances for early infestations and allows the billbugs to develop large populations when close to harvest.

The incidence and adult movement in the harvested areas can have potential implications for the management of *Sphenophorus* spp. on the sod farms. Traditionally, adults are not managed in harvested areas and are not considered a possible source for the pest problem. Based on the data presented in this manuscript, management spraying in the harvested area can not only possibly reduce new infestations within the newly developing sod in the harvested areas but can also reduce infestations in the nonharvested areas. Billbugs can spread to other noninfested regions or across boundaries through the movement of infested sods (Tashiro 1987). The domestic market is critical for sod producers, as it is a major segment of their business, but there are a few growers who export sod to other countries. As most exporting countries demand phytosanitary certification for various billbug species, all sod undergoes rigorous phytosanitary inspection before shipment (Gireesh and Joseph 2020). Because hunting billbugs deposit eggs inside the stolon (stem), the emerging first instars feed within the stolon (Gireesh and Joseph 2020). Infestations are not easily detected until the larvae molt into the second instar and break out of the stolon. Thus, at the time when the sod leaves the U.S., the sod is deemed free of billbugs, but larvae emerge out when it reaches the destination, which results in rejection of the shipment. Currently, growers sprig the turfgrass (sod) and repeatedly treat it with insecticides to

reduce larval emergence from stems. This process provides reasonable control but not enough to meet the export standards. Thus, the data from the current study show that timely control, especially in areas where the sod has been harvested, can reduce the risk of oviposition and reinfestation in the nonharvested sod.

In summary, the current study showed that adults of *Sphenophorus* spp. were present in harvested and nonharvested areas, although their activity was more prevalent in the nonharvested than in the harvested areas. These results also showed that *Sphenophorus* spp. adults continuously emerged in the harvested areas as well as in the nonharvested areas. Suppression of Sphenophorus spp. on sod farms is crucial for compliance with domestic and international markets. Previously, Sphenophorus spp. were routinely intercepted from sod shipped from outside states (Tashiro 1987), and international markets demand billbug free sod when exported from the US. The results implied that sod producers should refine their management strategy for billbugs in the field to reduce larval infested sods shipped to various destinations. Additionally, the population size of *Sphenophorus* spp. could be reduced by early adoption of management plans almost immediately after harvest of the sod. For effective management of Sphenophorus spp. in sod farm, insecticide sprays are necessary before the sod harvest on the fully grown sod and adjacent sod fields after harvest where sod is growing at various stages. Also, insecticide sprays immediately after the sod harvest would reduce the emergence of new adults. Contact insecticides such as pyrethroids are effective against emerging adults. Future research warrants the evaluation of stage-specific turfgrass management plans to reduce *Sphenophorus* spp. populations in sod fields.

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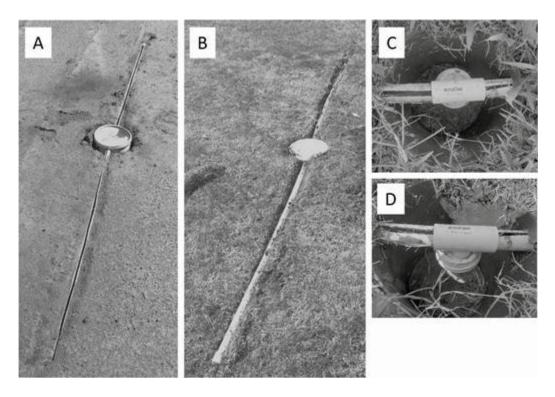
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Table 3.1. Details of the sod farm sites selected for weekly *Sphenophorus* spp. sampling in Marshallville and Fort Valley, Georgia, in 2019 and 2020.

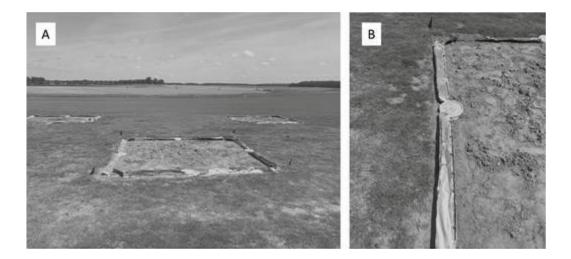
Year	Site	Grass genotype	Zoysiagrass cultivar	Location	GPS Coordinate
2019	1	Zoysiagrass <sup>a</sup>	'Emerald'	Marshallville, GA	32.431023, -84.009473
	2	Zoysiagrass <sup>b</sup>	'Zeon'	Marshallville, GA	32.426860, -83.893748
	3	Zoysiagrass <sup>b</sup>	'El Toro'	Fort Valley, GA	32.519937, -83.945832
	1	$Bermudagrass^b \\$	'TifTuf'	Marshallville, GA	32.436846, -83.884196
	2	$Bermudagrass^b \\$	'TifTuf'	Marshallville, GA	32.434454, -83.882362
	3	$Bermudagrass^b \\$	'TifWay'	Marshallville, GA	32.424725, -83.883139
2020	1	Zoysiagrass <sup>b</sup>	'El Toro'	Fort Valley, GA	32.520871, -83.945000
	2	Zoysiagrass <sup>b</sup>	'Zeon'	Marshallville, GA	32.428516, -83.890170
	3	Zoysiagrass <sup>b</sup>	'Zeon'	Marshallville, GA	32.424866, -83.887803
	1	$Bermudagrass^b \\$	'TifTuf'	Marshallville, GA	32.438719, -83.880321
	2	$Bermudagrass^b \\$	'TifTuf'	Marshallville, GA	32.410998, -83.992952
	3	Bermudagrass <sup>a</sup>	'TifWay'	Marshallville, GA	32.438970, -83.999231

Sampling was conducted at two sod producers in both years. <sup>a</sup>Sod farm 1: Insecticides are used as needed basis, chlorantraniliprole plus lambda-cyhalothrin for fall armyworm; *Spodoptera frugiperda* (J.E. Smith) (Noctuidae); fipronil for ant and mole cricket; and none for *Scapteriscus* spp. control. Insecticides are applied as a spot treatment.

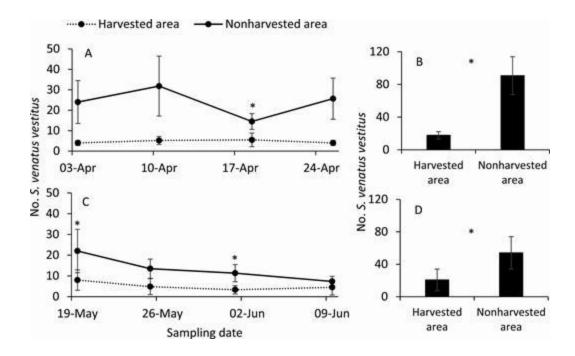
<sup>b</sup>Sod farm 2: Chlorantraniliprole for fall armyworm; *S. frugiperda*, fipronil for ant; and imidacloprid, dinotefuran, chlorantraniliprole for *Sphenophorus* spp. (in 'El Toro' fields only) control. Insecticides are applied as a spot treatment.



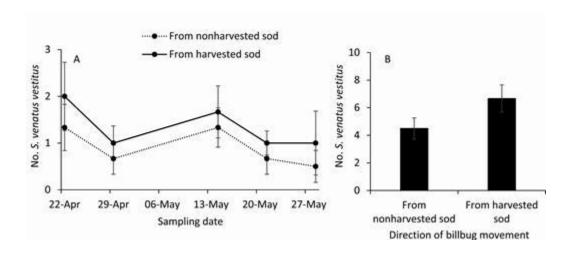
**Fig. 3.1.** A two-pipe linear pitfall trap deployed in the (A) harvested site and (B) nonharvested site, and (C) how the pipes are attached to adaptor and (D) how the collection cup is attached to the adaptor in the pail.



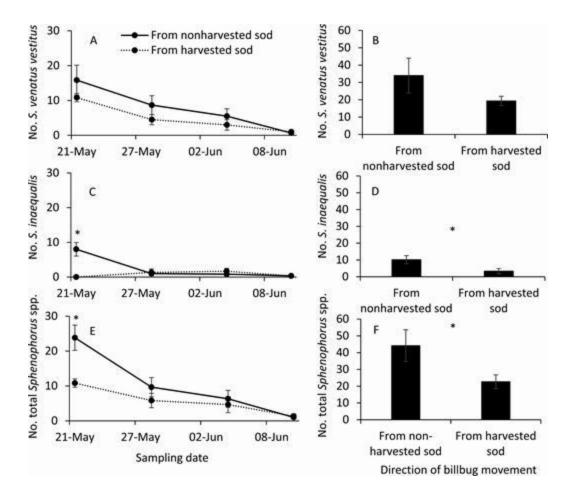
**Fig. 3.2.** A layout of the harvested square area to determine the direction of *Sphenophorus* spp. adult movement. (A) Four two-pipe linear pitfall traps were deployed on four sides of the harvested square, and (B) aluminum foil was attached to trap the movement of adults from a specific direction



**Fig. 3.3.** Mean ( $\pm$ SE) number of *Sphenophorus* spp. adults captured per two traps at harvested and nonharvested sites at (A) and (B) regular sampling intervals in 2019 and 2020, respectively. The adult captures were combined (C) and (D) in 2018 and 2019, respectively. The data points on the sample dates and bars with asterisks indicate significant differences (Tukey's HSD test, P < 0.05).



**Fig. 3.4.** Mean ( $\pm$ SE) number of *Sphenophorus* spp. adults captured per two traps from the harvested squares and surrounding nonharvested area (A) at regular sampling intervals in 2019 and (B) combined. The data points on the sample dates and bars with asterisks indicate significant differences (Student's t test, P < 0.05).



**Fig. 3.5.** Mean ( $\pm$ SE) number of adults captured per two traps from harvested squares and surrounding nonharvested area at (A, C and E) regular sampling intervals and (B, D and F) combined by sampling dates in 2020. (A and B) *S. venatus vestitus*, (C and D) *S. inaequalis* and (E and F) total *Sphenophorus* spp. (*S. venatus vestitus* + *S. inaequalis*). The data points on the sample dates and bars with asterisks indicate significant differences (Student's t test, P < 0.05).

## **CHAPTER 4**

# INFLUENCE OF ABIOTIC FACTORS ON WALKING BEHAVIOR OF HUNTING BILLBUG (COLEOPTERA: CURCULIONIDAE

To be submitted to Journal of Insect Behavior

Gireesh M, Joseph SV

**Abstract** The hunting billbug *Sphenophorus venatus vestitus* Chittenden (Coleoptera: Curculionidae) is an important insect pest of warm-season turfgrass. Larvae and adult S. venatus vestitus feed on turfgrass and affect normal grass growth and development. In sod farms and golf courses, management sprays are typically confined to affected areas because of the high insecticide and application costs. Understanding the walking behavior of S. venatus vestitus adults would help to refine management tactics. Thus, the objective of this study was to determine the influence of abiotic factors on the walking behavior of adult S. venatus vestitus. A series of laboratory, semifield, and field assays were conducted in 2019 and 2020. For the laboratory assays, field-collected S. venatus vestitus adults were acclimated at 15, 18, 21, 28, and 32 °C for 24 h, and the distances walked by these pre-acclimated adults were measured on sand and filter paper substrates using Noldus EthoVision XT software. For the semifield assays, the total and net distances walked by pre-acclimated adults were measured on a paved indoor surface. S. venatus vestitus males and females moved further when the temperature increased from 15 to 28 °C in the laboratory and semifield assays. For the field assays, field-collected S. venatus vestitus adults were not acclimated. The total and net distances walked by the adults were documented on a paved surface. Increases in temperature and relative humidity did not

**Key words** S. venatus vestitus, turfgrass, movement, temperature, IPM, sod farm

affect the distance moved by adults, but an increase in wind speed reduced the distance moved.

The hunting billbug, *Sphenophorus venatus vestitus* Chittenden (Coleoptera: Curculionidae) is an important insect pest of turfgrass (Dupuy and Ramirez 2016). In the U.S., turfgrass is an integral part of the urban and rural landscape (Monteiro 2017), covering approximately 20 million ha (Morris 2003), and the annual turfgrass production values are \$40-60 billion USD. Turfgrass is primarily produced on vast sod (turfgrass) farms. In Georgia, sod is produced on approximately 10,785 ha across 64 counties and is valued at \$118.3 million USD (Wolfe and Stubbs 2019). The sod is harvested from farms and planted in golf courses and residential and public landscapes. Although *S. venatus vestitus* can become a severe pest in all turfgrass systems, it is a persistent problem on sod farms, particularly on zoysiagrass [*Zoysia matrella* (L.) Merrill and *Zoysia japonica* Steudel] and bermudagrass [*Cynodon dactylon* (L.)] (Gireesh and Joseph 2020, 2021). *Sphenophorus venatus vestitus* oviposits into grass stems, and eggs hatch within 3-10 days (Dupuy and Ramirez 2016). The first and second instars feed within the stem, and the third instars leave the stem to feed on turfgrass roots (Huang and Buss 2009). At 25-28 °C, *S. venatus vestitus* completes development from egg to adult in 8-9 weeks (Huang and Buss 2009).

Sphenophorus venatus vestitus infestation affects the normal development of turfgrass. On golf courses and residential and public lawns, *S. venatus vestitus* feeding damage initially appears as isolated yellow spots in the turfgrass. With time, these individual spots coalesce to become noticeable brown patches. On sod farms, *S. venatus vestitus* feeding damage typically does not develop into brown patches; instead, it delays the growth and development of dormant turfgrass in spring and delays the scheduled harvest. Any delay in sod harvest affects the timely delivery of sod to customers. Additionally, *S. venatus vestitus* feeding on sod farms weakens the tight bonding between grass stems and roots, which poses a serious challenge for machine harvesting, as the sod does not hold together during harvesting.

Dispersal is an integral ecological process impacting the spatial and temporal distribution of organisms and the maintenance of populations (Bailey et al. 2020). The physical and structural characteristics of the environment play an important role in modifying insect movement (Grez and Villagran 2000). For instance, changes in landscape structure influenced the movement capacity of the tenebrionid beetle *Eleodes obsoleta* (Say) (Wiens et al. 1997, Grez and Villagram 2000); beetles on bare ground moved faster than individuals in the grass patches. Bykova and Blatt (2018) showed that mineral and organic soils favored the burrowing and movement of the carrot weevil Listronotus oregonensis (LeConte) relative to burrowing and movement in sand. Corneil and Wilson (2017) showed that light intensity and temperature influenced the behavior of the adult pales weevil Hylobius pales (Herbst). Adult H. pales moved up tree stems from the base when temperatures fell below 10 °C, and movement was not affected by light intensity. Joseph and Rijal (2019) observed that food-deprived fifth instar of the western tarnished plant bug, Lygus hesperus (Knight) traveled further with an increase in surface temperature. Similarly, adult Bagrada hilaris (Burmeister) moved further when starved and subjected to high-temperature conditions (Grettenberger and Joseph 2019). These studies suggest that insect dispersal and movement are influenced by biotic and abiotic factors. Thus, the factors influencing insect mobility should be identified and incorporated when developing an effective IPM strategy (Irwin 1999).

The walking behavior of adult *S. venatus vestitus* is still not well documented. Adult *S. venatus vestitus* is a nocturnal insect and is only reported to move by walking (Dupuy and Ramirez 2016). Previously, Gireesh and Joseph (2021) showed that adult *S. venatus vestitus* moved to and from harvested and nonharvested sod in a field, suggesting that adults emerging from the nonharvested, fully grown areas of sod could reinfest newly developing grass stems in

harvested areas. Sod farms encompass a vast hectarage in the southern USA. On sod farms and golf courses, management sprays are limited to affected areas due to high insecticide and application costs. Any knowledge of the walking behavior of *S. venatus vestitus* and the factors that influence its movement will immensely improve integrated pest management strategies by reducing insecticide use. Similarly, on established turfgrass, such as golf courses or lawns, knowledge of *S. venatus vestitus* mobility will help predict reinfestation windows when there is a resident *S. venatus vestitus* population in adjacent courses or lawns. Thus, the objective of the current study was to determine the influence of abiotic factors such as temperature, relative humidity, and wind speed on the walking capacity of male and female *S. venatus vestitus*.

#### **Materials and Methods**

General methods. In 2019 and 2020, experiments were conducted at the University of Georgia, Griffin campus. For all experiments, *S. venatus vestitus* adults were handpicked from turfgrass fields between 9 and 10 PM when the temperature was approximately 21.7 °C and relative humidity (RH) was 51.8%. *S. venatus vestitus* adults were not collected on rainy days. *Sphenophorus venatus vestitus* adults emerged from the soil and were mostly found copulating on the turfgrass surface. The adults were immediately sexed and temporarily stored in plastic containers. Males and females were identified by the presence of a groove or depression on the metasternum and the first two abdominal sterna (Johnson-Cicalese et al. 1990). *Sphenophorus venatus vestitus* adults were identified from other billbug species based on the large punctures (and similar size of the lateral pronotal punctures) on the outer surface of the profemur (Vaurie 1951, Johnson-Cicalese et al. 1990). The turfgrass sites were not sprayed with any insecticides, but fungicides and herbicides were applied as needed. These sites were under a regular mowing regime, with at least one mowing per week.

**Laboratory assay.** For the laboratory assay, field-collected *S. venatus vestitus* adults were transferred into 120 mL specimen cups with freshly cut bermudagrass (*Cyanodon* spp.) clippings. Adults of *Sphenophorus venatus vestitus* were acclimated at 15, 18, 21, 28, and 32 °C for 24 h by placing the specimen containers with adults in an environmentally controlled chamber (Percival Scientific, Perry, Iowa, USA) maintained at 16:8 L:D [photoperiod] and 50% RH. These acclimated adults were used in laboratory and semifield assays.

For the laboratory assay, S. venatus vestitus adults were individually transferred from the environmentally controlled chamber to 9 cm diameter Petri dish arenas. The assays were conducted on filter paper and sand substrates at room temperature (21 °C). For the filter paper assay, 9 cm diameter Whatman #1 filter paper (Whatman International Ltd, Maidstone, England) was used. For the assay with sand, 26.2 g of white, dry sand, with a particle size of 0.125-0.25 mm (topdressing sand, Butler Sand Company, Butler, GA) was used. The sand was spread inside the Petri dish to ~7 mm height. Thirty males and 30 females were used for each acclimated temperature treatment per substrate. The Petri dish arenas were placed on a glass surface inside a black wooden chamber (~60 cm tall × 30 cm wide, ×50 cm deep). Sphenophorus venatus vestitus adults are nocturnal (Huang and Buss 2009). Thus, the interior and exterior of the wooden chamber were painted black after sealing all cracks and crevices to prevent outside light from entering the chamber. The only light source inside the black wooden chamber was infrared lighting (AXTON, Infrared Illuminator, # AT8SB, North Salt Lake City, UT). Walking movement of adult S. venatus vestitus was videotaped using an Ethernet camera (acA1300-60 gm, Basler, Inc., Exton, PA) for 1 h. Total distance and average velocity moved by the adults were quantified using Noldus EthoVision XT software (Version 11.5, Noldus Information

Technologies, Wageningen, The Netherlands). Three *S. venatus vestitus* adults were individually assayed using EthoVision XT software.

**Semifield assay.** The effects of temperature on the mobility of *S. venatus vestitus* adults were further measured in a semifield environment in a darkroom. The floor of the room was concrete with no gaps. The method for quantifying the semifield walking behavior of S. venatus vestitus adults was modified and adapted from the methods used in Grettenberger and Joseph (2019). Field-collected S. venatus vestitus adults were sorted into males and females and introduced into an environmentally controlled chamber. The adults were exposed to 15, 18, 21, 28, and 32 °C for 24 h. The semifield assay was conducted at room temperature (21 °C). Once the acclimated adults were individually removed from the environmentally controlled chamber, they were immediately used for the assay by placing them on the concrete surface in the darkroom. At the start of the assay, each adult's position was marked on the concrete floor using a colored chalk. Thereafter, the position was marked every minute for up to 15 minutes. A total of 30 males and 30 females were assayed from each temperature treatment. After the assay, the distance between marked points at every minute was measured using a measuring tape, and the total distance moved was quantified. The net distance moved by the adults was determined after measuring the distance between the start position (at 0 minutes) and the final position (at the end of 15 minutes). An LED headlamp (Coast products, Inc. Portland, OR) was used to observe S. ventaus vestitus movement in the dark.

**Field assay.** The field assay was conducted at night between 9 PM and 10 PM on asphalt pavement at the University of Georgia, Griffin, GA. The paved surface had minimal cracks and the adult *S. venatus vestitus* could not hide. The surface temperature of the pavement was recorded at the beginning of the trial from three random spots using a laser thermometer

(infrared 12 10:1, model # 2267-20, Milwaukee Electric Tool Corporation, Brookfield, WI). Air temperature and wind speed were recorded before the assay via a handheld weather station (model # WM-4, Ambient Weather, Chandler, AZ) at approximately 1.2 m high. Light intensity was measured before each assay using a light meter (URCERI, Light Meter, MT-912 # 20190107278, Shenzhen, China). The field-collected S. venatus vestitus adults were sorted into males and females. The adults were individually marked with fluorescent markers (painters®, Elmer's products INC, Westerville, OH) on the pronotum before they were placed on the asphalt surface. The method used to determine walking behavior was adapted from Grettenberger and Joseph (2019). Once an adult was placed on the surface, its position was marked, and positions were further marked every minute using a colored chalk. The movement of fluorescent-color marked adults was tracked using an ultraviolet flashlight (BRIONAC, Zhejiang, China) for 15 minutes. For measurement, the same procedure used in the semifield assay was adopted, and the total and net distances moved by the adults were calculated. A total of 185 S. venatus vestitus adults (85 males and 100 females) were individually assessed. At the end of the assay, the surface temperature of the turfgrass foliage was recorded from three random spots. **Statistical analyses.** All analyses were performed using SAS software (SAS Institute 2017). For laboratory and semifield assays, total distance and net distance data were cube root transformed to satisfy normality assumptions (Shapiro-Wilk test; p < 0.05) before analysis of variance (ANOVA) using the PROC mixed procedure in SAS. Means were separated using the Tukey-Kramer test ( $\alpha = 0.05$ ). For the field study, the total and net distance data were cube root transformed, and multiple linear regression with surface temperature, wind speed and RH was performed using the PROC REG procedure in SAS. The association between total and net distances moved by S. venatus vestitus adults in the semifield assay was assessed using Pearson's correlation coefficient and the PROC CORR procedure in SAS. In the field, the associations between the surface and air temperatures, wind and relative humidity were also assessed in addition to the total and net distances moved by *S. venatus vestitus* adults using Pearson correlation coefficients.

#### **Results**

## Laboratory assay

Filter paper substrate. Temperature, sex and the interaction between temperature and sex significantly influenced the mobility of *S. venatus vetsitus* adults on filter paper (Table 4.1). To understand the effects of temperature, analysis was performed separately for each sex. For *S. venatus vestitus* males, the distance moved was significantly greater at 28 °C than at 15, 18, 21 and 32 °C (F = 11.8, df = 4, 115, p < 0.001; Fig. 4.1A). When the distances moved at 15, 18, 21 and 32 °C were assessed, males moved significantly farther at 15 °C than at 32 °C. However, there was no significant difference in the distance moved by males between 15, 18, and 21 °C or between 18, 21 and 32 °C. For *S. venatus vestitus* females, there was no significant difference in the total distance moved between 15, 18, 21, 28, or 32 °C (F = 0.8, df = 4, 116, P = 0.513; Fig. 4.1B).

The distance moved by male and female *S. venatus vestitus* was evaluated at various temperatures. Males traveled a significantly greater distance than females at 28 °C (F = 14.6, df = 1, 29, p = 0.006; Fig. 4.2A). However, there was no significant difference in distance moved between *S. venatus vestitus* males and females at 15 (F = 1.9, df = 1, 29, p = 0.169), 18 (F = 0.0, df = 1, 29, p = 0.905), 21 (F = 3.9, df = 1, 29, p = 0.056), or 32 °C (F = 1.9, df = 1, 29, p = 0.172) (Fig. 4.2A).

**Sand substrate.** There were significant effects of temperature and the interaction between temperature and sex on the mobility of *S. venatus vestitus* adults (Table 4.1). However, there was

not a significant effect of sex of *S. venatus vestitus* adults on the distance traveled in the sand substrate (Table 1). *Sphenophorus venatus vestitus* males moved significantly farther at 21 and 32 °C than at 15 and 18 °C (F = 5.8, df = 4, 116, p < 0.003; Fig. 4.1C). However, the distances traveled between 21, 28, and 32 °C or 15, 18 and 28 °C were not significantly different. Female *S. venatus vestitus* traveled significantly farther at 28 °C than at 15, 18 and 32 °C (F = 14.8, df = 4, 116, p < 0.001; Fig. 4.1D). However, the distances moved between 21 and 28 °C, 21 and 18 °C, 18 and 32 °C, or 15 and 32 °C were not significantly different (Fig. 4.1D)

The distances moved by *S. venatus vestitus* males and females was evaluated at various temperatures on sand substrates. *Sphenophorus venatus vestitus* males moved significantly greater distances than females at 15 (F= 8.8, df = 1, 29, p < 0.006) and 32 °C (F = 10.5, df = 1, 29, p = 0.003; Fig. 4.2B). At 28 °C, *S. venatus vestitus* females traveled a significantly greater distance than males (F = 4.9, df = 1, 29, p = 0.034; Fig. 4.2B). There was no significant difference between *S. venatus vestitus* males and females in distance moved at 18 (F = 2.2, df = 1, 29, p = 0.147) and 21 °C (F = 0.0, df = 1, 29, p = 0.877).

**Semifield assay.** Temperature had a significant effect on the total distance moved by *S. venatus vestitus* adults (Table 2). *Sphenophorus venatus vestitus* males moved significantly greater total distances at 21 and 28 °C than at 15 °C (F = 4.1, df = 4, 115, p = 0.003; Fig. 4.3A). However, the total distances moved by males between 21 and 28 °C, 18, 21, 28 and 32 °C, or 15, 18 and 32 °C were not significantly different. Females of *Sphenophorus venatus vestitus* traveled a significantly greater total distance at 21 °C than at 15 or 32 °C (F = 5.9, df = 4, 116, p = 0.002; Fig. 4.3B). However, there was no significant difference in the total distances traveled by females between 15 and 32 °C, 18, 21 and 28 °C or 15, 18, 28, and 32 °C. The sex of adult *S. venatus vestitus* and the interaction between sex and temperature did not significantly affect the

total distance traveled in the semifield assay (Table 4.2). Similarly, temperature, sex and their interaction did not significantly influence the net distance moved by *S. venatus vestitus* adults (Table 4.2; Fig. 4.3C and D). A strong positive association was noted between the total and net distance moved by *S. venatus vestitus* adults (r = 0.87, N = 299, p < 0.001).

**Field assay.** There was no significant relationship between the increase in surface temperature and the total distance ( $R^2 = 0.03$ , p = 0.427; Fig. 4.4A) or net distance ( $R^2 = 0.03$ , p = 0.458; Fig. 4.4C) moved by *S. venatus vestitus* adults. Similarly, changes in relative humidity were not significantly related to the total ( $R^2 = 0.01$ , p = 0.325) and net ( $R^2 = 0.02$ , p = 0.532) distances moved by *S. venatus vestitus* adults (Fig. 4.4B and E). The changes in the surface and air temperatures were significantly correlated (r = 0.39, R = 184, R = 0.001). Wind speed had a significant negative relationship with the total distance ( $R^2 = 0.03$ ; R = 0.031; Fig. 4.4C) and net distance ( $R^2 = 0.03$ , R = 0.054; Fig. 4.4F) traveled by *S. venatus vestitus* adults. Moreover, there was a significant association between the total distance traveled and net distance traveled by *S. venatus vestitus* adults (R = 0.81, R = 184, R = 0.001). Light intensity readings were zero lux during all the assays.

#### **Discussion**

The movement of insect pests determines their abundance and distribution in space and time (Mazzi and Dorn 2011). Long-distance movement is usually linked to the colonization rate of pests (Dingle 1996). In contrast, movements within a field or between habitat patches are related to pest reproductive success and effective utilization of resources. We sought to understand the walking behavior and dispersal capability of *S. venatus vestitus* adults in relation to abiotic factors such as temperature, relative humidity, and wind speed. The laboratory and semifield assays showed that temperature variations influenced the walking capacity of *S. venatus vestitus* 

adults. Males and females of *S. venatus vestitus* moved farther when the temperature increased from 15 to 28 °C. These results suggest that *S. venatus vestitus* adults can move long distances when the temperatures go beyond the minimum threshold. However, field data showed that the net and total distances moved by males and females of *S. venatus vestitus* were not related to temperature. Some individuals moved longer with an increase in temperature, but most were not influenced by temperature increases. Previous research on sod farms showed that overlapping generations of *S. venatus vestitus* are aggregated in the field, especially before the onset of winter (Gireesh et al. 2021). This and field results from the current study suggest that *S. venatus vestitus* tends to move shorter distances, seeking newly developing turfgrass shoots. Moreover, the night temperature (minimum temperature) in turfgrass production areas (e.g., Marshallville, GA) rarely exceeds 25 °C (Fig. 4.5).

In the laboratory and semifield settings, temperature affected the walking capacity of *S. venatus vestitus* adults. Before mobility was measured, adults were acclimatized to a specific temperature for a stipulated time. The distances moved by *S. venatus vestitus* males and females at 15 °C were comparable to the distances moved at higher temperatures (at least 18 or 21 °C). This suggests that *S. venatus vestitus* individuals are capable of walking at lower temperatures. The hardness of the substrate surface influenced the movement of male *S. venatus vestitus* especially; males walked more than two times further on filter paper than on sand. Although males and females of *S. venatus vestitus* moved a greater distance as temperature increased, they hardly moved away from the release point. Net distance moved by *S. venatus vestitus* adult was not affected by temperature, although total and net distance were strongly correlated.

In the field, temperature had no effect on *S. venstus vestitus* adult movement, regardless of sex. Although the exact reasons for this behavior are not clear, several factors could be

contributors. First, S. venstus vestitus adults were collected from a turfgrass field and were likely exposed to varied temperature regimes before collection. The adults hide within the turfgrass canopy or soil in some instances before they emerge at night. According to Ferro et al. (1990), variations in microhabitat temperature, humidity, wind speed, and radiation can affect insect mobility (Bernaschini et al. 2020, Rytteri et al. 2021). Additionally, a linear increase in movement with increasing temperature is not necessarily a general rule among insects. The flight capacity of the olive fly Bactrocera oleae Rossi was reduced when exposed to temperatures ranging between 35 and 37 °C (Roitberg et al. 2009, Martini and Stelinski 2017). Additionally, the plum curculio Conotrachelus nenuphar (Herbst) walks only until 20 °C, and then initiates flight above this temperature threshold (Prokopy et al. 1999, Martini and Stelinski 2017). Second, the age, mating status, and ovarian maturity of S. venatus vestitus females collected from the field were unknown, and these factors could affect walking behavior. When the effects of sex and mating status on self-directed dispersal by the whitefly parasitoid *Eretmocerus eremicus* Rose & Zolnerowich were studied, nonmated parasitoids flew 2.9 times longer than mated parasitoids (Bellamy and Byrne 2001). Gireesh and Joseph (2021) indicated that S. venatus vestitus adults are spatially aggregated in distribution in sod fields. It is possible that they do not have incentives to disperse as long as food and mates are available within shorter distances. Accessibility to food and mates could contribute to variations in walking behavior, and these factors warrants further research. Finally, S. venatus vestitus males are attracted by and orient toward host plant volatile organic compounds (VOCs) (Duffy et al. 2018). This controlled movement toward host plant VOCs helps males conserve energy rather than indulging in random walking to search for food and mates (Duffy et al. 2018). Host plant VOCs near the experimental

area could have influenced the orientation and walking behavior of *S. venatus vestitus* adults in our study.

The results show that wind speed affected the mobility of *S. venatus vestitus* adults, but relative humidity did not affect the mobility of adults. The *S. venatus vestitus* adults traveled less when the wind speed increased and moved ~1000 cm in 15 mins at lower winds (< 0.1 m/s). Previous studies have shown that the dispersal distance of the grasshopper *Chorthippus parallelus* (Zetterstedt) in a grazed pasture was reduced at wind speeds of more than 2.5 m/s (Gardiner 2006, Gardiner and Dover 2008). In another study, a wind speed of 2 m/s reduced the number of ovipositions made by the aphid parasitoid *Aphidius rosae* Haliday because females spent much more time resting than searching for hosts (Fink and Volkl 1995). Similarly, the effect of relative humidity on insect movement and dispersal has been widely studied (Zhang et al 2008, Vail and Smith 2001, Martini and Stelinski 2017), and changes in relative humidity did not affect the dispersal of the Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) (Martini and Stelinski 2017).

Overall, the results from assays conducted in all three settings suggest that temperature influenced the walking capacity of *S. venatus vestitus* adults. Even though temperature was not related to the walking capacity of *S. venatus vestitus* in the field, some individuals moved ~800 cm in 15 mins at ~30 °C. The impact of the variable microclimate of turfgrass on *S. venatus vestitus* adults before collection from the field may have affected their walking capacity. In addition, the ways that age, mating status of females, and specific physiology of adults affect walking capacity are not known. Based on our field data, ~15% of the total *S. venatus vestitus* adults tested in the field assay did not move at all. Additionally, *S. venatus vestitus* adults were less likely to move as the wind speed increased. Gireesh et al. (2021) showed that *S. venatus* 

vestitus adults and larvae are spatially aggregated in sod farms. If resources are readily available, insects tend not to move until they have entirely utilized the available resources (Bell 1990). These results will contribute to determining the population of *S. venatus vestitus* in a given area when adult densities are captured using in sampling method, such as pitfall trap, which can further utilized to determine the adult threshold for management decisions. More studies are warranted to determine the specific factors that adversely affect the movement of *S. venatus vestitus* in turfgrass production sites, golf courses, and lawns. The current study results will improve the integrated management approaches for *S. venatus vestitus* in residential and commercial settings.

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## **Conflicts of interest/Competing interests.**

The authors have no conflict of interest.

## Availability of data and material

Data will be available upon request.

## **Code availability**

Not applicable

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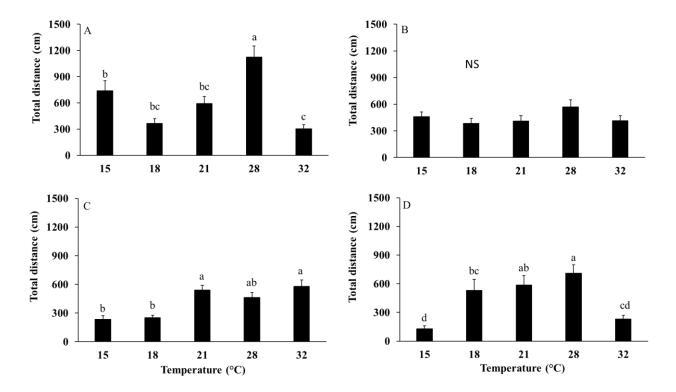
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**Table 4.1.** Effect of temperature and sex on the total distance moved by *S. venatus vestitus* adults in laboratory assay.

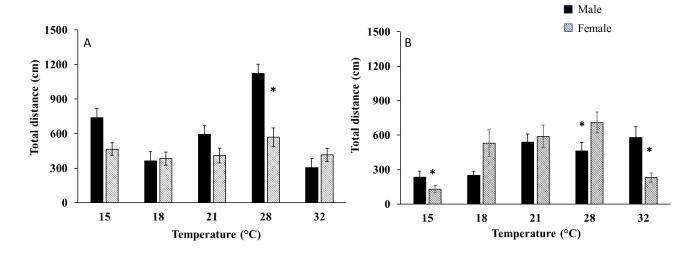
Substrate	Factor	Distance			
		F	df	p	
Filter paper	Temperature	7.80	4260	< 0.001	
	Sex	8.13	1, 260	0.004	
	$Temperature \times Sex$	4.02	4, 260	< 0.001	
Sand	Temperature	14.4	4, 261	< 0.001	
	Sex	0.02	1, 261	0.876	
	$Temperature \times Sex$	5.88	4, 261	0.002	

**Table 4.2.** Effect of temperature and sex on the distance moved by *S. venatus vestitus* adults in semi-field assay.

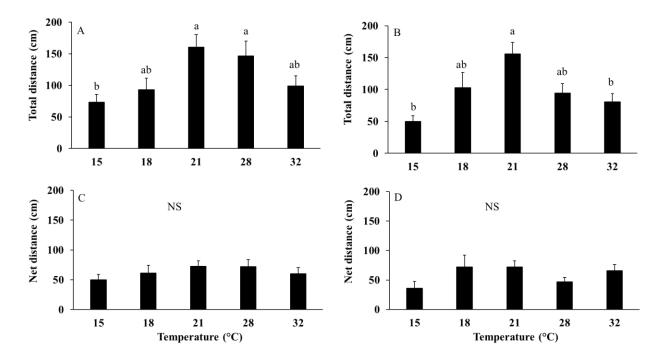
Factor	Total distance			Net	distance	
	F	df	p	F	df	p
Temperature	4.29	4, 261	0.002	2.01	4, 261	0.932
Sex	0.73	1, 261	0.394	0.33	1, 261	0.564
$Temperature \times Sex$	0.33	4, 261	0.855	0.34	4, 261	0.847



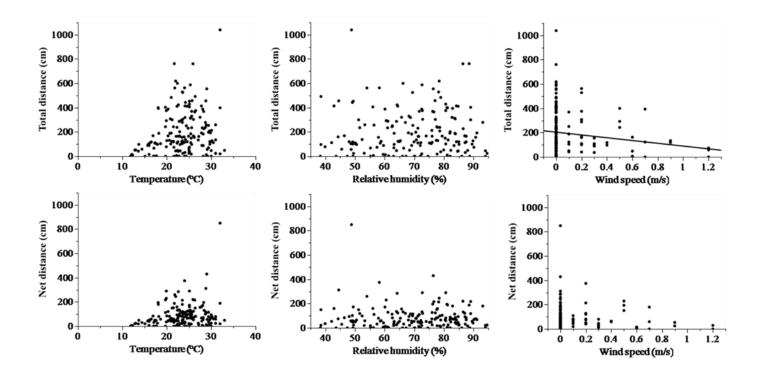
**Fig. 4.1.** Mean ( $\pm$  SE) total distance moved by males and females *S. venatus vestitus* on filter paper substrate (A and B) and on sand substrate (C and D) under various temperatures. Bars with the same letters are not significantly different (Tukey-Kramer Test,  $\alpha = 0.05$ ).



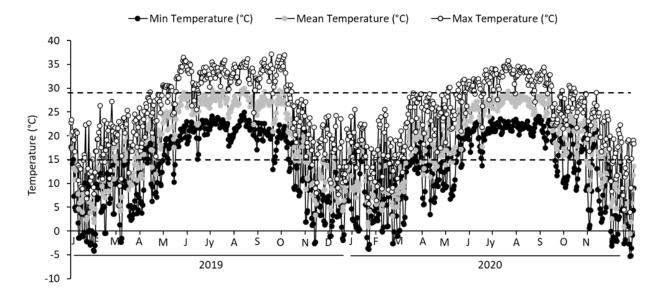
**Fig. 4.2.** Mean ( $\pm$  SE) total distance moved by *S. venatus vestitus* males and females on (A) filter paper and (B) on sand substrate. Significant effects at  $\alpha = 0.05$  are indicated with an asterisk.



**Fig. 4.3.** Mean ( $\pm$  SE) total distance (A and B) and net distance (C and D) moved by *S. venatus vestitus* males and females in semi-field under various temperature. Bars with the same letters are not significantly different (Tukey-Kramer Test,  $\alpha = 0$ .



**Fig. 4.4.** Linear regression analysis showing relationship between total and net distance walked by adult *S. venatus vestitus* at various temperature (A and D) relative humidity (B and E) and wind speed (C and F) in field. Males and females combined.



**Fig. 4.5.** Minimum, mean, maximum daily temperature (°C) of Marshallville, Georgia (USA) for from 1 Jan 2019 to 31 Dec 2020. http://www.prism.oregonstate.edu/documents/PRISM\_datasets.pdf

## **CHAPTER 5**

# SPATIAL DISTRIBUTION OF HUNTING BILLBUGS (COLEOPTERA: CURCULIONIDAE) IN SOD FARMS

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Simple Summary: The hunting billbug is the most dominant and damaging insect pest species of sod farms (where turfgrass is commercially produced) in Georgia (USA). The larvae feed within the turfgrass stem, and roots affect turfgrass growth. Hunting billbugs are usually managed using insecticides. However, the application of insecticides to entire sod fields is not an economically and practically feasible option. Thus, an improved sampling plan for larvae and adults is warranted to improve management decisions. The current study was aimed at understanding the spatial distributions of hunting billbug larvae and adults in sod farms using geospatial techniques. The larvae and adults were sampled using soil cores and pitfall traps, respectively. After evaluating two geospatial techniques, the distribution pattern of hunting billbug larvae and adults within the sod farms was aggregated. The presence of billbugs in samples collected at 4 m apart suggests active infestation. This information will help develop integrated pest management for hunting billbug in sod farms and reduce insecticide use, benefiting growers and the environment alike.

**Abstract:** The hunting billbug, *Sphenophorus venatus vestitus* Chittenden (Coleoptera:

Curculionidae), is an important turfgrass pest, especially in sod farms. *S. venatus vestitus* larvae feed on the stems and roots of turfgrass. Damaged turfgrass is loosely held together and poses a challenge for machine harvesting. Additionally, the normal growth of turfgrass is affected, especially after winter dormancy. Because *S. venatus vestitus* larvae are hidden inside the stems or under the soil, larval management is challenging. To improve sampling and management, the spatial distribution patterns of *S. venatus vestitus* larvae and adults were assessed at four sod farm sites with a history of *S. venatus vestitus* infestation in central Georgia (USA). The larvae were sampled by soil cores using a hole cutter, whereas adults were collected using pitfall traps for 7 d. The spatial distributions of larvae and adults was analyzed using SADIE and variograms. The SADIE and variogram analyses revealed a significant aggregation pattern for adults, whereas aggregated distributions were detected for larvae with variogram analyses. The average ranges of spatial dependence for larval and adult samples were 3.9 m and 5.4 m, respectively. Interpolated distribution maps were created to visually depict *S. venatus vestitus* infestation hotspots within the sod farms.

**Keywords:** *Sphenophorus* spp.; turfgrass; sampling plan; IPM; SADIE; variogram

#### Introduction

The hunting billbug, Sphenophorus venatus vestitus Chittenden (Coleoptera: Curculionidae), is a serious pest of warm-season turfgrass in the USA (Dupuy and Ramirez 2016). In Georgia, bermudagrasses (Cynodon dactylon (L). Pers), zoysiagrass (Zoysia spp.), St. Augustinegrass (Stenotaphrum secondatum (Walter) Kuntze), bahiagrass (Paspalum notatum Flugge), and centipedegrass (Eremochloa ophiuroides (Munro) Hack) are the major warm-season grasses and are produced on sod farms. These turfgrasses are grown over approximately 10,785 ha across 64 of 159 counties and are valued at \$118 million USD (Wolfe and Stubbs 2018). S. venatus vestitus is present at high densities in Georgia sod farms (Gireesh and Joseph 2020). Females prefer actively growing, thick stolons for oviposition, as eggs are inserted into the stolon (Dupuy and Ramirez 2016). The first instars feed within stems, and the late instar larvae leave the stolon and consume the roots (Huang and Buss 2009). The larvae go through five instars before pupating in the soil. Adults overwinter in protected areas of the soil, although the larval stages are also found in the soil during winter months (Dupuy and Ramirez 2016). In central Georgia, the adults emerge from the overwintering sites beginning in late winter, while overwintering larvae continue to develop in spring, and those adults emerge in late spring (Gireesh and Joseph 2020). The damage and problem from S. venatus vestitus feeding develop differently in various commercial turfgrass settings. In golf courses, because the adults and larvae of S. venatus vestitus consume on the roots, injury symptoms initially appear as chlorosis. Over time, the affected turfgrass develops brown patches (Doskocil and Brandenburg 2012). However, in sod farms, injury symptoms are rarely manifested because the sod is harvested rather quickly, e.g., within 1.5 years. Instead, the injured stolons and roots disintegrate during machine harvesting and pose a considerable challenge to growers (C. Carter, personal communication). In addition,

the stress from *S. venatus vestitus* feeding and oviposition affects the normal growth and development of zoysiagrass, especially when the grass breaks winter dormancy in spring (Dupuy and Ramirez 2016). The slow growth habit of zoysiagrass may contribute to the population density of *S. venatus vestitus* in sod farms. Any delay in the growth and development of zoysiagrass poses an economic challenge to growers, as the sod is not delivered at the scheduled times.

Implementation of a successful integrated pest management (IPM) program relies on determining population thresholds by using reliable pest monitoring tools (Rijal et al. 2016, UCIPM 2020). Mean- and variance-based models can be used to develop sampling plans for several arthropod pests (Taylor 1984, Young and Young 1990, Kuno 1991), but these models only use the frequency distributions of pest counts without considering the spatial locations of the pest population samples. Therefore, these models are not suitable for characterizing within-field population distributions or for developing sampling plans (Rijal et al. 2014, Reay Jones et al. 2019). Due to the lack of two-dimensional information for individual sample locations, the information derived from these mean-variance methods lacks many ecological interactions (Hardwood et al. 2001, Winder et al. 2019). Another benefit of spatial distribution sampling is to develop a visual representation of pest infestations in the field by creating prediction maps and kriging maps in variograms (Frank et al. 2011, Rhodes et.al 2011, Rijal et al. 2016,) and "red and blue" maps in SADIE (Perry 1995, Perry et al. 1999). This type of visual representation can be useful for site-specific pest management efforts.

There are many examples of how spatial distribution information can be used to understand the ecology and management of arthropod pests. A spatial distribution study of the annual bluegrass weevil *Listronotus maculicollis* Dietz (Coleoptera: Curculionidae) in golf courses showed

aggregates of adults and larvae along the edges of fairways (McGrow and Koppenhofer 2010). Additionally, this study suggested that *L. maculicollis* can spread to entire golf courses from an initially aggregated colonization. Similarly, previous studies have developed an efficient and quantitative sampling strategy to assess grape root borer, *Vitacea polistiformis* (Harris), (Lepidoptera: Sesiidae) infestations in Virginia vineyards (Rijal et al. 2014); alfalfa weevil, *Hypera postica* (Gyllenhal) and their natural predators; (*Coccinella septempunctata* L., *Adalia bipunctata* L., *Nabis americoferus* Carayon and *N. ferus* L.) (Shreshta et al. 2020); Kudzu bug, *Megacopta cribraria* [F.] and their egg parasitoid *Paratelenomus saccharalis* Dodd (Hymenoptera: Platygastridae) in soybean (Knight et al. 2017); thrips (Thysanoptera: Thripidae) in cotton; *Gossypium hirsutum* L (Reay Jones et al. 2019); and cereal leaf beetle, *Oulema melanopus* [L.] in wheat (*Triticum aestivum* L) (Reay Jones 2017). The spatial distributions of *S. venatus vestitus* in sod farms have not been studied. Obtaining this information could help develop an effective sampling plan and improve insecticide application strategies for *S. venatus vestitus* control.

On sod farms, *S. venatus vestitus* is managed by using insecticides (Gireesh and Joseph 2020). Because sod farms are composed of vast land areas that are under production, application of insecticides on entire sod fields can be logistically and economically impractical in all instances; thus, growers' resort to spotting the applications of insecticides based on the history of *S. venatus vestitus* incidence in a specific field. Here, an improved sampling method for *S. venatus vestitus* could be beneficial and will aid management decisions. Currently, there are no sampling plans for growers that guide *S. venatus vestitus* management decisions. The current plans mostly depend on visual inspections around the pavements for walking adult *S. venatus vestitus* that are conducted early in the morning, approximately one hour after sunrise. An understanding of how

S. venatus vestitus is spatially distributed within sod fields will help to develop an effective sampling strategy using available monitoring tools.

Several geospatial methods, such as spatial analysis by distance indices (SADIE) and variograms, can be used to assess insect spatial distributions (Sciarretta and Trematerra 2014, Silva et al. 2018, Winder et al. 2019, Shreshta et al. 2020, Ribeiro et al. 2020). Variograms are commonly used to analyze and model the spatial dependences among individuals in a population (Winder et al. 2019, Ribeiro et al. 2020). Spatial dependence (or spatial autocorrelation) can be used to define the sampling scales for independent samples and to quantify the spatial patterns of insect species (Williams et al. 1992, Rijal et al. 2014). SADIE is another advanced statistical method that has been used to estimate the spatial distribution patterns of insect species based on ecological count data (Mcgraw and Koppenhofer 2010, Winder et al. 2019). SADIE has also led to an improved understanding of pest dispersal (Ferguson et al. 2000, Kim et al. 2007, Mcgraw and Koppenhofer 2010), predator-prey dynamics (Mcgraw and Koppenhofer 2010, Winder et al. 2019, Shreshta et al. 2020), and the influence of habitat management on insect abundance (Donovan et al. 2007, Winder et al. 2019). The objective of the current study was to determine the spatial distributions of S. venatus vestitus in Georgia sod farms. We used variograms and SADIE to characterize the spatial distribution of *S. venatus vestitus* adults and *Sphenophorus* spp. larvae. More than 98% of Sphenophorus spp. adults sampled were S. venatus vestitus, while the remaining were S. cariosus Olivier and S. inaequalis Say. Thus, the larval samples could include other Sphenophorus spp. species, and there are no morphological keys available to easily distinguish billbug larvae.

#### **Materials and Methods**

## **Study Sites and General Method**

Four sod fields with a history of billbug infestations in Marshallville, Georgia, USA were selected for this study. In 2019, the turfgrass genotypes in the two sod field sites were a 'Zenith' (Z. *japonica*) zoysiagrass (designated as site 1; 32.3843, –3.9918) and a 'TifWay' bermudagrass (site 2; 32.4233, –85. 8816). In 2020, the turfgrass genotypes at two different sites were 'Zeon' (Z. *matrella*) zoysiagrass (site 3; 32.4241, –83.8872) and 'TifTuf' bermudagrass (site 4; 32.4425, –83.9978).

In 2019 and 2020, Sphenophorus spp. larvae were sampled, whereas S. venatus vestitus adults were sampled only in 2020. In 2019, larval sampling of *Sphenophorus* spp. was conducted between September and December. In 2020, larvae and adults were sampled in May and June. Based on the data from Gireesh and Joseph (Gireesh and Joseph 2020), the S. venatus vestitus adults continuously emerge in spring and summer, which indicates the occurrence of overlapping generations in late summer and fall. Therefore, multiple stages of S. venatus vestitus larvae are found in the sod fields in the fall. This also suggests that the adults and various stages of S. venatus vestitus larvae overwinter in central Georgia. Those overwintering larvae continue to develop and pupate and emerge as adults in late spring or summer in the following year (Gireesh and Joseph 2020). Thus, the larval samplings conducted in fall and spring were on the same overlapping generations of S. venatus vestitus. For site 1, the larval samples were collected in September because the grower was harvesting sod from other areas of the field. Adult S. venatus vestitus were sampled only at sites 2, 3, and 4 because the sod at site 1 was harvested immediately after larval sampling. As previously described, identification of larvae at the species level is challenging and was characterized as *Sphenophorus* spp. adults were identified to the

species level by using the morphological characteristics described in previous studies (Vaurie 1951, Johnson-Cicalese 1990). Three sites (2, 3, and 4) bordered a wood line, whereas site 1 bordered a dirt road on one side. The sod in all fields was fully grown and ready for harvest. Insecticides targeting *Sphenophorus* spp. control were not applied in 2019 and 2020 at any of the selected sites. The sites were subjected to routine mowing (twice a week), fertilizer and irrigation regimes.

#### **Sampling**

The sampling plan consisted of 90 sample points in a square grid, with ~3 m between any two sample points and covered a total of 27 m  $\times$  30 m (length  $\times$  width) of the field. Nine sampling points for sites 2, 3, and 4 were along the X coordinate and ten points were along the Y coordinate. For Site 1, ten sampling points were along the X coordinate, and nine points were along the Y coordinate. Larval sampling for sites 1 and 2 was initiated on 30 September and 15 October 2019, respectively, and was completed on 14 December for both sites (Table 1). Larval sampling for sites 3 and 4 was conducted in 2020. For site 3, sampling was initiated on 19 May and completed on 8 June 2020. For site 4, sampling began on 1 June and ended on 8 June 2020. For larval sampling, the soil was sampled ~10 cm deep and used a 10 cm diameter Par Aide Lever Action Hole Cutter (Par Aide product company, St. Paul, MN, USA). Similarly, two more samples were obtained in the following weeks from a single sampling point (a total of three soil cores were drawn from each sampling point). These three soil samplings from a single point were 1–2 cm apart. The soil samples were transported to the laboratory in sealed plastic bags. In the laboratory, larvae were extracted from the soil core samples contained grass roots, thatch, and soil. Adult sampling for site 2 was initiated on 26 May and completed on 8 June 2020. For site 3, sampling started on 27 May and was completed on 10 June 2020. Adult sampling for site

4 began on 10 June and ended on 24 June 2020. The adults were sampled using pitfall traps that were constructed by using 11.5-cm diameter and 7.5-cm deep clear plastic containers. The containers were partially closed with Styrofoam plates to prevent rainwater from entering the traps, and ethyl glycol was added, which acted as a preservative agent for the insects. Ninety traps were deployed at each site, and the traps were monitored weekly and were kept in place for three weeks after the date of installation. The trap contents were filtered using a sieve and were transported to the laboratory for further identification. Two geospatial methods, variograms and SADIE, were used to characterize the spatial distribution patterns of billbugs within the fields.

### Variogram Analysis

Variograms are a commonly used method for depicting the spatial dependency of sample points.

Spatial dependence is determined by developing an experimental semivariogram.

Mathematically, the semivariogram  $(\gamma)$  can be represented by

$$\hat{\gamma}(h) = \frac{1}{2} n(h)_{i=1} \sum_{n(h)} z(xi) - z(x_i + h)^2$$

where  $\hat{\gamma}(h)$  is the estimated semivariance for the entity of interest (z) at all points (xi), which are separated by lag distance (h), and n(h) is the number of sample pairs which are separated by lag distance h (Davis 1989).

All variogram models were created using the geostatistical software GS+ (Version 10, Gamma Design Software, LLC, Plainwell, MI). Variogram models have three parameters, range, sill (C<sub>0</sub> + C), and nugget (C<sub>0</sub>), and the values of these parameters determine the shape of the variogram. The semivariance value at which the variogram plot reaches a plateau is the sill, while the semivariance value at zero lag distance is called the nugget (Liebhold et al. 1983, Isaaks and Srivastava 1989). The best-fitting variogram models were used based on two criteria, the highest

 $y^2$  value or the lowest residual sum of squares (RSS) (Park and Tollefson 2005, Rijal et al. 2016). Curvilinear models (e.g., spherical, exponential, and Gaussian) indicate aggregation distribution patterns, which mean that neighboring sample points are spatially dependent or autocorrelated. Straight-line models (e.g., nugget and linear) represent non-aggregation or random distribution patterns with no evidence of spatial autocorrelation (Isaaks and Srivastava 1989, Schotzko and O' Keffee 1989, 1990). All models with evidence of spatial dependency have an additional parameter called "range". Range is the maximum distance between samples below which spatial autocorrelation is present (Liebhold et al. 1983, Dale and Fortin 2014), and the range value plays a critical role in determining the adequate sampling distance for an unbiased, independent sampling plan (Frank et al. 2011, Rijal et al. 2016, 2014, Hahn et al. 2017, Ribeiro et al. 2020). The nugget-to-sill ratio  $(C_0/C_0 + C)$  and nugget were used to determine the degree of aggregation (Trangmar et al. 1986), where ratios <0.25, 0.25–0.75, and >0.75 indicated strong, moderate, and weak aggregation, respectively (Fariaz et al. 2002, Rijal et al. 2014, Carvalho et al. 2020, Santos et al. 2020). After selection of the variograms, interpolated pest distribution maps of billbug infestations were generated to visually demonstrate the infestation hot spots in the fields using the kriging interpolation technique (Rijal et al. 2014, Gs<sup>+</sup>, Lima et al. 2018, Martins et al. 2018)

#### **SADIE Analysis**

SADIE was used to characterize billbug spatial distribution patterns and test whether the resulting distributions were statistically significant (Perry 1995, Perry et al.1999). Characterization of spatial distributions using SADIE has advantages, especially for ecological data that are collected from spatially referenced samples in which the likelihood of having zero counts at multiple sampling points is high (Madden and Hughes 1995, Perry 1998, Perry et.al 2002). SADIE, as an additional method, is useful for addressing some of the shortcomings of the

variogram method, such as no determinations of spatial structures at low pest density with many zero counts (Blom 2001).

SADIE measures the overall aggregation based on the distance to regularity (D), which represents the minimum total distance that individuals would need to move to achieve the same number (i.e., mean) for each sample point. The magnitude of D is assessed by a randomization test in which permutations of all observed counts among the sample points are performed (Perry and Dixon 2002). This assessment provides an index of aggregation, Ia, with an associated probability, pa. Aggregated, uniform, and random distribution patterns are indicated by Ia > 1, Ia = 1, and Ia < 1, respectively (Perry 1995). The associated probability (i.e., Pa < 0.025) determines whether the resultant distribution pattern is significantly different from randomness (Perry 1995, Reay-Jones 2012, Rijal et al. 2014). Furthermore, mean clustering indices that represent all units in a patch are denoted by  $v_i$  with an associated p-value,  $Pv_i$ . In contrast, mean cluster indices that represent all units in a gap are denoted by  $v_j$  with an associated p-value,  $Pv_i$ . Values of  $Pv_i$  and  $Pv_j < 0.0025$  indicate statistically significant gaps and patches, respectively. Calculations of the aggregation index and index of clustering in SADIE were carried out using SADIEShell (Rothamsted Experimental Station, Harpenden Herts, United Kingdom).

#### **Results**

#### **Variogram Analysis**

Variogram analyses were used to evaluate spatial aggregation for *Sphenophorus* spp. larvae (Table 5.1, Figure 5.1) and *S. venatus vestitus* adults (Table 5.2, Figure 5.2).

The development of an omnidirectional variogram revealed aggregation patterns of larvae at three (i.e., sites 2, 3, and 4) out of the four sites. These results were based on the variogram

model, high  $r^2$  and low RSS and nugget-to-sill ratio (C<sub>0</sub>/C<sub>0</sub> + C). Based on the  $r^2$  and RSS values, the linear model fitted best for site 1 (Figure 5.3A), the Gaussian model for site 2 ( $r^2$  = 0.64) (Figure 5.3B), the exponential model for site 3 ( $r^2$  = 0.03) (Figure 5.3C) and the spherical model for site 4 ( $r^2$  = 0.07) (Table 5.1, Figure 5.3D). For sites 2, 3, and 4, the nugget-to-sill ratios were <0.25, which indicated strong spatial aggregation among the larval samples.

Spatial aggregations were observed at all three sites (2, 3, and 4) for the adult *S. venatus vestitus* populations. Variogram analyses were conducted separately on adult data for each week and cumulatively (all three weeks combined) for all three sites. For the cumulative samples, the best-fitting variogram used the exponential model at all three sites  $(r^2 = 0.52, 0.08, 0.2 \text{ at sites } 2, 3, \text{ and } 4, \text{ respectively})$  (Table 5.2, Figure 5.4A–C). Spatial aggregation was detected in all three sampling weeks for sites  $2 (r^2 = 0.003, 0.5, \text{ and } 0.13)$  and  $3 (r^2 = 0.24, 0.82, \text{ and } 0.003)$ , whereas at site 4, spatial aggregation was not evident in the second sample (Table 5.2). The best-fitting model for site 2 was the spherical model for the first week, whereas the exponential model fitted well for the two following sampling weeks (Table 5.2). At site 3, the best-fitting models were exponential models for all three sampling weeks. At site 4, the spherical model was the best-fitting model for the first week, whereas the linear and exponential models fitted well for the following two weeks. The nugget-to-sill ratios were <0.25, which indicated a high degree of aggregation for all three sites for the cumulative data and for all weekly sampling data for sites 2 and 3 (Table 5.2). For site 3, the nugget-to-sill ratio was <0.25 for the second week of sampling.

The range values that were produced by variogram analyses and indicated aggregation distributions have implications for developing sampling methods for *Sphenophorus* spp. or *S. venatus vestitus*. For *Sphenophorus* spp. larvae, the range values for these sites were between 3.82 and 4.11 m (Table 5.1). The interpolated maps that were developed by kriging based on

selected variogram models for *Sphenophorus* spp. larvae are shown in Figure 1. For *S. venatus* vestitus adults, the range values were between 2.13 and 7.11 m (Table 5.2). The interpolated maps that were developed by kriging based on selected variogram models for adult *S. venatus* vestitus are shown in Figure 5.2.

#### **SADIE Analysis**

Based on the aggregation index, the spatial aggregation of *Sphenophorus* spp. larval samples were not significant for any of the three sites (Table 5.3). Significant spatial aggregations were observed at all three sites for *S. venatus vestitus* adult sampling (p < 0.025) (Table 5.4). The weekly analysis of *S. venatus vestitus* adult samples using SADIE detected a significant aggregation pattern in at least one of the sampling weeks for sites 2 and 4. Moreover, at site 3, significant aggregation patterns for adult *S. venatus vestitus* were observed for all three sampling weeks (Table 5.4).

#### **Discussion**

The spatial distribution of an insect is an inherited trait but can be influenced by behavior and various environmental factors (Taylor 1984, Nestel et al. 1995). The results based on variogram and SADIE analyses showed that the adult populations of *S. venatus vestitus* followed spatially dependent distributions in the sod farms. The variogram results for *Sphenophorus* spp. larvae showed that they were spatially aggregated in 3 out of 4 sites studied. Although *S. venatus vestitus* mostly overwinter as adults (Dupuy and Ramirez 2016), multiple larval stages of *S. venatus vestitus* were found in the sod farms in central Georgia. The larval stages sampled in fall or winter and spring are likely from the same overlapping generations because adult emergence was continuous from late winter to summer in the central Georgia sod farms (Gireesh and Joseph

2020). This suggests that the distribution of larvae sampled in winter and spring in the current study is comparable and not different. Moreover, larval distribution from September samples showed no distinct pattern, possibly because of low larval densities, which affected our ability to compare differences in larval sampling in fall and spring.

A previous study showed that *S. venatus vestitus* is the dominant billbug species that causes damage (>98% of *Sphenophorus* spp. collected) in sod farms (Gireesh and Joseph 2020). Thus, although the larval stages were not identified at the species level in the current study, they were most likely *S. venatus vestitus* larvae and, hereafter, are referred to as *S. venatus vestitus* larvae. Likewise, *S. venatus vestitus* adults were most abundant in the fully grown sod fields (Gireesh and Joseph 2020). They frequently move from harvested to nonharvested areas of sod fields and vice versa. This suggests that they are likely to colonize newly harvested sod fields and remain aggregated after the sod is harvested. These results are consistent with those for another weevil species, *L. maculicollis*, where *L. maculicollis* in golf courses was initially found to be aggregated at the edges of golf courses and then eventually dispersed throughout the entire course (McGraw and Koppenhofer 2010). *L. maculicollis*, however, overwinters in leaf litter offsite and moves into golf courses during spring every year. Thus, knowledge of the aggregated distributions of *S. venatus vestitus* in sod farms will help in the development of more effective IPM.

Understanding spatial distributions helps to predict and manage pest populations by implementing accurate sampling plans and decision-making processes (Ribeiro et al. 2020). When using variograms to analyze the spatial distribution data, the range value of the variogram has a significant role for site-specific IPM efforts (Weisz et al. 1995, Ifoulis and Savopoulou-Soultani 2006, Carvalho et al. 2020). The average range value of the selected variograms in our

study was 3.9 m (i.e., the cumulative mean of all three sites) for the larval S. venatus vestitus distributions and 5.4 m (the cumulative mean of all three sites) for the adult S. venatus vestitus distributions. Range values can be used either to create hotspot maps for site-specific management (Duarte et al. 2015, Baek and Lee 2021) or to obtain individual samples to understand the threshold values for insecticide treatments (Martins et al. 2018). In the current study, if the range value was used for making S. venatus vestitus hotspot maps, the distance between two samples should be less than 3.9 m and 5.4 m for larvae and adults, respectively. Hotspot maps indicate those areas with high degrees of infestation and therefore, they help with information-based decision-making for pest management (Duarte 2015). However, developing distribution maps may not be feasible for sod growers because they require many sample points and substantial technical skills to process the raw data for map construction (Karimzadeh et al. 2011, Shreshta et al. 2020). When the range values are used to obtain unbiased samples, the distances between two sampling points should be greater than the average range values for both larvae and adults (Williams et al. 1992, Frank et al. 2011, Baek and Lee 2021). S. venatus vestitus larvae are hidden in the soil, and thus, their infestations in soils can be determined if soil samples are collected using a hole cutter at 4.0 m (average range value = 3.9 m) distances to capture larvae. Because the larval samples were mainly collected in winter and spring, further research is warranted to determine the larval distribution of *S. venatus vestitus* in summer and early fall. Similarly, the prevalence of S. venatus vestitus adults can be determined if they are collected by deploying pitfall traps at 5 m (the average range value = 4.7 m) distances at 7 d intervals. This information can be used for pest management decisions.

The variogram and SADIE analyses showed inconsistent results, in which both *S. venatus vestitus* larvae and adults showed aggregations in the variogram analyses. However, only for *S.* 

venatus vestitus adults did the data support aggregation when using SADIE analysis. This discrepancy may be due to the variations in which the spatial weights are calculated for individual sample points (Anthanassiou et al. 2010, Kamdem et al. 2012). SADIE measures spatial dependence based not only on relative positions but also on the absolute sampling positions of the counts (Xu and Madden 2003, Reay-Jones 2017). As a result, spatial aggregation is sometimes not observed due to the higher values of isolated individual sampling points. In contrast, variogram analysis includes these higher values, which can contribute to the aggregated distribution patterns of insect populations (Williams et al. 1992, Rijal et al. 2016). Therefore, the use of more than one geospatial technique is preferred to address this discrepancy between methods when determining spatial distribution patterns (Karimzadeh et al. 2011). A previous study combined variogram and SADIE to generate prescription maps for the bean leaf beetle, Cerotoma trifurcata Forster (Coleoptera: Chrysomelidae) (Park and Krell 2005). Another study used semivariograms and SADIE to understand the spatiotemporal patterns of *Ricania* shantungensis (Hemiptera: Ricaniidae) in chestnut fields (Baek and Lee 2021). A previous study investigated and differentiated various statistical methods and found that no single method could completely identify all spatial characteristics of the dataset (Perry and Dixon 2002, Anthanassiou et al. 2010). Moreover, combining both global and local methods provide clarity for various aspects of spatial patterns and thereby provides an exact elucidation of spatial heterogeneity (Anthanassiou et al. 2010, Quieroz et al. 2010, Kamdem 2012). While variograms revealed the spatial dependences among the larval and adult populations in our study, SADIE detected significant aggregation patterns only for adults. Similar discrepancies in results when using variograms and SADIE have been reported in previous studies (Karimzadeh et al. 2011, Rijal et al. 2014). The main aim of combining several geospatial methods should be to provide better

accuracy of results and not to validate the results of one method over the other (Perry et al. 2002, Queiroz et al. 2010).

This is the first study that shows the spatial distributions of *S. venatus vestitus* in sod farms. Although most previous studies have used adult S. venatus vestitus sampling, larval sampling remains challenging (Johnson-Cicalese 1990, Huang and Buss 2009, Dosckoil and Brandenburg 2012). Moreover, in sod farms and golf courses, S. venatus vestitus larvae cause more economic damage than adults (Dosckoil and Brandenburg 2012). Thus, understanding the distribution patterns of S. venatus vestitus larvae is critical to developing an effective strategy for addressing this pest from a management standpoint. The current practice of larval sampling using hole cutters requires more labor and time and has still not been demonstrated to be an efficient method for determining Sphenophorus spp. larvae distributions (Dosckoil and Brandenburg 2012). Based on our study, we suggest that soil samples using a 10-cm hole cutter should be taken 4.0 m apart at ~10 cm depth to indicate the prevalence of aggregated patches of S. venatus vestitus larvae in the field, especially in the winter and spring months. Further research is warranted to determine the minimum number of samples per sod field to quantify S. venatus vestitus and develop thresholds for management decisions. This information can be used for spot applications or site-specific management of S. venatus vestitus larvae and S. venatus vestitus adults in sod farms and can reduce insecticide use and application costs.

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writing—review, and editing. All authors have read and agreed to the published version of the manuscript.

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**Table 5.1.** Variogram models and parameters representing the spatial distribution patterns of *Sphenophorus* spp. larvae at four sites in Marshallville, Georgia (USA) in fall and winter 2019 and spring 2020.

Site †	Sampling Time	Range (m) ‡	Model §	r <sup>2</sup>	C <sub>0</sub> ‡	$C_0 + C^{\ddagger}$	$C_0/C_0 + C^{\ddagger}$
1	Fall	$=\P$	Linear	0.003	_	_	_
2	Winter	3.82	Gaussian	0.64	0.009	0.133	0.060
3	Spring	3.9	Exponential	0.03	0.003	0.142	0.020
4	Spring	4.11	Spherical	0.07	0.001	0.120	0.008

Site  $^{\dagger}$  1, 'Zenith' zoysiagrass (*Z. japonica*); Site 2, 'TifWay' bermudagrass (*Cynadon* spp.); Site 3, 'Zeon' zoysiagrass (*Z. matrella*); and Site 4, 'TifTuf' bermudagrass (*Cynadon* spp.). Three-week samples were combined to form a cumulative sample at each site.  $^{\ddagger}$  variogram parameters; range, nugget (C<sub>0</sub>), sill (C<sub>0</sub> + C), and nugget-to-sill ratio (C<sub>0</sub>/C<sub>0</sub>+C). § Spherical and exponential are curvilinear models (indicating aggregation distribution) Linear, and straight-line models (aggregation not observed). ¶ Aggregation not observed.

**Table 5.2.** Variogram models and parameters representing the spatial distribution patterns of *Sphenophorus venatus vestitus* adults at three sites in Marshallville, Georgia (USA), in 2020.

Site †	Date	Range (m) ‡	Model §	$r^2$	C <sub>0</sub> ‡	$C_0 + C^{\ddagger}$	$C_0/C_0 + C^{\ddagger}$
2	May 26th	3.50	Spherical	0.000	0.001	0.600	0.001
	June 2nd	3.39	Exponential	0.500	0.650	14.80	0.040
	June 9th	3.72	Exponential	0.130	1.310	16.78	0.070
	Combined †	7.11	Exponential	0.529	1.300	43.09	0.030
3	May 27th	6.50	Exponential	0.240	0.040	7.760	0.005
	June 3rd	4.50	Exponential	0.820	0.010	4.970	0.002
	June 10th	2.13	Exponential	0.030	0.001	0.630	0.001
	Combined	4.2	Exponential	0.080	1.060	19.02	0.050
4	June 10th	3.72	Spherical	0.947	0.030	16.19	0.001
	June 17th	_ ¶	Linear	0.101	-	-	-
	June 24th	5.97	Exponential	0.828	0.060	0.700	0.080
	Combined	4.98	Exponential	0.200	0.690	28.73	0.020

Site  $^{\dagger}$  2, 'TifWay' bermudagrass (*Cynadon* spp.); Site 3, 'Zeon' zoysiagrass (*Z. matrella*); and Site 4, 'TifTuf' bermudagrass (*Cynadon* spp.). Threeweek samples were combined to form a cumulative sample at each site.  $^{\ddagger}$  variogram parameters; range, nugget ( $C_0$ ), sill ( $C_0 + C$ ), and nugget-to-sill ratio ( $C_0/C_0 + C$ ). § Spherical and exponential are curvilinear models (indicating aggregation distribution) Linear, and straight-line models (aggregation not observed). ¶ Aggregation not observed.

**Table 5.3.** Parameters for the spatial distribution patterns of *Sphenophorus* spp. larvae using SADIE at four sites in Marshallville, Georgia, in 2019 and 2020. Sites 1 and 2 were sampled in fall and winter 2019, and sites 3 and 4 spring 2020.

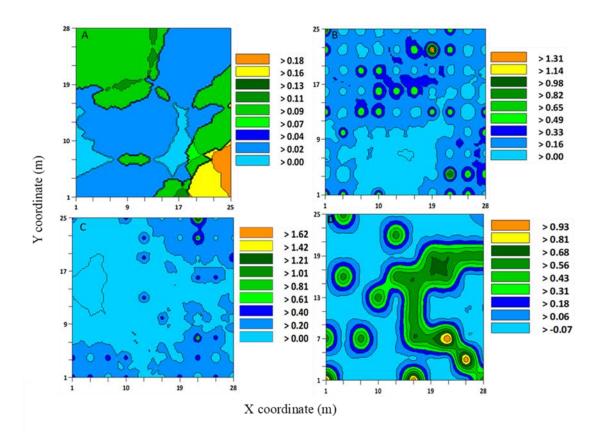
Site †	Ia ‡	PIa ‡	$v_j$ $\S$	$v_i$ $\P$	$Pv_j$ §	$Pv_i$ ¶
1	1.153	0.157	-1.157	1.073	0.154	0.272
2	0.900	0.715	-0.899	0.934	0.713	0.610
3	1.070	0.304	-1.063	1.024	0.326	0.387
4	1.015	0.386	-1.019	1.056	0.396	0.304

Site † 1, 'Zenith' zoysiagrass (*Z. japonica*); Site 2, 'TifWay' bermudagrass (*Cynadon* spp.); Site 3, 'Zeon' zoysiagrass (*Z. matrella*); and Site 4, 'TifTuf' bermudagrass (*Cynadon* spp.). Three-week samples were combined to form a cumulative sample at each site.  $Ia^{\ddagger}$  index of aggregation,  $PIa^{\ddagger}$ ; with an associated probability, significant aggregation at p < 0.025;  $v_j^{\$}$ ; mean clustering indices representing all units in a patch,  $Pv_j^{\$}$ , with an associated probability.  $v_i^{\$}$ ; mean cluster indices representing all units in a gap,  $Pv_i^{\$}$ , with an associated probability.

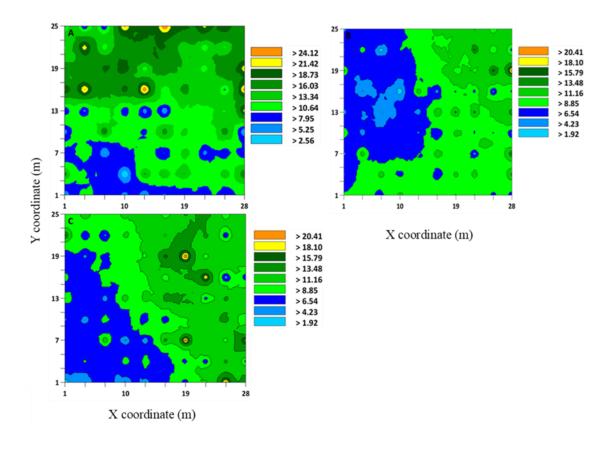
**Table 5.4.** Parameters for the spatial distribution patterns of *Sphenophorus venatus vestitus* adults using SADIE at three sites in Marshallville, Georgia (USA) in 2020.

Site †	Date	Ia ‡	PIa <sup>‡</sup>	$v_j$ §	$v_i$ ¶	$Pv_j$ §	$Pv_i$ ¶
2	May 26th	1.613	0.002 **	-1.572	1.609	0.003 **	0.002 **
	June 2nd	1.283	0.051	-1.236	1.286	0.083	0.051
	June 9th	1.105	0.215	-1.118	1.146	0.208	0.158
	Combined †	1.740	<0.001 **	-1.864	1.699	<0.001 **	0.001 **
3	May 27th	1.842	<0.001 **	-1.723	1.757	<0.001 **	<0.001 **
	June 3rd	1.456	0.014 *	-1.332	1.472	0.030 **	0.005
	June 10th	1.864	<0.001 **	-1.837	1.573	<0.001 **	0.004 **
	Combined	1.518	0.007 **	-1.509	1.587	0.006 *	0.003 **
4	June 10th	1.071	0.266	-1.084	1.055	0.238	0.279
	June 17th	1.379	0.027	-1.438	1.204	0.011 *	0.084
	June 24th	1.286	0.056	-1.358	1.176	0.026	0.118
	Combined	1.728	0.001 **	-1.612	1.451	0.002 **	0.011 *

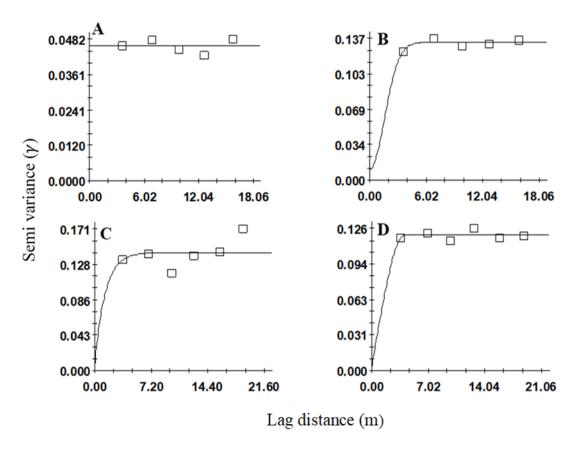
Site<sup>†</sup> 2, 'TifWay' bermudagrass (*Cynadon* spp.); Site 3, 'Zeon' zoysiagrass (*Z. matrella*); and Site 4, 'TifTuf' bermudagrass (*Cynadon* spp.). Three-week samples were combined to form a cumulative sample at each site.  $Ia^{\ddagger}$ ; index of aggregation,  $PIa^{\ddagger}$ , with an associated probability, significant aggregation at p < 0.025. vj §; mean clustering indices of all units in a patch,  $Pv_j$  §, with an associated probability. vi ¶; mean cluster indices of all units in a gap,  $Pv_i$  ¶, with an associated probability. \* Significant at  $p \le 0.025$ , \*\* Significant at  $p \le 0.005$ .



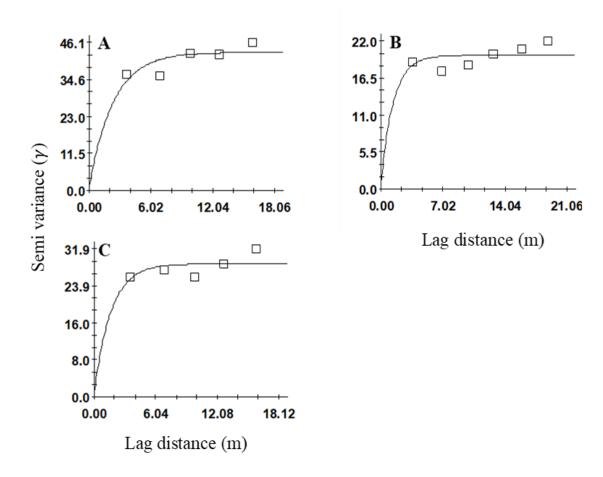
**Fig 5.1**. Interpolated map that was developed using the kriging based on variogram models of *Sphenophorus* spp. larvae from sod field sites (A) 1, (B) 2, (C) 3, and (D) 4 in Marshallville, Georgia (USA) in 2019 and 2020. Three-week samples were combined to form a cumulative sample at each site. Sites 1 and 2 were sampled in fall and winter 2019, and sites 3 and 4 spring 2020.



**Fig 5.2.** Interpolated map that was developed using the kriging based on variogram models of *Sphenophorus venatus vestitus* adults from three sod field sites, (A) 1, (B) 2, and (C) 3, in Marshallville, Georgia (USA) in 2020. At each site, three individual samples were combined (A–C).



**Fig 5.3.** Variogram models showing the spatial distributions of *Sphenophorus* spp. larvae in sites (A) 1, (B) 2, (C) 3, and (D) 4. Sites 1 and 2 were sampled in fall and winter 2019, and sites 3 and 4 spring 2020.



**Fig 5.4**. Variogram models showing the spatial distributions of *Sphenophorus venatus vestitus* adults in sites (A) 2, (B) 3, and (C) 4.

# **CHAPTER 6**

# A SURVEY ON MAJOR INSECT PESTS AND MANAGEMENT PRACTICES ADOPTED BY THE COMMERCIAL TURFGRASS INDUSTRY IN GEORGIA

To be submitted to Journal of Entomological Science

**Abstract** Because turfgrass is maintained in various settings, such as golf courses, athletic fields, and commercially produced in sod farms, it is critical to understand the major insect pests and adopted management practices. A survey was conducted to determine the major pests and current management practices in the commercial turfgrass industry. Of 35 respondents, 93.9% were from Georgia, and 6.1% were from Alabama. A significantly greater number of respondents represented golf courses (65.8% of 37 respondents) than sod farms (28.9%), landscape maintenance and installation companies (2.6%), and public lawns (2.6%). The respondents (n =35) identified fall armyworm (Spodoptera frugiperda JE Smith; 32.9%), white grubs (Phyllophaga spp.; 20.7%), and mole crickets (Orthoptera: Gryllotalpidae; 17%) as major pests than billbugs (Sphenophorus spp.; 8.5%), chinch bugs (Blissus spp.; 0%) and others (20.7%). A significantly greater percentage of respondents (n = 25) indicated that their pest management decisions were based on previous experience (51.4%) than on university extension (8.6%), scouting (17.1%), and internet resources (17.1%). Of 35 respondents, 66.7% applied insecticides 2-5 times than < 2 (8.3%) or > 5 (25%) times for insect pest management. Among nonchemical tools (n = 27), most respondents opted to do nothing (71.4%) than practice biological control (0%) or host plant resistance (25%) or other tools (3.6%). For Sphenophorus spp. control (n =21), significantly greater respondents applied insecticide sprays in spring (50%) and summer (28.6%). The respondents (n = 23) primarily used pyrethroids (52.2%) than other classes of insecticides (47.8%).

Keywords: IPM, sod farms, golf courses, Sphenophorus spp., damage, fall armyworm

Turfgrass is an inseparable component of urban, suburban, and rural landscapes in the U.S. (Monteiro 2017). It is planted in recreational facilities, such as golf courses, athletic fields, and public lawns. In the U.S., turfgrass (sod) production is valued at \$40-60 billion USD annually and covers approximately 20 million ha (Morris 2003). In Georgia, sod is produced on approximately 10,785 ha across 64 counties and is valued at \$118.3 million USD (Wolfe and Stubbs 2019).

In Georgia, although both cool-season or warm-season grasses are grown, warm-season grasses are more widely planted. The warm-season grasses are better adapted to the conditions of most of Georgia. They require temperatures ranging between 26 and 35 °C for growth and development, and their growth is inhibited at temperatures below 10 °C (Vittum 2020). The major warm-season grasses planted in Georgia include bermudagrasses [*Cynodon dactylon* (L). Pers], zoysiagrass [*Zoysia* spp.], St. Augustinegrass [*Stenotaphrum secondatum* (Walter) Kuntze], bahiagrass [*Paspalum notatum* Flugge], and centipedegrass [*Eremochloa ophiuroides* (Munro) Hack] (Potter and Braman 1991, Hanna et al. 2013). These turfgrass species are planted based on geographical location, type of facility (e.g., golf courses or parks or residential settings), and type of activity the grass is being used. Regardless, aesthetic appearance and ease of management of turfgrass are critical considerations for turfgrass selection and planting. Any discoloration of turfgrass can quickly become unacceptable in any setting, especially golf courses and sod farms whose revenues entirely depend on the health and quality of turfgrass (Beard 1972, Dupuy and Ramirez 2016).

Turfgrass presents unique ecological conditions, and several arthropods are adapted to survive and thrive in the various turfgrass systems. Turfgrass is managed differently depending on the needs and priorities. For example, turfgrass in sod farms is in production mode where the

grass is grown within two years then harvested and sold. In golf courses, they are typically maintained for several years, and management practices vary by specific area within the course. Thus, the occurrence, abundance, diversity, and distribution (spatial and temporal) of arthropods are subjected to various factors, including the type of turfgrass system. In Georgia, several species of arthropod pests invade turfgrass. The major pests include mole crickets, *Neoscapteriscus vicinus* Scudder and *Scapteriscus borellii* Giglio—Tos (Potter and Braman 1991 and Vittum 2020), white grubs, such as Japanese beetle, *Popillia japonica* Newman (Potter and Braman 1991), hunting billbug, *Sphenophorus venatus vestitus* Chittenden (Gireesh and Joseph 2020), black cutworm, *Agrotis ipsilon* (Hufnagel) (Held and Potter 2012), fall armyworm, *Spodoptera frugiperda* JE Smith, several species of sod webworms (Lepidoptera: Pyralidae), southern chinch bug, *Blissus insularis* Barber, bermudagrass mite, *Eriophyes cynodoniensis* (Sayed) (Huang 2008), and rhodesgrass mealybug, *Antonina graminis* (Maskell) (Joseph and Hudson 2019).

Although many herbivorous arthropod pests can invade turfgrass, not all the pests equally invade all the turfgrass systems. Similarly, the current management practices adopted against major pest species problems can vary by turfgrass genotype and the system. Most arthropod pests are managed using insecticides that can cause exposure to nontargets, including predators, parasitoids, and pollinators. The major objective of this survey was to determine the major pests and management approaches adopted by various turfgrass systems in Georgia. The information generated will shape the focus of research and extension efforts and allocation of resources.

There is a growing need to develop turfgrass management practices that protect the community and environmental health (Held and Potter 2012, Thompson and Kniffin 2017).

#### **Materials and Methods**

Survey design. A survey questionnaire was developed to collect information about the major pests and their management from various turfgrass systems in Georgia. The survey was conducted from May to September 2020 using Qualtrics (Provo, UT), an online survey tool under the subscription of the University of Georgia. Before the release of the survey, the questions were reviewed by the extension specialist at the Department of Entomology, University of Georgia. An Institutional Review Board (IRB) at the University of Georgia has reviewed the questionnaire and has exempted it from approval as there was no personal information requested in the survey (IRB#PROJECT00002269). A total of 15 questions were organized into three sections (Table 1). The first group of questions (1-5) was mostly on facility type, grass type grown, facility location and size, and major pest problems. The second group of questions (6-12) focused on current IPM approaches adopted against turfgrass pests. The last set (13-15) included questions specific to billbugs (*Sphenophorus* spp.). This pest was previously recognized as a severe pest in sod farms based on repeated one-on-one interactions with the producers in recent years.

**Survey distribution.** The survey was initially distributed to members of turfgrass and ornamental industry associations, such as Georgia Urban Agriculture Council (GUAC) and Golf course Superintendents of America (GCSAA) through email list-serves. These members include sod producers, golf course superintendents, and landscape installation and maintenance company managers, mainly from Georgia. Some participating facilities were also located in Alabama. Sod producers and golf course superintendents were also contacted through phone calls as reminders to complete the survey. The responses obtained from 12<sup>th</sup> May to 3<sup>rd</sup> September were included in the analysis.

**Statistical analysis.** The questions with multiple choices were converted into categorical data. Each question with multiple-choice data was analyzed using nominal logistic regression (JMP SAS 2019). When there was a significant effect in the likelihood ratio test for each question, the responses were compared by examining the odds ratio. The analyses were conducted for choices with n = 0 after adding 0.2 for all the choices to establish homogeneity.

## **Results**

Turfgrass facility, location, grass genotype, and major pests. A total of 37 respondents representing various turfgrass facilities participated in the survey. Among the total respondents, two respondents did not provide location information. Of 35 respondents, 89.1% (n = 33) and 5.4 % (n = 2) were from Georgia and Alabama, respectively (Fig. 6.1). There were significantly more respondents from golf courses (n = 25) compared to sod farms (n = 11), landscape maintenance and installation companies (n = 1) and public lawns (n = 1)  $(\chi^2 = 57.9, df = 3, P <$ 0.001; Fig. 6.2A). When respondents from sod farms, landscape maintenance and installation companies, and public lawns were compared, significantly more respondents were from the sod farms than from the other two turfgrass systems (Fig. 6.2A). However, there was no significant difference among respondents between landscape maintenance and installation companies and public lawns. When asked about species of turfgrass grown, a significantly greater percentage of respondents had bermudagrass (n = 31) compared to zoysiagrass (n = 23), centipedegrass (n = 8), and other turfgrass species (n = 8), such as tall fescue (Festuca arundinacea Schreb), Paspalum spp., and bentgrass (*Agrostis palustris* Huds.) ( $\chi^2 = 35.2$ , df = 3, P < 0.001; Fig. 6.2B). Among zoysiagrass, centipedegrass, and other turfgrass species, zoysiagrass was planted significantly greater than centipedegrass and other turfgrass species (Fig. 6.2B). A significantly greater number of respondents had facilities between 12 and 81 ha (n = 25) followed by 81 - 201 ha (n = 25) 6) than facilities ranging < 12 (n = 1), 201 – 404 ha (n = 1), 404 – 2023 (n = 2), and > 2023 ha (n = 2) ( $\chi^2 = 74.2$ , df = 6, P < 0.001; Fig. 6.2C). However, there was no significant difference among respondents operating between the smallest (< 12 ha) and largest turfgrass facilities (> 2023 ha).

A significantly greater percentage of respondents identified fall armyworm (n = 25) as a major turfgrass pest than mole crickets (n = 14), billbugs (n = 7), and chinch bugs (n = 0) ( $\chi^2 = 47.2$ , df = 5, P < 0.001; Fig. 6.2D). Among white grubs (n = 17), mole crickets, chinch bugs, billbugs, and other turfgrass pests (ants, nematodes, cutworms, and sod webworms; n = 17), a significantly greater percentage of respondents indicated that mole crickets are a problem than billbugs and chinch bugs (Fig. 6.2D). Among fall armyworm, white grubs, and other turfgrass pests and between white grubs and other pests, there was no significant difference in the percentage of responses. Similarly, there was no significant difference in the percentage of respondents who selected among white grubs, mole crickets, and other turfgrass pests and between billbugs and chinch bugs (Fig. 6.2D).

Current IPM approaches. When the damage becomes evident on turfgrass, a significantly greater percentage of respondents preferred employing a management tactic (n = 18) than deploying traps (n = 6), seeking help from extension agents (n = 3), checking online or other approaches (seeking multiple options, n = 2) ( $\chi^2 = 26.7$ , df = 5, P < 0.001; Fig. 6.3A). There was no significant difference in respondents choosing options, such as deploying traps, seeking help from extension agents, checking online, and other options when the turfgrass damage becomes noticeable (Fig. 6.3A). For the question on the number of insecticide applications per year for pest management in turfgrass, a significantly greater number of respondents indicated that they spray 2-5 times per year (n = 24) than spray one-time (n = 3), 5-10 (n = 7) and > 10 (n = 2) times

per year ( $\chi^2 = 44.5$ , df = 3, P < 0.001; Fig. 6.3B). However, there was no significant difference among the categories 1, 5-10, and > 10 times per year indicated by the respondents (Fig. 6.3B). For pest management decisions, a significantly greater percentage of respondents prefer spraying either by the calendar (n = 5), when insects were detected (n = 5), when damage was detected (n = 5)= 10), or using other approaches (n = 6), such as spraying based on regular monitoring and scouting than adopting university extension recommendations ( $\chi^2 = 5.6$ ; df = 4, P = 0.018; Fig. 6.3C). However, the percentages of responses were not significantly different among respondents spraying by the calendar, when insects were detected, when damage was detected, or other approaches, such as spraying based on regular monitoring and scouting (Fig. 6.3C). For the question on strategies employed for insecticide resistance agaisnt turfgrass pests, a significantly greater percentage of respondents adopted rotation of insecticides (n = 17) compared to nonchemical tactics, such as release of nematodes, (n = 0), not using insecticide control (n = 1), not adopting any measures (n = 6) and other approaches (n = 1), such as treating insecticide only once ( $\chi^2 = 46.9$ , df = 4, P < 0.001; Fig. 6.3D). There was no significant difference among respondents who adopted nonchemical tactics, no insecticide use, follows no measures, and uses other approaches, such as treating only once (Fig. 6.3D).

When comes to insecticide selection for pest management, a significantly greater percentage of respondents considered the efficacy of insecticide (n = 24) than cost (n = 9), applicator safety (n = 10), and other attributes (n = 4), such as environmental safety ( $\chi^2 = 24.3$ , df = 3, P < 0.001; Fig. 6.4A). However, there was no significant difference in respondents who chose cost, applicator safety, and environmental safety (Fig. 6.4A). When the respondents were asked about the nonchemical options adopted in their facilities, a significantly greater percentage of respondents adopted no specific measures (n = 20) followed by using resistant turfgrass

cultivars (n = 7), biological control (n = 0) or other approaches (n = 1), such as cultural control ( $\chi^2 = 49.8$ , df = 3, P < 0.001; Fig. 6.4B). When the respondents were asked about the source of pest biology and management information, a significantly greater number of respondents indicated that the source of such information was from university extension (n = 15), peers working in the same business (n = 16), industry and turfgrass related associations (n = 18) compared to trade shows (n = 6) and other sources, such as internet (n = 2) ( $\chi^2 = 25.6$ , df = 5, P < 0.001; Fig. 6.4C). However, there was no significant difference in the percentage of respondents who consulted the university extension, peers working in the same business, industry, and turfgrass-related associations, and between tradeshows and other resources, such as the internet (Fig. 6.4C).

Current IPM approaches for *Sphenophorus* spp. Three questions were asked specifically on IPM approaches adopted for the management of *Sphenophorus* spp. When asked if insecticide application timing for *Sphenophorus* spp. control was based on turfgrass phenology, a significantly greater percentage sod farm respondents indicated that they do not follow turfgrass phenology (n = 8) than spraying immediately after harvest (n = 1), at growing stages of turfgrass (n = 2) and ready to harvest (n = 2) ( $\chi^2 = 22.9$ , df = 4, P < 0.001; Fig. 6.5A). A significantly greater percentage of respondents preferred to spray during spring (n = 14) for managing *Sphenophorus* spp. than during fall (n = 6) or before spring (n = 0) ( $\chi^2 = 22.8$ , df = 3, P < 0.001; Fig. 6.5B). There was no significant difference between spring and summer (n = 8) or between fall and before spring in response to insecticide spray timing for *Sphenophorus* spp. (Fig. 6.5B). Moreover, a significantly greater number of respondents use pyrethroids (n = 12) than neonicotinoids (n = 4), diamides (n = 2) and other insecticides (n = 5), such as carbaryl for *Sphenophorus* spp. control ( $\chi^2 = 12.7$ , df = 3, P = 0.005; Fig. 6.5C). However, there was no

significant difference in the respondents choosing among neonicotinoids, diamides, and other insecticides (carbaryl) for *Sphenophorus* spp. control (Fig. 6.5C).

#### **Discussion**

Respondents in the current survey were primarily representing golf courses across Georgia. Although the respondents representing sod farms were relatively fewer than those from golf courses, sod producers represented a large hectarage (Fig. 6.2A). Based on the survey, most of the respondents grow or maintain bermudagrass genotypes in their facilities. Bermudagrass cultivars are typically planted in golf courses (Waldo et al. 2021) and are produced in sod farms in large areas. The second most widely grown turfgrass genotype was zoysiagrass. Zoysiagrass is increasingly produced in sod farms in Georgia (Waltz 2021). The demand for zoysiagrass has increased significantly as more golf courses, and residential lawns have been shifting to this turfgrass genotype (Patton 2009, Patton et al. 2017). Among other turfgrasses, a few respondents indicated that they grow or maintain centipedegrass, tall fescue, paspalum, and bentgrass. Bentgrass is primarily planted in the putting greens of golf courses, and other grasses except centipedegrass are planted in fairways of golf courses. Clearly, the survey suggests that arthropod issues on bermudagrass and zoysiagrass should be given high priority for future research and extension programming.

Most of the respondents identified fall armyworm, white grubs, and mole crickets as the major pest problems in their facilities. A few respondents (n = 7) identified billbugs as a major turfgrass pest problem. Recently, billbugs were reported to be a major issue in sod farms (Gireesh and Joseph 2020). A respondent from a landscape maintenance and installation company growing centipedegrass reported billbugs as a problem. None of the respondents identified chinch bugs as a major turfgrass pest, which is typically a pest in St. Augustinegrass

(Stenotaphrum secondatum (Walter) Kuntze) and are not widely planted in commercial sites in Georgia. Understanding of current status of economic pests in turfgrass facilities is critical as the integrated pest management (IPM) research, and extension efforts can be appropriately prioritized to meet clientele needs.

Results show that most respondents spray insecticides multiple times (2-5 times) every year. Respondents indicated that insecticide application decisions are driven mainly by the knowledge of prior pest issues in the facility. This suggests that most insecticide use is likely preventative sprays as the tolerance to arthropod pest injury in the facilities is almost zero. A very few respondents employed regular scouting of pests to determine the insecticide application timing. Most respondents depend on the incidence of damage, pest, or calendar-based (preventative application) for pest management decisions. The results also suggest that a low number of respondents sought extension recommendations for pest management. Most of the respondents adopted the insecticide rotation approach to delay insecticide resistance against pests, but many others adopted no specific approaches. Turfgrass pest management can be achieved by adopting nonchemical options, such as host plant resistance for fall armyworm (Singh and Joseph 2020), using entomopathogenic nematodes for white grubs control (Guo et al. 2020), entomopathogenic nematodes for mole crickets (Barbara and Buss 2006) and biological control agents for billbugs (Dupuy and Ramirez 2019), all of which can be effective alternatives for addressing insecticide resistance. However, based on our results, none of the respondents preferred to use any of these nonchemical tactics to prevent insecticide resistance among turfgrass pests. Efficacy of insecticides against the pest was the most important criteria for choosing insecticide than insecticide cost, applicator safety, environmental safety, and residual activity of insecticide. This suggests that the tolerance of pest infestation is extremely low for the turfgrass industry, and aesthetic appearance is the most valued attribute of the industry. To reduce the use of insecticide, some respondents were considering planting resistant turfgrass varieties based on the current survey. However, none of the respondents considered biological control an effective way to reduce synthetic insecticides. Even though the exact reason for not using biological control tactics is unclear, this result shows the existing gaps in education about the benefits of using biological control tactics in pest management. More research is warranted to develop or refine reliable biological control tactics comparable to efficacy of insecticide in turfgrass systems. Most of the respondents sought universities, peers working in the same business, industry professionals, and industry associations alike for information on biology and management of pests.

For billbug management, most of the respondents did not consider turfgrass phenology for insecticide application timing. Gireesh and Joseph (2020) showed that the billbugs were abundant when the turfgrass was fully grown or ready to harvest in sod farms, and their activity was noticed even after sod harvest (Gireesh and Joseph 2021). Respondents preferred to use insecticides in spring and summer rather than in fall or before spring. Although respondents use a wide range of insecticides such as pyrethroids, neonicotinoids, diamides, and carbamate (carbaryl), most of them indicated that they use pyrethroids for billbug control. These insecticides can negatively affect nontarget organisms, such as beneficial arthropods present in the turfgrass (Smith and Stratton 1986, Oliver et al. 2015, Joseph et al. 2021).

In summary, the current survey indicated that the respondents, mostly from golf courses and sod farms, identified fall armyworm, white grubs, and mole crickets as major turfgrass pests. Other major turfgrass pests identified by the respondents include nematodes, sod webworms, cutworms, and billbugs. This suggests that the research and extension efforts should include

programs to address fall armyworm, white grubs, and mole crickets problem. The survey also indicated that management of the major pests is driven mainly by insecticide use and biological control tactics are rarely used. The survey also indicated that the respondents use insecticides multiple times a year, which suggests that there may be opportunities to incorporate cultural and biological control tactics to reduce the insecticide use in turfgrass. Those respondents who chose not to use insecticides for pest management needs tended not to implement any control measures. These turfgrass facilities might already be in conservation mode or are ideal grounds for conserving beneficial arthropods. More research and extension efforts are warranted to improve integrated pest management approaches in turfgrass systems across Georgia.

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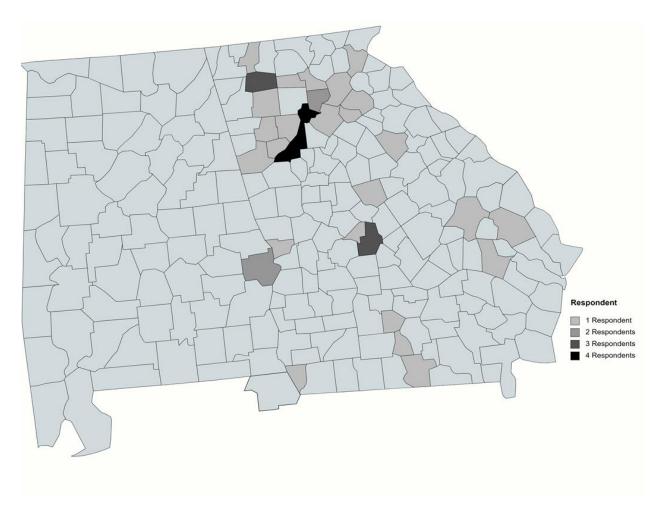
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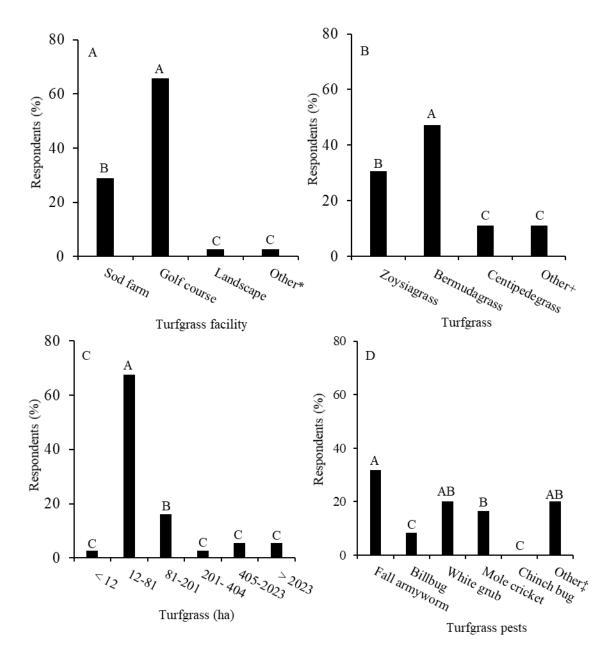
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**Table 6.1.** Survey questionnaire with percentage responses to specific questions, n = 37

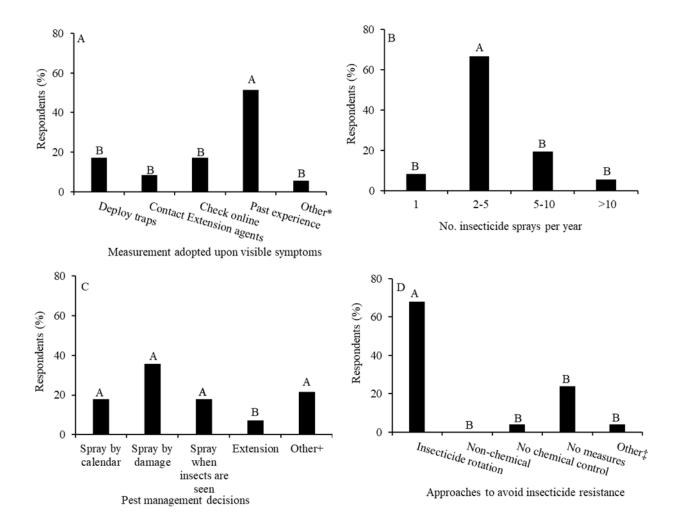
No.	Questions	Response rate (%)
1	What type of turfgrass facility are you associated with?	100
2	Where is your facility located?	94.95
3	What type of turfgrass is grown at your facility? (Check on multiple choices)	100
4	How many acres of turfgrass (sod, golf course or landscapes under supervision)	94.95
5	Three major pests of turfgrass in your facility?	94.95
6	Steps taken when damage become evident on turfgrass (Check on multiple choices)	67.56
7	How many insecticide sprays are applied per year in your facility?	94.95
8	How do you make decisions on pest management?	72.97
9	Approaches taken to address insecticide resistance against pests in the turfgrass	71.42
10	Which attribute would you consider before choosing an insecticide for pest management? (Check on multiple choices)	72.97
11	Alternative approaches taken to reduce the use of chemical pesticides (Check on multiple choices)	72.97
12	How do you get information about pest's biology and management options (Check on multiple choices)	72.97
13	If sod farm, what stage (s) of turfgrass development insecticide are applied for billbug control.	32.43
14	Insecticides used to control billbugs.	62.16
15	What time of year are insecticide applications made for billbug control? (Check on multiple choices	56.75



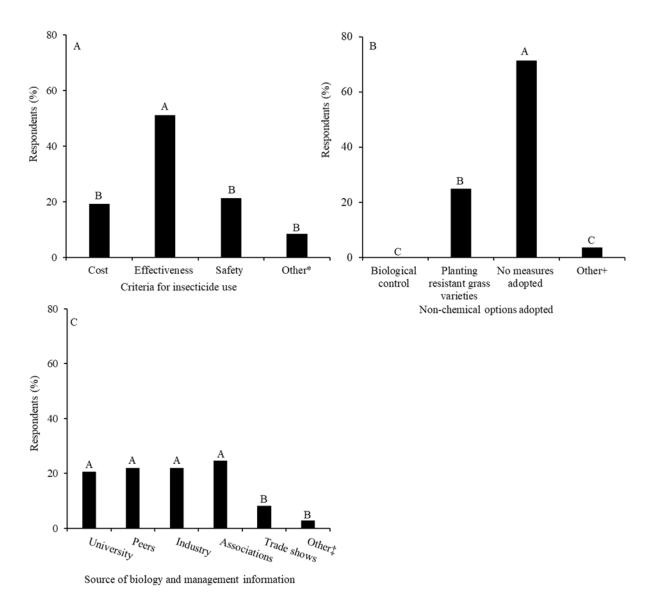
**Fig. 6.1.** U.S. counties where the survey respondents operate (n = 37). -three and two respondents were represented from Georgia and Alabama, respectively. Two participants did not provide location information. The interactive map was created using mapchart.net.



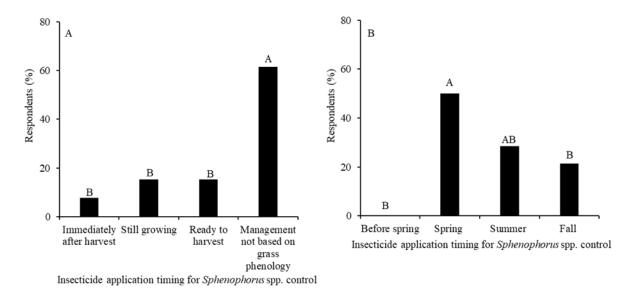
**Fig. 6.2.** The percentage of survey respondents (A) representing various turfgrass facilities (n = 37), (B) turfgrass genotype planted (n = 37) and (C) size of turfgrass facility in ha (n = 35) and (D) major turfgrass pests (n = 35). \*Other facility included public lawn (n = 1), \*Other genotypes planted were tall fescue (n = 4), paspalum (n = 1), bentgrass (n = 3), \*Other pests include ant (n = 5), nematode (n = 3), sod webworm (n = 1), cutworm (n = 4), Fire ant and cutworm (n = 2), cutworm and nematode (n = 1).

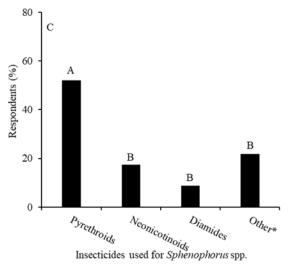


**Fig. 6.3.** The percentage of survey respondents responding to (A) steps taken when the turfgrass damage becomes evident (n = 25), (B) the number of insecticide application per year (n = 35), (C) decisions taken for pest management (n = 27) and (D) approaches taken to address insecticide resistance against turfgrass pests (n = 25). \*Other approaches include deployment of traps, internet resource, and past experience (2); \*Others include pray by the calendar, when damage is noticed and when insects are seen (n = 1) spray based on regular monitoring, threshold met (n = 1), scouting periodically to determine when to spray (n = 1), first application by the calendar, then by scouting and curative control (n = 1), spray when above the threshold and visible damage (n = 1), the combination of all mentioned as options (n = 1); ‡Others include only treated once (n = 1).



**Fig. 6.4.** The percentage of survey respondents responding to (A) selection attributes for insecticide use in their facility (n = 27), (B) alternate approaches adopted to reduce the use of chemical pesticides (n = 27) and (C) source of pest biology and management information (n = 27). \*Others include cost-effectiveness and applicator safety (n = 1), environmental safety (n = 1), residual activity (n = 1), \*Other best management practices (n = 1). \*Others include all the above options (n = 1), and internet (n = 1).





**Fig. 6.5.** The percentage of survey respondents responding to (A) insecticide application timing based on turfgrass phenology for *Sphenophorus* spp. control in sod farms (n = 13), (B) insecticide application timing for *Sphenophorus* spp. control (n = 28) and (C) insecticides used for *Sphenophorus* spp. (n = 23). \*Other responses include do not manage billbugs (n = 1), use of carbaryl (n = 1), use of all the insecticide classes mentioned in the multiple-choice options (n = 1), pyrethroids, and neonicotinoids (n = 1), use of carbaryl, bifenthrin, and imidacloprid (n = 1).

## **CHAPTER 7**

#### **SUMMARY**

The billbugs, *Sphenophorus* spp. (Coleoptera: Curculionidae), are important pests in sod farms in Georgia. In 2018 and 2019, adult billbugs were sampled from five zoysiagrass sod field sites in central Georgia. Four linear pitfall traps were used per site from February to December each year, and the traps were checked at weekly intervals. The data show that > 98% of the sampled billbugs were the hunting billbug, *Sphenophorus venatus vestitus* Chittenden, whereas the nutgrass billbug, *S. cariosus* Olivier; uneven billbug, *S. inaequalis* Say; and vegetable weevil, *Listroderes difficilis* Germain was the minor species. Seasonal billbug capture was influenced by turfgrass phenology (e.g., early-growth-stage, late-growth-stage or fully grown turfgrass). The numbers of *Sphenophorus* spp. collected were significantly greater in the fully grown turfgrass than in the early- or late-growth-stage turfgrasses. Significantly greater densities of billbug were found in *Zoysia matrella* (L.) Merrill ('Zeon') and the *Z. matrella* × *Z. pacifica* (Goudswaard) M. Hotta & S. Kuroki hybrid ('Emerald') than in the *Z. japonica* (Steudel) cultivars 'El Toro' and 'Zenith'. Similar numbers of male and female billbugs were collected from the sod field sites.

We documented the movement activity of billbugs during the early stages of sod development. In 2019 and 2020, adult billbugs were sampled from harvested and nonharvested areas of sod farms by using linear pitfall traps. Although a significantly greater number of billbug adults were captured from the nonharvested sod, the data showed that adults were present in the harvested sod area. To understand the direction of billbug movement in both harvested and nonharvested sod, a square area was selected, and the sod inside the square was removed. Linear

pitfall traps were deployed along the perimeter of square areas to collect adults from outside and inside the square.

In 2020, a significantly greater number of billbug adults were collected in the traps from the nonharvested areas outside the square than from harvested area inside the square. In contrast, in 2019, adult captures were similar in both areas. The data documented the activity of billbugs in the areas where the sod was harvested, posing a risk of infestation for both strips of nonharvested grass in the harvested area and the adjacent, nonharvested sod fields near harvest.

We determined the influence of abiotic factors on the walking behavior of adult *S. venatus* vestitus. A series of laboratory, semi-field and field assays were conducted in 2019 and 2020. For the laboratory assays, field-collected *S. venatus vestitus* adults were acclimated at 15,18, 21, 28, and 32 °C for 24 h, and the distance walked by these pre-acclimated adults was measured on sand and filter paper substrates using Noldus EthoVision XT software. For the semi-field assay, the total and net distance walked by pre-acclimated adults were measured on the paved indoor surface. *S. venatus vestitus* males and females moved further when the temperature increased from 15 to 28 °C under laboratory and semi-field assays. For the outdoor assay, field-collected *S. venatus vestitus* adults were not acclimated. Therefore, the total and net distance walked by adults were documented on a paved surface. The increase in temperature and relative humidity did not affect the distance moved by adults, but the increase in wind speed reduced the distance moved.

To improve sampling and management, the spatial distribution patterns of *S. venatus* vestitus larvae and adults were assessed at four sod farm sites with a history of *S. venatus vestitus* infestation in central Georgia (USA). The larvae were sampled by soil cores using a hole cutter, whereas adults were collected using pitfall traps for 7 d. The spatial distribution of larvae and

adults was analyzed using SADIE and variograms. The SADIE and variogram analyses revealed a significant aggregation pattern for adults, whereas aggregated distributions were detected for larvae with variogram analyses. The average ranges of spatial dependence for larval and adult samples were 3.9 m and 5.4 m, respectively. Interpolated distribution maps were created to visually depict *S. venatus vestitus* infestation hotspots within the sod farms.

In 2020, an online survey was developed to determine the major pests and adopted management practices from various turfgrass systems mostly in Georgia. A questionnaire composed of 15 questions, organized into three sections 1) facility type, grass type grown, facility location and size, major pest problems and insecticide usage, 2) current IPM approaches against turfgrass pests, and 3) current management approaches for *Sphenophorus* spp. control was asked to turfgrass professionals. Thirty-seven respondents participated from Georgia (93.9%) and Alabama (6.1%). A significantly greater percentage of respondents was from the golf courses (65.8 %) than sod farms, landscape maintenance, and public lawn. A significantly greater percentage of respondents (31.8%) consider fall armyworm a major turfgrass pest compared to mole cricket, billbug, and chinch bug. When turfgrass damage becomes noticeable, a significantly greater percentage of respondents addressed the problem based on previous experience than seeking help from extension agents, deploying traps, and checking online resources. For *Sphenophorus* spp. control in sod farms, a significantly greater percentage of respondents applied insecticides in spring, regardless of turfgrass phenology.

The results indicate that *S. venatus vestitus* is the dominant billbug species in Georgia sod farms, and their seasonal abundance was influenced by turfgrass phenology. Based on our results, *S. venatus vestitus* adults were active in both harvested and nonharvested areas of the field. Also, *S. venatus vestitus* adults continuously emerged in the harvested areas and

nonharvested areas. These findings could refine the current management of *S. venatus vestitus* on the sod farms. In addition, our data showed that temperature variations, in general, influenced the walking capacity of *S. venatus vestitus* adults. The results will improve the integrated management approaches for *S. venatus vestitus* in residential and commercial settings. Also, our results indicate that *S. venatus vestitus* larvae and adults within the sod farms were found aggregated. This information will help develop IPM for *S. venatus vestitus* in sod farms and reduce insecticide use, benefiting growers and the environment. Finally, our data showed that the turfgrass facilities, mostly from golf courses and sod farms, identified fall armyworm, white grubs, and mole crickets as major turfgrass pests management is driven mainly by insecticide use. These findings indicate that more research and extension efforts are warranted to improve the IPM approaches in turfgrass systems across Georgia.