

ABSTRACT

DOSKOCIL, JOSEPH PAUL. The Biology and Ecology of Hunting Billbug in North Carolina Turfgrass (Under the direction of Rick L. Brandenburg.)

The objectives of this research project focused on the biology and habits of the hunting billbug, *Sphenophorus venatus vestitus* Chittenden. The specific scopes of the study were: a) determination of billbug species composition, life cycle, damaging life stage and distribution within the soil profile of turfgrass in North Carolina, b) evaluation of a novel technique to monitor and quantify the movement and behavior of surface and subterranean turfgrass pests, c) quantification of the movement and behavior of hunting billbugs using the previously mentioned technique, d) evaluation of commercially available pesticides for the control of billbugs through field bioassays and evaluation of the contact and ingestion toxicity of these compounds with laboratory bioassays and e) investigations seeking potential presence of attractive compounds produced by either sex of the hunting billbug, host-plant volatiles, and the potential attraction of either sex of hunting billbug to synthetically-produced aggregation pheromones of *Rhynchophorus* spp. and *Metamasius* spp. weevils.

Life history studies were conducted at field sites located near Burgaw in Pender County, Angier in Harnett County, Raleigh in Wake County, Charlotte in Mecklenburg County, and Hendersonville in Henderson County, North Carolina. Linear pitfall trapping yielded six species of billbug in the turfgrass systems in these areas, with hunting billbug, making up 99.7% of the beetles collected. Collections from cupcutter samples suggested that hunting billbugs have two overlapping generations per year and

overwinter as both adults and medium-sized larvae. Field studies indicate that adult hunting billbugs are capable of damaging warm season turfgrasses.

Billbug movement and behavioral quantification was evaluated in the laboratory at North Carolina State University and on the grounds of the University Faculty Club golf course in Raleigh. The addition of a Radio Frequency Identification (RFID) tag did impact survivability, but it was concluded that survival was sufficient to justify further evaluation of the utility of the technique. There were no differences in monthly movement patterns of adult hunting billbugs on golf courses from May through October and regardless of sex. The average distance moved by tagged billbugs was greater on greens compared to roughs.

Field evaluation of insecticides for billbug control took place in Burgaw, NC with laboratory evaluation of compound toxicity conducted at the facilities of North Carolina State University. Field evaluations of a bifenthrin and imidacloprid combination, Allectus™, in the field at Burgaw, NC, suggests it is effective at reducing adult billbug populations when applied in Sep and May with a second application in Sep. LD₉₅ and LC₉₅ data collected in the laboratory showed significant differences between lethal doses of neonicotinoids and pyrethroids or combination products which contain pyrethroids.

Weevil responses varied by treatment with multiple treatments receiving 80% response or better. Treatments assessed included: bermudagrass, bermudagrass plus either or both sexes, pheromone alone, pheromone plus bermudagrass, and pheromone plus bermudagrass plus either or both sexes of weevils.

The results of these studies have provided a more in-depth understanding of the biology and ecology of the hunting billbug in North Carolina turfgrass systems. In addition, a new approach to monitoring surface and subterranean insects was successfully implemented. Finally, a baseline for compound application timing in the field along with contact and ingestion toxicities of these compounds to adult hunting billbugs are provided, and potential compounds for monitoring billbug via pheromone trapping have been identified.

The Biology and Ecology of Hunting Billbug in
North Carolina Turfgrass

by
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A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Entomology

Raleigh, North Carolina

June, 2010

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DEDICATION

This dissertation and the works present within are dedicated to my best friend and partner in life Kelly R. Duskocil and our son Daniel J. Duskocil.

BIOGRAPHY

Joseph Paul (Jake) Duskocil was born the first of three children on 1 December 1981 in Dallas, Texas. He received his elementary and secondary education in Maypearl, Texas, graduating as Valedictorian of his class from Maypearl High School in 2000.

Jake attended Texas A&M University in College Station, graduating in 2004 with a Bachelor of Science degree majoring in both Agronomy and Entomology. He moved on to attend Oklahoma State University, graduating in 2007 with a Masters of Science in Entomology and a thesis titled: Evaluating the Occurrence, Seasonal History, Species Composition and Impact of *Phyllophaga* and *Cyclocephala* Grubs Infesting Bermudagrass (*Cynodon* spp.) in Oklahoma. The author began the pursuit of his Doctorate of Philosophy degree in January 2007 under the supervision of Dr. Rick L. Brandenburg.

On 13 September 2008, he married Kelly Renee Bloom in Girard, Ohio.

On 3 June 2009, his son Daniel James Duskocil was born in Raleigh, North Carolina.

ACKNOWLEDGMENTS

I would like to thank Bayer Environmental Science and the Department of Entomology at North Carolina State University for providing the financial support for this research. I am grateful to Dr. Rick Brandenburg for taking me into his lab and providing perspective on the project, science, and life. In Dr. Brandenburg's lab, Brian Royals and Brenda Watson for getting me on my feet and providing priceless assistance on countless occasions, they are the glue that holds it all together. My committee members Drs. Clyde Sorenson, Yasmin Cardoza, Nate Royalty, and Lane Tredway provided great insight and technical expertise which greatly contributed to the betterment of this project and advancement of my education. Additionally, thank you to the two gentlemen that stepped up and stood in for the defense Drs. Nick Hamon and Larry Grand.

From the Department of Statistics Drs. Consuelo Arellano and Kevin Gross provided expertise in experimental design and data analysis. The cooperation and assistance of the golf course superintendents and managers of the sod farms was critical without them I would not have been able to conduct my research. Alfred Wooten at Quality Turf (Burgaw, NC), Bob Ericson at North Carolina State University Turf Research Facility (Raleigh, NC), Mitch Clodfelter at Cowan's Ford Golf Course (Cowan's Ford, NC), and David Bradley at Turf Mountain Sod (Hendersonville, NC)

THANK YOU!

Last but definitely not least to my wife Kelly for the company on all the road trips checking traps, the late nights helping collect beetles, and the support and understanding along the way, I could not have done it with out you by my side.

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LITERATURE REVIEW

Genus *Sphenophorus*

Billbugs, *Sphenophorus* spp. (Coleoptera: Curculionidae), as described by Vaurie (1951) are weevils, black to deep red in color that vary in length from 4 to 20 mm. The common name comes from a description of the characteristic snout, found on adults, that has chewing mouthparts at the tip. The snout is at least half the length of the pronotum. The body of adults is hard and robust, often with pronotum and elytra covered with an opaque muddy coating; the tip of the abdomen is exposed beyond the elytra. Antennae are inserted at the base of the snout, large and geniculate, with a long scape and bulbous antennal club. *Sphenophorus* can be separated from other related genera by several characters: the relative separation of the coxae; shape of the mesoepimeron, metaepimeron, and intercoxal process; the inward bend of the claw segment at the apex of the tibiae; and the amount and arrangement of setae on the underside of the third tarsal segment. Adults of several billbug species can be distinguished from each other by the markings on their pronotum and elytra (Shetlar 1989). Characteristics to distinguish different species of billbug larvae have not been published.

The genus *Sphenophorus* contains 71 species, 50 of which occur only in the United State or Canada. Billbug species are widespread occurring from the Atlantic to the Pacific Coasts and from southern Canada into Mexico (Vaurie 1951). Eight species are known to be pests of turfgrasses: the hunting billbug, *S. venatus* Say; the bluegrass billbug, *S. parvulus* Gyllenhal; the small billbug, *S. minimus* Hart; the uneven billbug, *S. inaequalis* Say; *S. apicalis* LeConte; the Denver billbug, *S. cicatristriatus* F.; *S. coesifron*

Gyllenhal; and the phoenix billbug, *S. phoeniciensis* Chittenden (Johnson-Cicalese et al. 1990).

Life Cycle

In general, adult grass infesting billbugs emerge from overwintering sites during the spring to feed. Females deposit eggs inside of holes which they have chewed into the base of a grass stem. Eggs are oblong, clear to creamy white, and hatch within 6-14 days (Kelsheimer 1956). The larvae are white, legless and have a brown to red head capsule. Upon hatching the larvae feed first within the stem and tiller of grasses until they become too large to remain in the stem. Larvae then move to the ground and begin feeding from outside the plant, chewing on tillers and roots, damaging the crown. Development extends throughout the summer with 3-6 instars (Vaurie 1951, Tashiro 1987). The average time from egg hatch through pupation is approximately 30 days (Oliver 1984). This information is based on the biology of the bluegrass billbug the most well documented species. Extensive research on hunting billbug biology and ecology has not been conducted. In Arkansas, the hunting billbug is suspected to have one to two generations per year (Young 2002), and in Florida hunting billbugs can have up to six overlapping generations per year (Huang and Buss 2008).

Distribution and Host Range

Most of the research reports available focus on the bluegrass billbug because it causes considerable injury to Kentucky bluegrass (*Poa pratensis* L.) and perennial ryegrass (*Lolium perenne* L.). For example, Turner in (1955) recorded *S. parvulus* as a pest of orchard grass in Virginia with one generation occurring per year and large larvae

the most damaging life stage. Similarly, Johnson-Cicalese et al. (1990) reported one generation of *S. parvulus* per year in cool season turfgrasses in New Jersey. Tashiro and Personius (1970) also found this species destroying lawns in New York, with as many as 50 adults counted on sidewalks within a 5 minute period. In Utah rangeland wheat grass, it was found to have overlapping life stages in the field during summer months (Hanson 1987).

Sphenophorus venatus was first described by Say in 1831 in the genus *Calendra*. The common name “hunting billbug” for *Sphenophorus venatus vestitus* comes from the translation of *venatus* which means “the chase, hunting” (Vaurie 1951). Satterthwait (1919) described *S. venatus* as destructive to forage and grain crops. He found their range to be from Maine to Florida and Wisconsin to Texas, and he presented the first extensive host-plant list for this insect, containing host plant species from which eggs, larvae, pupae, and adults were collected. He noted timothy (*Phleum pratense* L.), bermudagrass (*Cynodon dactylon* [L.] Pers.), wheat (*Triticum* spp. L.), American Great Bulrush (*Scirpus validus* Vahl.), and yellow nutsedge (*Cyperus esculentus* L.) as *S. venatus*' preferred hosts. Other host plants from which *S. venatus* has been recorded include: corn (*Zea mays* L.) (Satterthwait 1919), zoysiagrass (*Zoysia* spp.) (Kelsheimer 1956), orchardgrass (*Dactylus glomeratus* L.) (Kamm 1969), crabgrass (*Digitaria* spp. L.), signal grass (*Brachiaria decumbens* Stapf), St. Augustine grass (*Stenotaphrum secundatum* Kuntze), centipedegrass (*Eremochloa ophiuroidesi* [Munro] Hack), barnyardgrass (*Echinochloa crusgalli* Beav.), sugarcane (*Saccharum officinarum* L.),

bahiagrass (*Paspalum notatum* Flugge), and leatherleaf fern (*Polypodium scolieri* Hook. And Grev.) (Oliver 1984).

The hunting billbug was first described as a serious pest of zoysiagrass sod in Florida (Kelsheimer 1956). In 1960 it was introduced into Hawaii and California by infested sod from the Southeastern United States (Tashiro 1987). Brussell and Clark (1968) showed *S. ventatus* as a pest of zoysiagrass lawns and sod farms in Kansas, and in a review by Oliver (1984), the hunting billbug was described as primarily damaging zoysiagrass and bermudagrass lawns. More recently, it was reported as a pest of turfgrass in the northeastern United States (Johnson-Cicalese et al. 1990), and is the most common species found in warm-season turfgrasses in the Southeastern United States (Young 2002, Huang and Buss 2009).

Damage

Billbugs cause damage which is frequently misdiagnosed as disease, drought, fertility, or other factors (Potter 1998). Billbug damage in cool season turfgrasses has been well documented; adult males notch the stems and blades of grass, and adult females chew holes into stems for oviposition (Shetlar 1995). Small larvae hollow out the stems, leaving frass (Potter 1998), and large larvae can cause severe damage by feeding on crowns, stolons, and roots. This damage initially results in yellow areas in turfgrass which grow larger and coalesce into larger patches of brown or tan turfgrass (Shetlar 1995). Billbug injury can be identified by pulling on the stems of damaged turfgrass and inspecting for signs of chewing or for sawdust-like frass. Affected stems may also break off easily as a result of feeding by billbug larvae (Tashiro 1987). Late

instars, which cause the majority of damage, are most abundant in mid-summer.

However, larvae are not easily found and may require digging 5-7 cm in the soil to locate them (Young 2002).

Although the hunting billbug has been found on many grasses, damage is most apparent in zoysiagrass. It occasionally causes severe damage in bermudagrass, especially when plants are stressed, but the grass's rapid growth allows bermudagrass to repair quickly, masking the symptoms of billbug presence (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 year after year, but billbug damage in bermudagrass, *Paspalum* and zoysiagrass has not been characterized (Doskocil and Brandenburg 2008).

Management

Managing billbug populations can be a challenge because the larvae are hidden within leaf sheathes and stems of the host plant (Potter 1998). Monitoring for adult billbug activity during the spring can help determine presence and whether a management strategy should be implemented. Tashiro and Personius (1970) suggest that when 15 to 25 adults can be collected from paved surfaces in a one-minute period by one person, management is warranted. Pitfall traps have been used to estimate adult billbug densities (Lawrence 1982, Johnson-Cicalese et al. 1990, Young 2002, Huang and Buss 2009). Inspecting traps several times a week and counting the collected adults can provide an indication of when management strategies are needed (Potter 1998).

Cultural Control - Limited research has been conducted looking at traditional cultural management tactics for billbugs such as irrigation, mowing height, and fertilization, but one study reports Kentucky bluegrass suffers from greater injury when mowed at higher heights and maintained under low nitrogen levels (Bishop et al. 1981). Resistant cultivars may reduce the vigor of phytophagous insects, making them more susceptible to natural enemies and other management strategies. Resistant varieties can also help manage more than one insect pest (Campbell et al. 1984). Host plant resistance was reported in several bluegrass cultivars to have the fewest number of bluegrass billbugs present including 'Park', 'Nebraska Common', and 'South Dakota certified' (Kindler and Kinbacher 1975 and Lindgren et al. 1981). Rye grass varieties 'Pennet' and 'Regal' were reported to exhibit minimal damage when billbugs were present (Ahmad and Funk 1983). In New Jersey, Ahmad and Funk (1983) observed significant differences in the damage caused by billbugs in perennial ryegrass trials. Differences in resistance to bluegrass billbug have been observed in range grasses (Asay et al. 1983) and Kentucky bluegrass (Kindler and Kinbacher 1975, Lindgren et al. 1981, Kindler et al. 1982). Varieties with fine stems such as 'Touchdown' or tough tissues such as 'Aquila', 'Geary', 'Nugget', and 'Park' are reported to have better resistance to billbugs (Bruneau et al. 1987). Kentucky bluegrass, creeping bentgrass, and warm-season grasses do not have endophytes but vary in their resistance to billbugs as a result of other physiological characteristics (Young 2002).

Biological Control - Several parasitoids, nematodes, and fungal pathogens have been associated with billbug populations. The presence of *Neotyphodium* spp.

endophytic fungi found in fescue and ryegrass have been associated with resistance to many insects, including billbugs. Endophytic fungi live within plant tissue and produce toxins that effect insect and other herbivore feeding on the colonized plants (Johnson-Cicalese and White 1990). Resistance found in perennial ryegrass cultivars is enhanced by the presence of a *Neotyphodium* endophyte (Ahmad et al. 1986). The survival of billbug adults is reduced when feeding on endophyte-colonized tall fescue, and fewer eggs are deposited (Johnson-Cicalese and White 1990). Further experimentation in the field showed that the development and survival of billbug larvae may also be inhibited by the presence of an enophyte (Murphy et al. 1993). Overseeding existing stands of Kentucky bluegrass with 35% endophytic varieties of perennial ryegrass may reduce bluegrass billbug larval populations and damage (Richmond et al. 2000).

With the exception of nematodes, no attempts have been made to use biological management programs for billbugs in turfgrass. Several parasitoids of billbug eggs, larvae, and adults have been reported. *Anaphes (Anaphoidea) calendrae* Gahan, a mymarid wasp, has been recorded parasitizing eggs of several species of billbugs including; *S. parvulus*, *S. minimus*, and *S. callosus* (Satterthwait 1931). There is only one record of a parasitoid of larvae, the wasp *Zavipio belfragei* Cresson [renamed *Vipio belfragei* (Cresson)] (Satterthwait 1919), and only one for adults, the tachinid fly *Strongygaster triangulifer* (Young 2002). Young (2002) also reported that only 0.5% of all adults dissected had parasites or mites, and suggested that parasitoids have little influence on billbug populations.

Three species of nematodes have been used against billbugs: *Steinernema carpocapsae*, *S. feltae*, and *Heterorhabditis bacteriophora* (Niemczyk and Shetlar 2000). These authors suggested that treatments were most effective while the larvae inhabited the crowns of the plants.

Beauveria spp. is the only pathogen reported in natural billbug populations (Johnson-Cicalese 1988). This fungus has been found infecting larvae of *S. venatus confluens* in orchardgrass in Oregon (Kamm 1969), *S. callosus* in corn stalks in North Carolina (Wright et al. 1983), where it caused 30% mortality of *S. coesifrons* in Georgia (Morrill and Suber 1976). Biopesticides containing *Beauveria* spp. have been marketed, but little information is available concerning the level of billbug control they provide (Potter 1998).

Chemical Control - Insecticides have traditionally been the primary tool for the reduction of subterranean insect pests in turfgrass systems (Shetlar 1995). Numerous studies have evaluated chemical control of *Sphenophorus* spp. in field situations imidacloprid, provided 98% control (Anderson et al. 2004), chlorantraniliprole provided 91% control, bifenthrin 82%, clothianidin 94% (Eickhoff et al. 2007) thiamethoxam 90% when evaluated in Kentucky bluegrass in Nebraska (Pierson et al. 2008) and bifenthrin and clothianidin combination granular preformed better than soluble concentrate when evaluated in Pennsylvania (Heller et al. 2008). Only two studies have been conducted in warm season turfgrass; Walker and Royer 2002 found thiamethoxam granular to be more effective than the wettable granular in Oklahoma bermudagrass and Buss et al. (2004)

accounted for billbug presence in trials of trichlorfon and imidacloprid. Only those studies conducted by Buss et al. (2004) occurred in the southeastern United States.

Knowledge of an insect's life cycle and seasonal activity is essential to maximize the efficacy of insecticides. Insecticide applications are recommended in the spring as a preventative approach to control adults in areas with consistent billbug pressure (Shetlar 1995). Timing can be complicated by geographic region dependant upon multiple generations and the presences of multiple species. Larval control can be difficult because they spend the majority of their time burrowing within grass stems and are sheltered from contact insecticides, like pyrethroids. They become more susceptible to contact poisons once they have moved to the root zone (Potter 1998). Reducing the larval population has been viewed as a curative and alternative approach to reduce the ensuing overwintering adult population (Niemczyk 1983). In this case a systemic insecticide, like imidacloprid or halofenozide, is needed to deliver the toxin to the larvae inside the stem (Potter 1998).

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Journal of Economic Entomology
Horticultural Entomology
Doskocil and Brandenburg: Billbug species,
life cycle and damaging stage.

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Determination of Billbug (Coleoptera: Curculionidae) Species Composition, Life Cycle
and Damaging Life Stage in North Carolina Turfgrass.

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ABSTRACT

In the southeastern United States, hunting billbug adults, *Sphenophorus venatus vestitus* Chittenden, are often observed, but our knowledge of their biology and ecology is limited. Field surveys and experiments were conducted to determine the species composition, life cycle, damaging life stage and distribution within the soil profile of billbugs in turfgrass in North Carolina. Linear pitfall trapping revealed six species of billbug, with the hunting billbug making up 99.7% of all beetles collected. Data collected from cupcutter sampling suggest that hunting billbugs have two overlapping generations per year in North Carolina and overwinter as both adults and medium-sized larvae. Field experiments provide evidence that adult hunting billbugs are capable of damaging warm season turfgrasses.

Keywords: *Sphenophorus venatus vestitus*, hunting billbug

INTRODUCTION

Over the past ten years, reports of billbugs in warm season turfgrasses have increased (R.L.B. personal observation). Billbugs (Coleoptera, Curculionidae) are well known insect pests of turfgrasses in many parts of the world (Tashiro 1987). Over 60 species of billbugs exist in the United States with pest activity historically located in areas where cool season turfgrass is grown (Niemczyk and Shetlar 2000). In the southeastern United States, our knowledge of billbug biology and ecology is limited. In Florida, where ten different billbug species have been recorded in turfgrasses, the hunting

billbug, *Sphenophorus venatus vestitus* Chittenden, was the predominate species collected. There, the hunting billbug can have up to six overlapping generations per year, and adults are nocturnal (Huang and Buss 2008). Similar information on billbug species composition, life cycle, and distribution within the turfgrass and soil profile is lacking for the cool and warm season turfgrass transition zone areas such as North Carolina.

Billbugs cause damage which is often misdiagnosed as disease, drought, fertility, or other factors (Potter 1998). Billbug damage in cool season turfgrasses has been well described; adults notch the stems and blades of grass, and females chew holes into stems for oviposition (Shetlar 1995). Small larvae hollow out the stems, leaving frass (Potter 1998) and large larvae can cause severe damage by feeding on crowns, stolons, and roots. Damage caused by larvae can result in yellowing areas that grow larger and coalesce into patches of brown or tan turfgrass (Shetlar 1995). On warm season turfgrass in North Carolina, the most severe symptoms appear during late spring and early fall in the same areas year after year, but billbug damage in bermudagrass, paspalum and zoysiagrass has not been thoroughly characterized (Doskocil and Brandenburg 2008). The objectives of this study were to determine the species composition, abundance, seasonal activity, life-cycle, damaging stage, and distribution of billbug adults and larvae within the turfgrass and soil profile in North Carolina turfgrass systems.

MATERIALS AND METHODS

Species Composition, Abundance, and Activity. Five locations across North Carolina with a history of billbug presence were selected from a range of turfgrass

species for billbug species composition surveying: Western NC- Turf Mountain Sod Farm, Hendersonville, NC, bluegrass *Poa pratensis* L.; Cowan's Ford Country Club, Charlotte, NC, bermudagrass, *Cynodon dactylon* (L.) Pers.; Central NC- Hidden Valley Golf Course, Angier, NC, bermudagrass; North Carolina State University Faculty Club, Raleigh, NC, bermudagrass; and Southeastern NC- Quality Turf sod farm, Burgaw, NC, zoysiagrass, *Zoysia japonica* L. Linear pitfall traps (similar to Lawrence 1982) were placed at each location. Each trap consisted of 2 m long polyvinyl chloride (PVC) pipe, 5 cm in diameter, with a 2.5 cm slit cut lengthwise across the top and with a cap fastened at one end. A 0.96 L plastic cup (Sweetheart® Maximizers™, PFS Sales, Raleigh, NC) with a hole cut into the side, 7.5 x 7.5 cm, for pipe insertion was placed over the open end of the PVC pipe. A lid was placed on top of the cup and small holes were made in the bottom to allow for drainage. Two traps were placed perpendicular to each other so that the slit along the length of the trap was even with the soil surface, one meter apart at the edge of an area of known billbug presence. Collection from traps occurred weekly from Mar through Oct and monthly from Nov through Feb. Collection occurred Jun 2007 through Dec 2007 at the Angier site, Jun 2007 through Nov 2009 at the Raleigh and Charlotte sites, and Feb 2008 through Nov 2009 at the Burgaw and Hendersonville sites. All adult billbugs collected were placed into vials with 70% ethanol for preservation. Beetles were identified to species in the laboratory using morphological characters (Vaurie 1951). Species abundance by collection date and location was compiled and recorded to determine seasonal activity. Voucher specimens have been deposited in the North Carolina State University Entomology Museum.

Distribution in the Turfgrass-Soil Profile. Two locations were selected to examine seasonal occurrence and distribution of billbug life stages in the turfgrass and soil profile; North Carolina State University Faculty Club, Raleigh, NC, where the sod was bermudagrass grown on Cecil clay loam soil; and Quality Turf sod farm, Burgaw, NC, where the sod was zoysiagrass grown on Norfolk fine sandy loam soil. Sampling occurred weekly from Mar through Oct and monthly from Nov through Feb. The sampling period occurred from Mar 2008 through Nov 2009 in Burgaw, and Mar 2009 through Nov 2009 in Raleigh.

In 2008, eight, 10.2 cm dia x 15.2 cm depth, cupcutter samples were taken randomly, during each sampling in areas which exhibited symptoms of billbug damage. In 2009, the protocol was modified by taking 20 cupcutter samples in a sequential manner per sampling. Initially, sampling areas were chosen randomly; the same manner as in 2008. If no billbugs were found in a given sample, a new area was selected. If larvae were found, four additional samples were taken in the immediate area of that sample; once five samples were taken in an immediate area, a different area was selected. This was repeated until a total of 20 cupcutter samples had been taken.

All samples were processed in the field at the time of collection. Cupcutter samples were divided into four segments; turfgrass and thatch, 0-5 cm, 5-10 cm, and 10-15 cm in the soil profile. The turfgrass and thatch section was processed by tearing the turfgrass and thatch apart in a tub and searching the contents for billbugs. Soil segments were sifted into a tub and searched for billbug presence. The number and size of recovered larvae were recorded for each segment along with the number of pupae.

For the purposes of this study, larvae were placed into one of three categories; small, medium, and large. This is because billbug larval size has not been characterized. Small refers to neonate larvae and those of similar size, within 1-2 instars from egg hatch; medium refers to larvae noticeably larger which have matured through 2-3 instars; large refers to larvae larger than medium larvae which are in their last 2 instars before pupation. Data from both locations were compiled along with pitfall trap data of adult activity and presence to develop a comprehensive overview of the billbug life cycle in North Carolina turfgrasses.

Damaging Life Stage. Two locations were selected for determination of billbug damaging life stage. Trial I was conducted at Quality Turf sod farm, Burgaw, NC, in zoysiagrass grown on Norfolk fine sandy loam soil, while Trial II was conducted at North Carolina State University's Lake Wheeler Turf Research Facility, Raleigh, NC, in bermudagrass grown on Cecil clay loam soil. Areas of turfgrass which had no known history of billbug presence in these respective locations were selected. Arenas were constructed by driving a large cylinder of polyvinyl chloride pipe (15 cm dia x 20 cm length) to a depth of 15 cm into established stands of turfgrass (Potter 1982). This resulted in an enclosed turfgrass arena with a 5 cm barrier above the turfgrass surface and a 15 cm barrier below the surface.

On 13 Aug 2009, adult hunting billbugs were collected manually at night prior to initiation of the trial from the North Carolina State University Faculty Club Golf Course in Raleigh, NC. Billbugs were kept in groups of ten in plastic graduated 30 mL medicine cups (Solo®, Highland Park, IL) until introduction into the arenas the following morning.

Billbug larvae were collected from the same location as the adults on 14 Aug 2009. Bermuda turfgrass was cut into strips with a sod cutter and medium to large larvae were collected manually from the bare soil and sod. Larvae were held in 28.3 L storage containers (57.9 x 42.4 x 17.8 cm Rubbermaid® Snaptoppers™, Newell Rubbermaid Inc., Fairlawn, OH), between layers of moist paper towel out of direct sunlight, until introduction to arenas. Larval collection occurred from 6 to 10 am and introduction to arenas was complete by 12 pm. Adult populations of 6, 10, and 16 beetles were introduced into arenas and allowed to settle into the turf. One cm holes were made through the turfgrass 2 cm deep into the soil using the end of a Sharpie pen (Sanford Corp., Atlanta, GA); one larva was placed into each hole and covered with soil. Treatment populations of 3, 5, and 8 larvae per arena were used. After introduction, arenas were covered with fiberglass screen (Phifer INC., Tuscaloosa, AL) and the screen was secured with a 0.91 m cable tie (Cable Ties Plus Inc., Kingston, MA). Treatments along with an untreated control, a covered arena with no billbugs, were replicated four times at each location in a RCB.

Billbugs were introduced at the Trial I location, Burgaw, NC, on 14 Aug 2009. The Trial II location in, Raleigh, NC, had two introductions; first on 17 Aug 2009 and on 9 Sep 2009 in different areas of the same location. Collection for the first introduction of Trial II occurred 16 Aug 2009 for adults and 17 Aug 2009 for larvae. For the second introduction of Trial II adults were collected on 8 Sep 2009 and larvae were collected on 9 Sep 2009.

Trial I was evaluated on 10 Sep 2009. The PVC barrier was removed from the ground to allow digital photographs to be taken and the grass height to be recorded for each arena. Grass height was measured from the soil surface to top of grass blades within the arena using a metric ruler. Digital photographs were taken with a Lumix DMC-LZ5 (Panasonic Corp., Secaucus, NJ) set on simple mode and with the aid of a light box. The light box was consisted of a 64 L opaque plastic tub (40.4 x 41.9 x 60.7 cm, Rubbermaid[®], Roughneck[™], Newell Rubbermaid Inc., Fairlawn, OH) with a hole (5 x 7.6 cm) cut in the center of the bottom. Six, 30.4 cm, white fluorescent battery operated lights (Model No. 17406, General Electric[®], Fairfield, CN) were attached to the inside of the box with adhesive Velcro (Velcro[®], Sticky Back[®], Levitt Industrial Textile, Hicksville, NY) in the corners and the center of the longest sides. The light box was placed top side down over the arena, and the lens of the camera guided through the hole in the bottom. Images were analyzed with Sigma Scan Pro (Systat Software Inc., San Jose, CA) digital imaging software to provide non-discriminate empirical data of the grass color in each plot. Images were resized to 680 x 500 pixels and threshold values were set at; hue 50-130, saturation 20-100. The turfgrass and soil it enclosed were then removed with a shovel and placed into a collection tub where the contents were searched for billbug life stages in a manner similar to that described in the life stage distribution protocol. All life stages found were recorded. The first introduction of Trial II was evaluated on 13 Sep 2009 while the second introduction was evaluated on 30 Sep 2009 for the second infestation.

All data were analyzed using Proc GLM and with means separated with Tukey's HSD at $p < 0.05$ (SAS Institute 2003).

RESULTS

Species Composition, Abundance, and Activity. From 2007 through 2009, 21,326 billbugs were collected from five locations. Six species were identified in this order of abundance: *S. venatus vestitus* > *S. minimus* > *S. inaequalis* > *S. callosus* > *S. rectus* > *S. parvulus* (Table 1). *Sphenophorus venatus vestitus* was collected at all five locations, representing >99% of all beetles collected, and was the only species recorded at the Charlotte and Raleigh locations in bermudagrass. *S. minimus*, *S. rectus*, and *S. parvulus* were recorded only at the Hendersonville location, in bluegrass. *S. callosus* was recorded only at the Angier location, in bermudagrass. *S. inaequalis* was recorded at both the Burgaw and Angier locations in zoysiagrass and bermudagrass, respectively.

As measured by linear pitfall trapping, adult hunting billbug activity varied from location to location and year to year (Figures 1, 2, and 3).

Distribution in the Turfgrass-Soil Profile. From 2008 to 2009 at two locations 162 immature billbugs were collected from 1276 cupcutter samples, soil volume 1.58 m^3 (Table 2). Results from immature life stage sampling revealed trends in presence throughout the year (Figure 4).

Damaging Life Stage. All larval treatments were found to have no significant impact on turfgrass color (Figures 5 and 6) or height (Figures 7 and 8) when compared to the control in both zoysiagrass and bermudagrass plots. In contrast, all adult treatments

were found to significantly reduce turfgrass greenness (Figures 5 and 6) and height (Figures 7 and 8) when compared to the control in both zoysiagrass and bermudagrass plots. Differences in larval treatment rates did not result in a significant difference in either color or height among treatments. At the conclusion of the study 81% of adults and 21% of larvae used were recovered from the zoysiagrass plot. Three pupae were found in larval treatments and no larvae were found in adult treatments. In the bermudagrass plot 87% of adults and 27% of larvae were recovered. Two pupae were found in larval treatments and no larvae were found in the adult treatments.

DISCUSSION

Hunting billbug was the most abundant species recorded in North Carolina turfgrasses. Peaks in adult activity occurred in late spring and from late summer to early fall. Pitfall trapping only monitored adult activity and provides no insight into immature life stage occurrence. Little work has been done to document other life stages of billbugs in the field (Johnson-Cicalese 1990). We were only able to find low numbers of larvae on a consistent basis at two locations over two field seasons, but these data did help determine that hunting billbugs have two overlapping generations per year in North Carolina, with both medium-sized larvae and adults overwintering.

Immature presence in the soil profile (Table 2) and adult seasonal activity (Figures 1, 2, and 3) present a picture which does not match reports in the literature of larvae being the damaging life stage. Peaks in adult activity coincided with billbug damage symptoms observed in warm season turfgrasses (Doskocil and Brandenburg

2008). Damage is often noticed in the spring; affected turfgrass is slow to recover from winter dormancy. In late summer and fall damage presents itself as a “dry patch” which does not respond to additional watering (Doskocil and Brandenburg 2008). Closer examination of these areas reveals grass that is easily pulled free from the crown and roots. The stems break off with no trace of soil, indicating damage which occurred above the soil surface (J.P.D., personal observation). This is unlike damage from larvae of Scarabaeid white grubs which results in turfgrass that can be pulled loose and rolled up like carpet, a result of the root system being severely damaged (Watschke et al. 1995). Information from literature indicates that young billbug larvae feed in and on the crowns of grass while late instars feed on the root system below the surface (Doskocil and Brandenburg 2008). During our survey of immature billbug life stages, very few larvae were found above the soil surface in the turfgrass and thatch where damage is occurring. The majority of larvae were found below the soil surface (Table 2).

Data from the damaging life stage studies indicate that adult hunting billbugs are capable of reducing the greenness and height of bermudagrass and zoysiagrass when compared to the control (Figures 5, 6, 7 and 8). At the conclusion of the study no immature life stages were found in adult treatments, suggesting that the damage and resulting symptoms were caused only by adults. Larvae were not found to reduce the greenness or height of the grass when compared to the control (Figures 5, 6, 7, and 8). This may be due to the fact that larval recovery in both bermudagrass and zoysiagrass plots was poor, less than 30%. Poor recovery is not uncommon in studies which introduce subterranean pest into artificial arenas; white grub larvae have also been

reported to experience poor recovery when relocated for damage threshold studies (Potter 1982). While we were not able to quantify any damage caused by larval presence, this does not suggest that they are incapable of causing damage. More likely, our larval population was not maintained at high enough levels in the treatment arenas to cause damage. However, the numbers used for treatments in this trial equaled or exceeded any populations we have encountered in the field, and it is important to note that in severely damaged areas we never recovered significant numbers of larvae or pupae.

In this study we were able to characterize the species composition and seasonal activity of billbugs in North Carolina turfgrasses. Additionally we were able to determine the life cycle and damaging life stage of hunting billbug in bermudagrass and zoysiagrass. The information gained by this study provides a better understanding of the life history billbugs in North Carolina, a key step in developing an effective and comprehensive management strategy of this turfgrass pest.

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Footnotes

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Table 1. Billbug species abundance by location for the collection period May 2007 through Nov 2009. Grass type surveyed found beneath location.

Species	Location					Total Billbugs
	Raleigh	Charlotte	Burgaw	Hendersonville	Angier	
	bermudagrass	bermudagrass	zoysiagrass	Bluegrass	bermudagrass	
<i>S. venatus vestitus</i>	6832	13922	331	95	84	21264
<i>S. inaequalis</i>	0	0	10	0	9	19
<i>S. minimus</i>	0	0	0	28	0	28
<i>S. rectus</i>	0	0	0	4	0	4
<i>S. parvulus</i>	0	0	0	1	0	1
<i>S. callosus</i>	0	0	0	0	10	10
Total	6832	13922	341	128	103	21326

Table 2. Total number of immature billbugs found in the turfgrass, thatch, and soil profile by section from Raleigh and Burgaw, NC during 2008 and 2009 in a total of 1276 cupcutter samples constituting a soil volume of 1.58 m³.

Life stage	Turfgrass and thatch	Soil depth (cm)			Total
		0-5	5-10	10-15	
Small larvae	3	30	0	0	33
Medium larvae	5	44	5	0	54
Large larvae	3	43	13	1	60
Pupae	0	6	8	1	15
Total	11	123	26	2	162

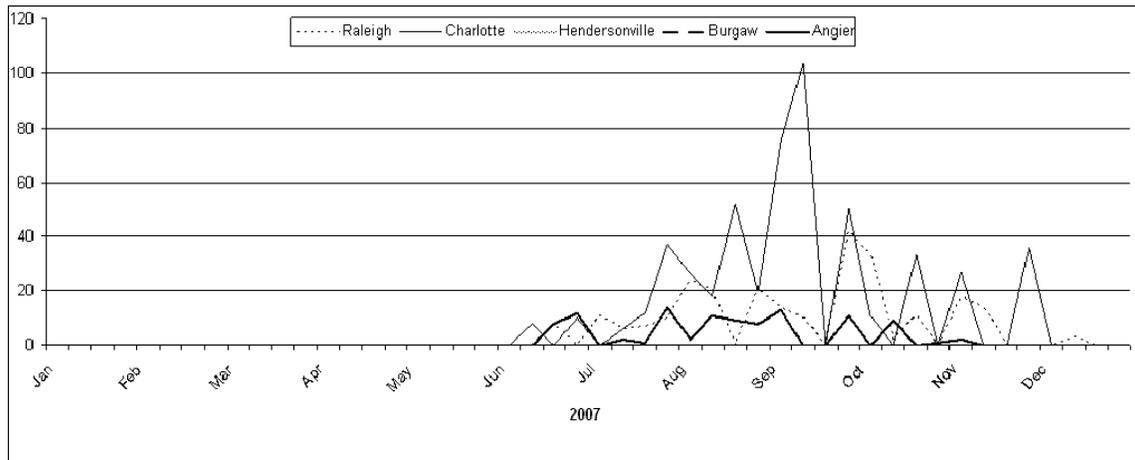


Figure 1. Total number of *Sphenophorus* spp. adults collected from two linear pitfall traps in Raleigh, Charlotte, and Angier, NC from Jun 2007 through Dec 2007.

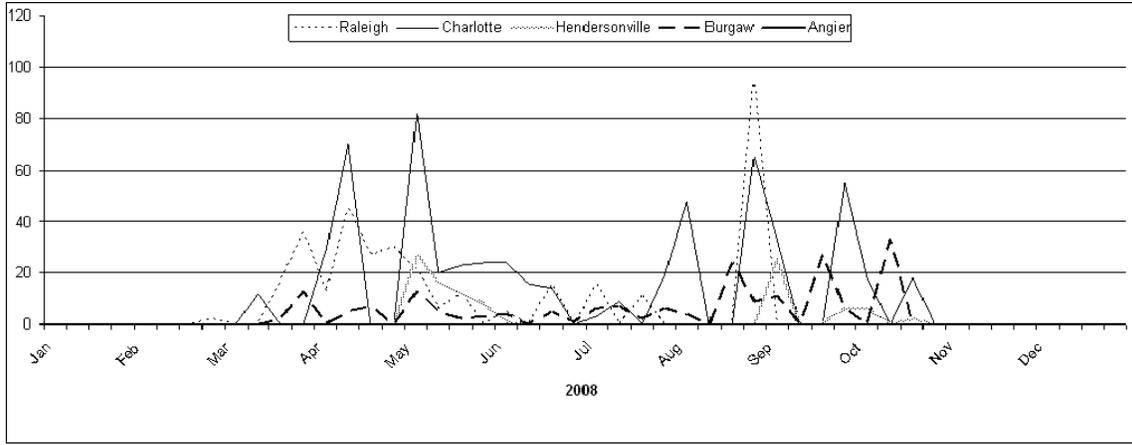


Figure 2. Total number of *Sphenophorus* spp. adults collected from two linear pitfall traps in Raleigh, Charlotte, Hendersonville, Burgaw, and Angier, NC from Jan 2008 through Dec 2008.

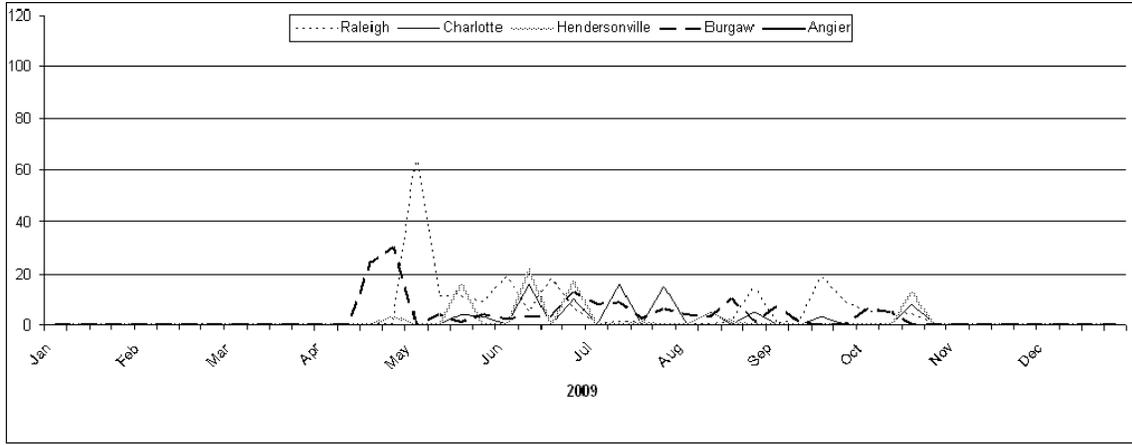


Figure 3. Total number of *Sphenophorus* spp. adults collected from two linear pitfall traps in Raleigh, Charlotte, Hendersonville, Burgaw, and Angier, NC from Jan 2009 through Nov 2009.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Small larvae						█	█	█	█	█		
Medium larvae	█	█	█	█		█	█	█	█	█	█	█
Large larvae		█	█	█	█		█	█	█			
Pupae				█	█		█	█	█			
Adults	█	█	█	█	█	█	█	█	█	█	█	█

Figure 4. The seasonal occurrence of *Sphenophorus venatus vestitus* life stages from Raleigh and Burgaw, NC during 2008 and 2009. These data were combined to graphically represent the presence of each life stage throughout a calendar year; as found through the field sampling at these locations.

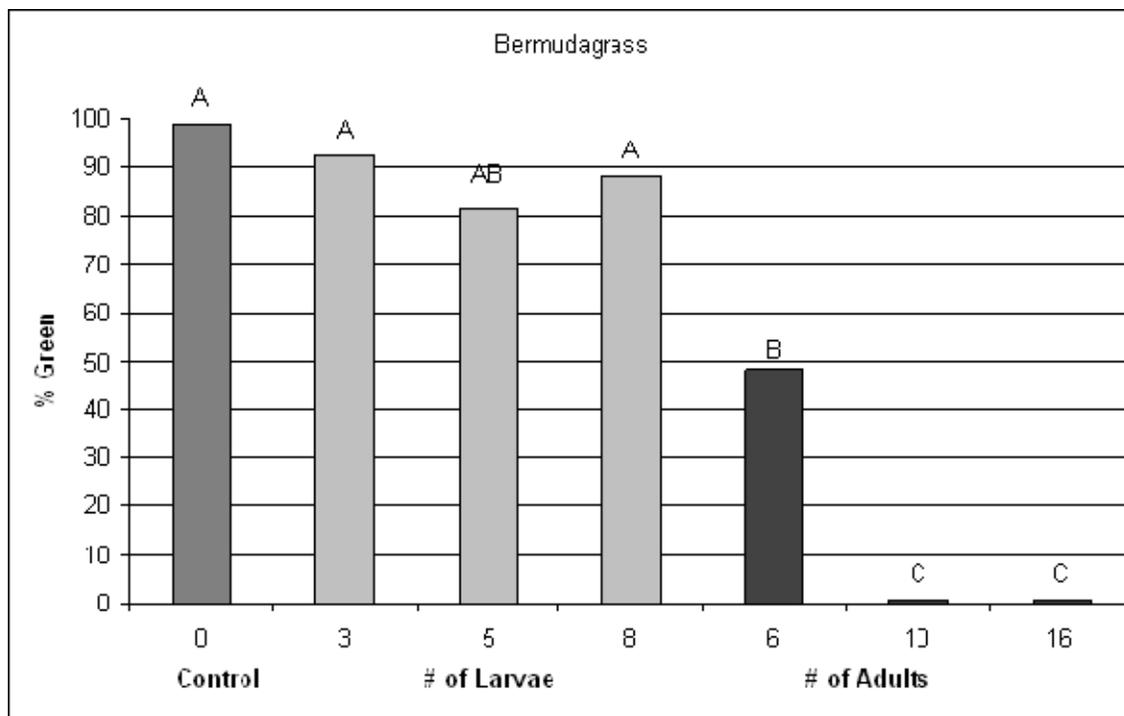


Figure 5. Percent greenness of bermudagrass at the conclusion of the damaging life stage study using *S. venatus vestitus*. Larvae and adults were introduced into 15 cm dia PVC arenas. Study was conducted Sep 2009, at North Carolina State University Lake Wheeler Turf Research Facility, Raleigh, NC. Data were analyzed with Proc GLM, Tukey's HSD at $p < 0.05$.

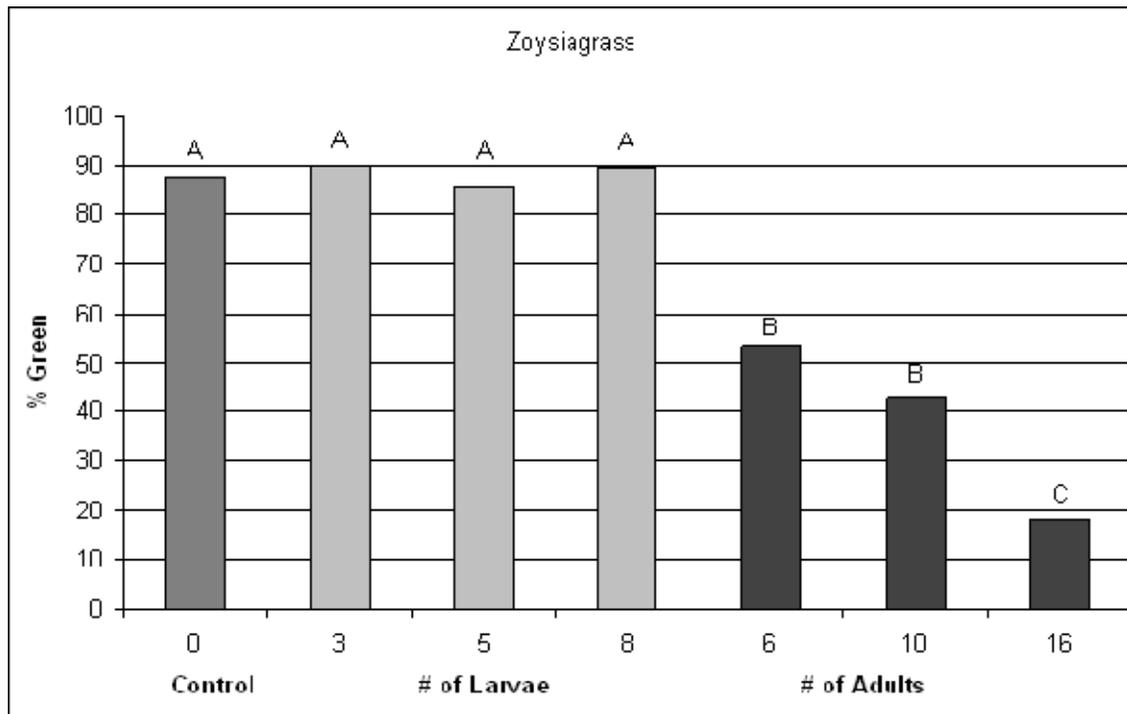


Figure 6. Percent greenness of zoysiagrass at the conclusion of the damaging life stage study using *S. venatus vestitus*. Larvae and adults were introduced into 15 cm dia PVC arenas. Study was conducted Sep 2009, at Quality Turf sod farm, Burgaw, NC. Data were analyzed with Proc GLM, Tukey's HSD at $p < 0.05$.

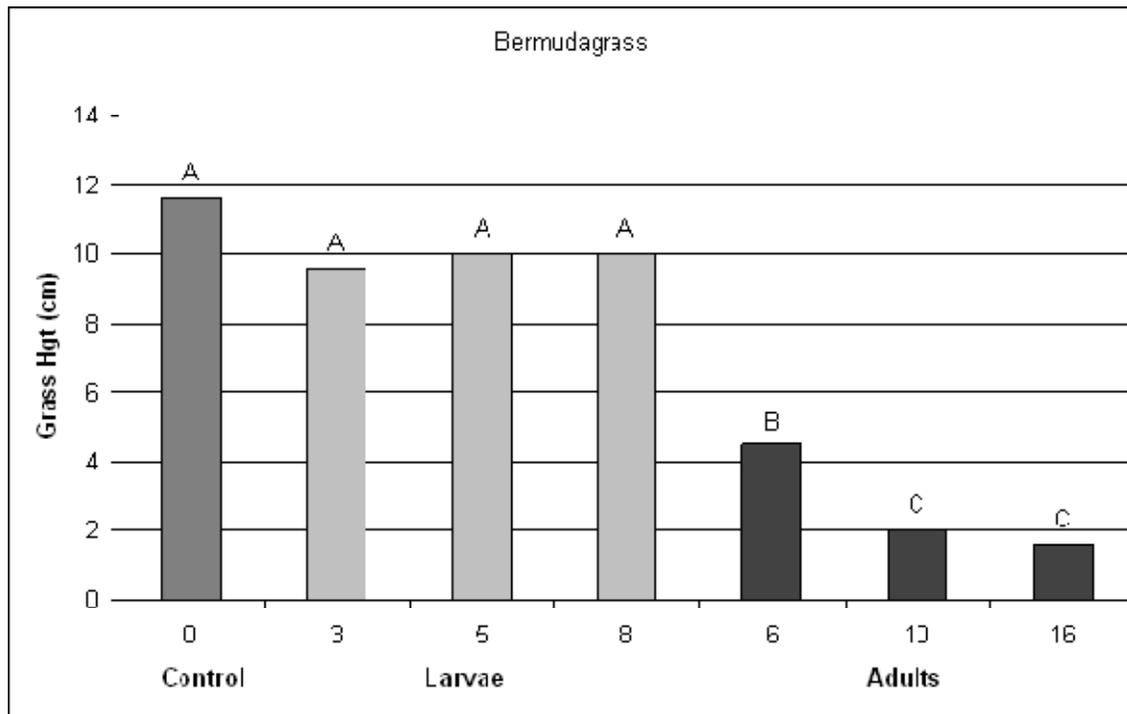


Figure 7. Grass height of bermudagrass at the conclusion of the damaging life stage study using *S. venatus vestitus*. Larvae and adults were introduced into 15 cm dia PVC arenas. Study was conducted Sep 2009, at North Carolina State University Lake Wheeler Turf Research Facility, Raleigh, NC. Data were analyzed with Proc GLM, Tukey's HSD at $p < 0.05$.

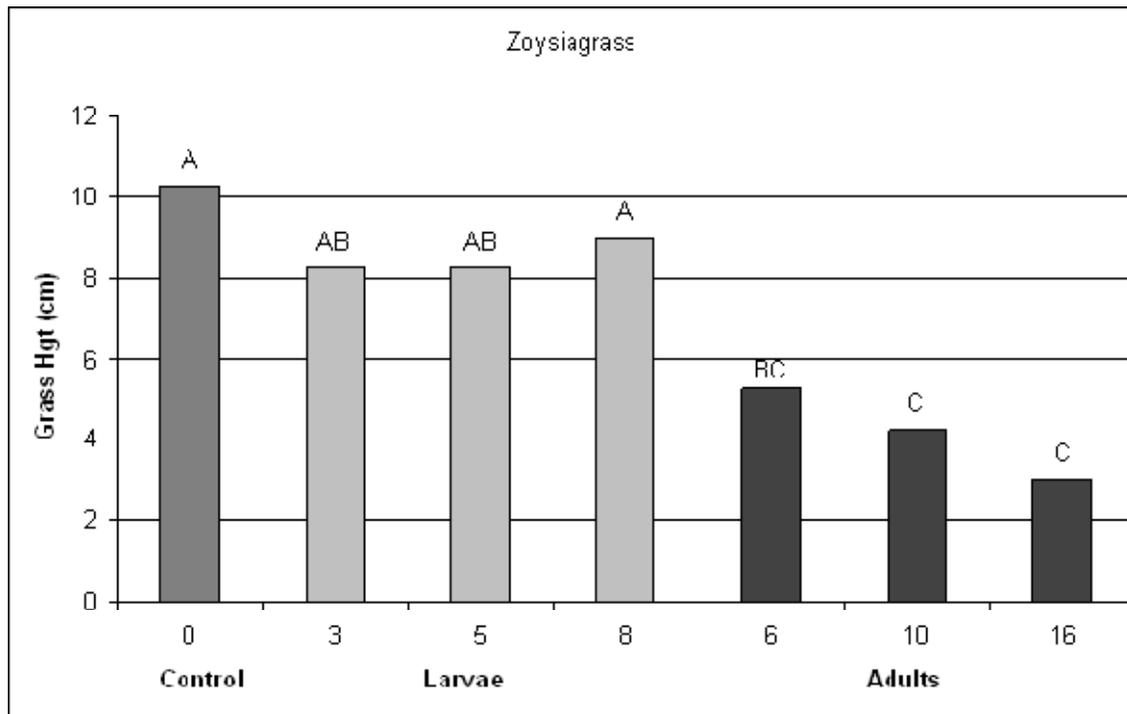


Figure 8. Grass height of zoysiagrass at the conclusion of the damaging life stage study using *S. venatus vestitus*. Larvae and adults were introduced into 15 cm dia PVC arenas. Study was conducted Sep 2009, at Quality Turf sod farm, Burgaw, NC. Data were analyzed with Proc GLM, Tukey's HSD at $p < 0.05$.

A Novel Approach to Monitoring Insects

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Radio Frequency Identification Tagging: A Novel Approach to Monitoring Surface and
Subterranean Insects

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ABSTRACT

Radio frequency identification (RFID) transponders were attached to hunting billbugs, *Sphenophorus venatus vestitus* Chittenden (Coleoptera: Curculionidae) and implanted in the abdomen of tawny mole crickets, *Scapteriscus vicinus* Scudder (Orthoptera: Scapteriscus), to evaluate a technique for monitoring and quantifying the movement and behavior of these turfgrass pests. While the addition of the transponder did impact survivability, there was sufficient survival to justify further evaluation of the utility of the technique. Our data suggest that RFID marking can be a useful technique for elucidating the location and behavior of soil surface-dwelling and subterranean insects.

Key Words: RFID, mark- relocation, *Scapteriscus vicinus*, *Sphenophorus venatus vestitus*.

INTRODUCTION

Effective pest management strategies require an understanding of the pests' biology, ecology, and behavior. Insect dispersal and behavior monitoring techniques include mark and recapture, the use of colored or numbered tags (Southwood 1987), or video recording (Wratten 1994). However, these techniques become more challenging to apply when the insect of interest is difficult to observe, as is the case for subterranean insects.

Hunting billbugs, *Sphenophorus venatus vestitus* Chittenden., are important pests of warm season turfgrasses that cause damage frequently misdiagnosed as disease, drought, fertility problems, or other factors (Potter 1998). Such misdiagnosis is often due to the lack of information about the insect's biology and ecology (Johnson-Cicalese et al. 1990). The immature life stages are difficult to locate in the soil profile and the adults spend the majority of their time concealed below the turfgrass surface in the thatch layer (Shetlar 1995). These factors make it difficult to locate and observe individual billbugs and render the study of their biology and ecology challenging.

Mole crickets are some of the most important soil-dwelling turfgrass pests in the southeastern United States (Brandenburg 1997). The herbivorous tawny mole cricket, *Scapteriscus vicinus* Scudder, damages turfgrass by feeding on roots and shoots, and through extensive soil tunneling (Brandenburg et al. 2000). Mole crickets live primarily underground and are quite mobile, making them difficult to monitor and to control. Alterations in mole cricket behavior have been observed in response to treatments with insecticides and entomopathogenic fungal conidia (Villani et al. 2002, Thompson and Brandenburg 2005). Changes in behavior included reduced surface activity and avoidance of insecticide treated areas. Understanding the subterranean movement of mole crickets may facilitate the development of improved, cost-effective, and environmentally-sound control practices.

Recent advances in Radio Frequency Identification (RFID) technology, including improvements in strength of signal and size of transmitters, have increased the potential for its use in monitoring insects (Streit et al. 2003). RFID technology consists of three

basic components: 1.) an antenna or coil, 2.) a transponder (the tag) and 3.) a transceiver (decoder). The wireless sensor technology is based on the detection of electromagnetic signals. The antenna emits radio signals that activate the passive transponder. The activated transponder sends its unique code and data set back to the transceiver, via the antenna, where the data record is acquired and decoded. The effective read range between antenna and tag varies depending upon power output, radio frequency used, and the size of transponder (Domdouzis et al. 2007).

Limited work has been done on the use of RFID technology for tracking the movement of insects (Streit et al. 2003 and Robinson et al. 2009). These previous experiments were conducted using social insects in easily observable environments. The colony-forming nature of these insects allowed the establishment of receiver locations at fixed points, such as nest entrances, where the insects were likely to return (Sumner et al. 2007). Such locations also permitted the marked insect to consistently come in close proximity to the receiver with no obstructions between the transponder it carried and the receiver. While these studies produced valuable information on the behavior of social insects, to date, work has not been conducted using RFID technology for mark-relocation experimentation specifically to track soil-dwelling insects.

The objectives of the research reported in this paper were: 1.) to evaluate insect survivability and mobility with the addition of a RFID transponder, 2.) to evaluate the utility of RFID technology as a method for relocating tagged and released insects, and 3.) to monitor the localized movement of soil-dwelling insects in both insecticide treated and untreated plots.

MATERIALS AND METHODS

Billbugs

Adult hunting billbugs were collected by hand at night on 29 May 2008, 5 Jun 2008, 8 Jun 2008, and 9 Jun 2010 from the North Carolina State University Faculty Club golf course in Raleigh, NC. Billbugs were kept in groups of ten in plastic graduated 30-ml plastic medicine cups (Solo®, Highland Park, IL) until the following morning.

Average beetle weight was 35 mg, and length varied between 7-9 mm. Forceps were used to restrain individual beetles, on either side of the abdomen and thorax, while a drop of quick-set adhesive, 1/2 the size of the beetle pronotum, (Impact-tough formula, Gorilla Glue Co., Cincinnati, OH) was placed on the dorsal side of the thorax. An 8 mm x 2.12 mm, 67mg, RFID transponder, TXP148511B (Biomark®, Inc., Boise, ID), was placed length-wise on top of the beetle and the glue was allowed to harden. Additional glue was added to the top and sides of the tag, encasing it to ensure its attachment. A round, wooden toothpick was used to remove excess glue before it hardened.

Survivability - Survivability of billbugs was evaluated by holding both tagged and untagged beetles in plastic petri dishes (95 mm diameter, Fisher Scientific, Pittsburgh, PA) with moistened paper towel for 12 d. Six beetles were tagged on 6 Jun 2008, 8 on 9 Jun 2008, and 3 on 10 Jun 2008. Each tagged beetle had an untagged counterpart, collected on the same date, for a total of 34 individuals. Dishes were checked daily and mortality recorded. Data were transformed (*square root of $X + 0.5$*) prior to analysis for treatment effect using Proc GLM and means were separated using Tukey's

HSD values through use of Statistical Analysis System version 9.1 program (SAS Institute 2003).

Mobility - Mobility was evaluated by placing tagged and untagged beetles in an arena with a smooth wooden runway (175x5x7cm). Eight beetles were tagged on 30 May 2008, six on 6 Jun 2008, eight on 9 Jun 2008, and eight on 10 Jun 2010. Each tagged beetle had an untagged counterpart for a total of 60 individuals. Tagged and untagged individuals in each pair were assessed simultaneously in adjacent runways separated by a 6.2 cm tall wooden barrier. Experiments were conducted between the hours of 10 am and 4 pm each day. Each beetle was initially covered with an opaque plastic vial lid for five minutes prior to release in an attempt to acclimate the insect to the runway. Once the cap was removed, the distance traveled by each beetle was measured in cm at five minute intervals for 15 minutes. Data were transformed and analyzed in the same manner as described above.

Field Utility - Field studies were conducted with beetles that were collected, tagged, and returned to the field within the same night to the location of original collection. Beetles were collected at the same site and in the same manner as those used in the laboratory assay. Cohorts of ten beetles, five male and five female, were tagged on each of the following dates: 10 May, 25 May, 29 Jun, 14 Jul, 4 Aug, 25 Aug, 14 Sep, and 6 Oct 2009. The location of collection and number of beetles collected from each location varied. Ten beetles were collected from and returned to the rough on 10 May, 25 May, and 29 Jun. On 14 Jul four beetles from the green and six from the collar were collected, tagged, and returned. On 4 Aug, 25 Aug, and 6 Oct five beetles from the

rough, three from the green and two from the collar, and on 14 Sep five from the rough, two from the green and three from the collar were collected, tagged and returned. The unique ID from each RFID transponder was recorded to ensure proper identification of each beetle so that individual movement could be monitored. Using a BP Portable Antenna System and FS2001F-ISO Reader (Biomark®, Inc., Boise, ID), beetles were located the morning immediately following tagging and at 24 h intervals afterward for two weeks there after. When monitoring, the last known point was scanned first; if the transponder was not located, sweeping movements in 2 to 3 m arcs were made from the last known point out in every direction up to 15 m away. Once the transponder was located, the distance covered since last location was measured as a straight line distance (cm). Current position was marked with a numbered utility flag and previous positions were marked with turf paint. Data were compiled to determine the percentage of tagged individuals relocated the day immediately following tagging and the percentage which moved during the two week monitoring period. Additionally, data were analyzed to determine the maximum, minimum and average movement by a single tagged beetle in a 24 h period.

Mole Crickets

Large (2.5 – 3.8 cm) tawny mole cricket nymphs were collected from golf course fairways in southeastern North Carolina using flushes of soapy water (Short and Koehler 1979) on 13 Sept 2008, 17 Sept 2008, 23 Aug 2009 and 15 Sept 2009. Crickets were rinsed immediately with water after collection to remove residual soap and placed in a 68

L tub (Rubbermaid® Newell Rubbermaid Inc., Atlanta, GA) filled with native soil. Prior to transponder implantation crickets were anesthetized for one minute with CO₂ in a 1.2L plastic food-storage container (Rubbermaid® TakeAlongs, Newell Rubbermaid Inc., Atlanta, GA). Individual crickets were removed from the anesthesia container and placed on a clean paper towel; a 2-3 mm slit was made under the spiracles on the 6th and 7th abdominal segments using a sterile X-Acto knife (x3201, Elmer's Products Inc., Columbus, OH). A sterile RFID transponder, 12.5mm x 2.07mm, 104 mg, (TX1411SST, Biomark, Inc., Boise, ID) was inserted into the incision with sterile forceps, any exuding hemolymph and fat body were blotted away with a clean paper towel, and the incision was closed using a drop of quick-set adhesive (Gorilla Glue Co., Cincinnati, OH) applied with a clean, round, wooden toothpick. All tools were sterilized with 70% alcohol between procedures. Crickets were allowed to recover in individual one liter Ziploc® containers (12.1 x 12.1 x 9.3 cm, SC Johnson, Racine, WI) filled with 7.6 cm of native soil with a lid placed on top.

Survivability - Survivability of tagged mole crickets was determined with a bioassay of CO₂ anesthetized and tagged, CO₂ anesthetized and sham-operated, CO₂ anesthetized only, and control crickets. Sham operations were similar to tagging a cricket except that the RFID transponder was only inserted half its length into the incision and then removed before the incision was sealed. Ten crickets were used for each treatment. Crickets were placed individually in a one liter Ziploc® container (12.1 x 12.1 x 9.3 cm, SC Johnson, Racine, WI) that was filled with 7.6 cm of soil with a lid (perforated with five 0.635 cm holes) placed on top. The soil was sifted daily to assess survivability for

three weeks after initial cricket treatment (08 October 2009 – 29 October 2009). Data were transformed and analyzed in the same manner used in the billbug protocol described above.

Field Utility - Field studies were conducted at Sandhills Research Station, Jackson Springs, NC, involving 22, 3 m by 3 m plots (2008) and 26, 1 m by 1 m plots (2009) in a bermudagrass, *Cynodon dactylon* (L.) Pers planting. Both years there were two separate test sites used in field studies. Thirty-two plots were treated with fipronil (Chipco Choice™, Bayer Environmental Science, Research Triangle Park, NC) and 16 plots were untreated, to evaluate the utility of this technique in an insecticide treated environment. Fipronil was applied using a shaker jar at a rate of 97.6 kg/ha of product (0.0157 kg ai/ha). Plots were irrigated in compliance with the standard management procedures for bermudagrass established by the Sandhills Research Station superintendent. For all studies, crickets were tagged in the laboratory as described above and held for at least two days before release. This was to ensure that crickets survived the initial trauma of the tagging procedure before being released into the field. The unique ID from each RFID transponder was recorded before cricket placement, and cricket movement was monitored using the BP portable antenna system and FS2001F-ISO reader (Biomark, Inc., Boise, ID). A 2.54 cm diameter x 7.68 cm deep core was taken from the middle of each plot, and the marked cricket was placed head first into the hole. The extracted soil was placed over the cricket to minimize above ground relocation. When relocating the tagged individuals, the release point was first scanned with the antenna; if the cricket was not found, scanning started in the eastern corner of the plot

with the antenna swept across the width of the plot. Scanning continued in this fashion until the entire area of the plot was evaluated. If the cricket was not found in the plot where it was released, the adjacent plots were scanned in a manner similar to that used in the original plot. If the cricket was not found in adjacent plots than the perimeter of the test area was scanned. If the cricket was not found after all areas were scanned, it was recorded as not found. Turf paint was used to mark the location of each cricket found, and different color turf paint was used for each day. Approximate location and transponder ID were recorded on a plot plan. In 2008, movement was monitored 1 d (Sept 22 and Sept 24) and 7 d (Sept 30) or 8 d (Sept 29) after cricket release. In 2009, movement was monitored 1d (Aug 28 and Sept 22) and 7 d (Sept 03 and Sept 28) after cricket release. Movement was measured by recording the “east-west” and “north-south” (x,y) movement, in cm, from the origin, producing grid coordinates for the relocated cricket. These coordinates were used to determine the straight line distance (cm) of movement. Data were transformed and analyzed in the same manner used in the billbug protocol described above.

RESULTS

Billbugs

Survivability - Survivability between tagged and untagged billbugs differed statistically ($F=12.89$; $df=1, 32$; $p < 0.05$). Untagged individuals survived longer, 9.5 days on average, while tagged individuals survived an average of 7.6 d.

Mobility - When billbugs were placed in an arena to observe potential hindrance of movement due to tagging, no statistical difference was found in the average distance traveled over 15 minutes when compared to untagged individuals ($F=2.18$; $df=1, 58$; $p < 0.05$) (Table 1).

Field Utility - In field studies, 68 billbugs were tagged and 57 of them (84%) were relocated with the receiver the following day. Thirty five of the relocated beetles (61%) had moved from the release site. The average distance traveled in any one 24 h period was 40.8 cm, with minimum of 4 cm and maximum of 316 cm (Table 2).

Mole Crickets

Survivability - Survivability of mole crickets differed among treatments ($F=4.39$; $df= 3, 35$; $p < 0.05$). Sham-operated and control treatment groups survived the longest with no significant difference between them (22 d and 21.4 d respectively). Tagged (13.5 d) and CO₂ (13 d) treated crickets survived significantly fewer number of days with no difference between these treatments. Untagged crickets survived significantly longer (21.4 d) than tagged crickets (13.5 d). Survivability of tagged and CO₂ treated crickets decreased throughout the duration of the experiment; from 90% and 100% survival respectively after day one to 50% and 55% survival respectively after two weeks. The sham-operated and control crickets had high survivability throughout the duration of the experiment; 100% survivability for both treatments after day one and after two weeks. The majority of mortality in all treatments was seen one week after treatment (Figure 1).

Field Utility - In field studies, 48 mole crickets were tagged with 40 crickets (83%) relocated with the receiver the first day after release. Six (15%) moved from the release site. One week after release 37 crickets (77%) were relocated with the receiver and 10 (27%) moved from where they were found on day one. Of the crickets relocated with the receiver on day seven, only one was not relocated during day one monitoring. Crickets were successfully located, and movement of mole crickets was observed, in both treated and untreated plots.

DISCUSSION

We determined that the survivability of tagged insects was significantly reduced for both species. On average tagged billbugs survived two days fewer than their untagged counterparts. This may be due in part to the added stress of carrying an object which is approximately the same size as the beetle and double its body weight. The addition of the adhesive could also have reduced longevity. Even with special attention, it was difficult to prevent adhesive from spreading to areas off the elytra and pronotum, such as onto the plural walls of the thorax and abdomen where it could cover the spiracles. The effect of the adhesive on the cuticle is unknown. Future experimentation could include beetles treated with glue and no transponder to differentiate the adverse effects of the glue from those of the transponder. Data from lab experimentation showed that adult billbugs can survive approximately one week in an artificial environment with no food source. The field monitoring period was extended to two weeks in order to

capture potential movement should survival be longer under natural conditions with a food source.

The disparity between weights of the 8 mm RFID transponder (68 mg) and a billbug (35 mg) did not significantly hinder the rate of movement between tagged and untagged billbugs in an arena (Table 1). In the field, billbugs were relocated with the reader 84% of the time one day after their initial release. The portable antenna used for field relocation has a read range sensitivity of (+/-) 4 cm. This could account for some of the variation in recorded movements. Additionally, variation between the minimum and maximum distances moved could be attributed to the location of beetle collection and replacement i.e. rough, green, or collar. Beetles located in the rough moved shorter distances than those on the green and collar (Dorskocil, et al. unpublished). Billbug movement in the field (Table 2) confirms that tagged beetles are capable of movement in their natural environment and that they can be relocated with this technology.

On average, tagged mole crickets survived eight days fewer than untagged crickets. Potential factors contributing to mortality include, but are not limited to, organ displacement within the body cavity from the addition of the transponder, and potential infection. The high mortality seen in the CO₂ treated crickets could be due to overexposure from residual CO₂ not removed from the treatment container. All crickets anesthetized with CO₂ were treated in the same container. Ten tagged crickets were treated first, and then ten sham-operated treated second, and lastly ten CO₂ treated crickets. However, survival appears sufficient to allow for the evaluation of subterranean insect movement given a sufficient number are originally tagged to provide for a

minimum number necessary for monitoring. Future studies on movement of tagged versus untagged crickets should be considered.

Mole crickets were relocated with the receiver 83% of the time one day after their initial release and were relocated with the receiver 77% of the time one week after release even if they moved (Table 2). This suggests that the technology is applicable for relocating previously tagged and released insects. Mole crickets which were relocated, but which did not move from the original location of release, could either be alive but not moving from the location of release, or they could have died. Soapy-water flushing to assess cricket survivability was unsuccessful due to the large size of the nymphs and their ability to avoid the flushing. Of the tagged crickets, 66% were placed in treated plots. This could have had an impact on their movement. Soil moisture and irrigation was consistent across all plots, and thus should have influenced subterranean movement equally in treated versus untreated plots. Crickets which moved from the original release point could be exhibiting avoidance behavior if they were released in a treated plot, while those that did not may have succumbed to the insecticide. Movement could also indicate natural tunneling to feed away from the origin of release. Crickets which were not located could have been out of the scanned area or too deep in the soil profile to be detected. Preliminary lab studies found that a tag can be located as much as 17.78 cm deep in the soil if the transponder was parallel to the receiver and 27.94 cm if the transponder was perpendicular to the receiver. Tawny mole crickets have been reported to go as deep as 70 cm (Brandenburg et al. 2002) in the soil profile. Refined techniques

to implement RFID transponders, including techniques to reduce the likelihood of infection, may improve two-week survivability of mole crickets above the current 40%.

While mortality occurred, our data suggest that RFID technology is a useful method for locating soil surface-dwelling and subterranean insects. The survivability of tagged individuals of both species was great enough to allow for data collection in field studies. The intervals with which both insects were monitored could have influenced movement recorded in the field. Increased frequency of monitoring could provide a clearer picture of dispersal and movement of tagged individuals. Currently, these protocols are tailored for insects limited in their range of mobility, but as technologies improve the potential uses could expand.

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Table 1. Billbug movement rate in an arena recorded as cm per five minutes. Rate recorded in three periods at five, ten, and fifteen minutes of observation. Data were transformed (*square root of X + 0.5*) prior to analysis for treatment effect using Proc GLM and means were separated using Tukey's HSD, (F=2.18; df=1, 58 ; p < 0.05).

Billbug Movement (cm) in Arena				
Interval/Time Period	(1) 0-5	(2) 5-10	(3) 10-15	Average
Tagged	34.2	27.1	22.4	27.9 a
Untagged	57.6	39.4	19.5	38.8 a

Table 2. The recovery and movement of tagged insects with the minimum, maximum, and average distances traveled (cm).

Insect Movement in Field Studies						
Insect	No. of Individuals			Distance Traveled (cm)		
	Tagged	Recovered	Moved	Min.	Max	Average
Billbug	68	57	35	4	316	40.8
Mole Cricket	48	40	6	-	-	-
Day 1						
Mole Cricket	48	37	10	-	-	-
Day 7/8						

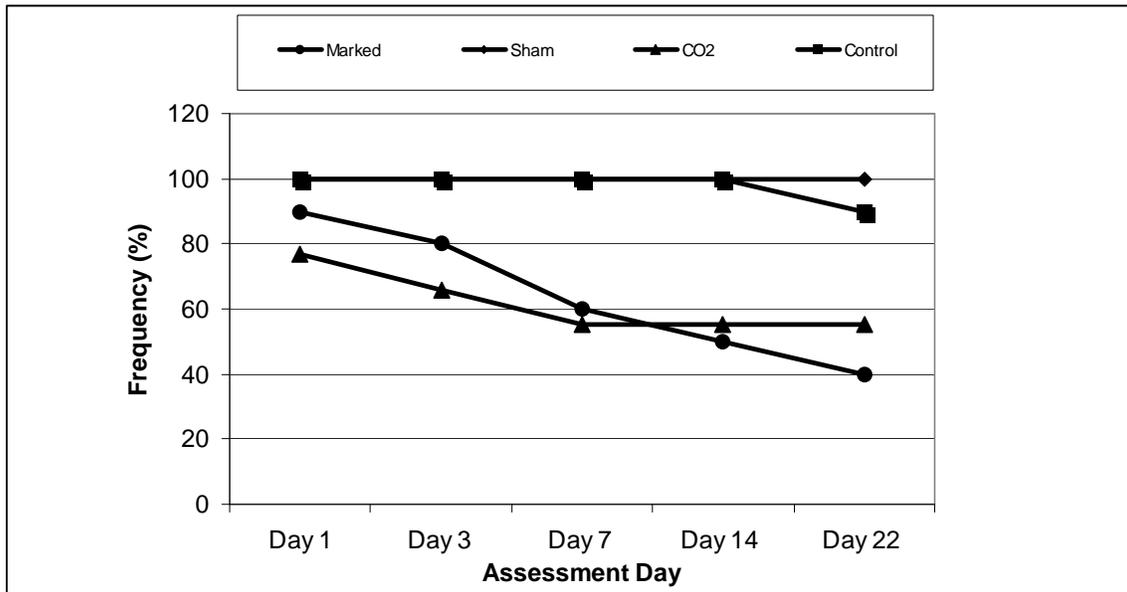


Figure 1. Survival frequency (%) for marked, sham-operated, CO₂ treated, and control mole crickets over time.

Journal of Economic Entomology

Dr. Rick L. Brandenburg

Horticultural Entomology
Doskocil et al.: Tracking hunting billbugs
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Tracking the Movement of Hunting Billbug, (Coleoptera: Curculionidae) on a
Bermudagrass Golf Course with the Use of Radio Frequency Identification Tagging.

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ABSTRACT

A sound understanding of pest biology and behavior is critical for the development of effective pest management strategies. The collection of these data is often difficult when the pest under investigation is cryptic in its behavior and inhabits concealed environments. Radio frequency identification tags (RFID) were attached to hunting billbugs, *Sphenophorus venatus vestitus* Chittenden, in an effort to monitor and quantify the movement of this turfgrass pest. Field experimentation showed no difference in movement patterns between male and female beetles or monthly from May through October, but the average distance moved by tagged billbugs was greater on greens when compared to those in the rough. RFID tagging proved valuable in characterizing the movement patterns of hunting billbugs in bermudagrass and could prove to be a useful technique in uncovering cryptic behaviors of other surface and subterranean insects.

Key words: *Sphenophorus venatus vestitus*, RFID tagging, mark-relocation

INTRODUCTION

In regions dominated by warm season turfgrass, the hunting billbug, *Sphenophorus venatus vestitus* Chittenden, has become an emerging pest. While there have been numerous studies on bluegrass billbug, *Sphenophorus parvulus* Gyllenhal, in cool season turfgrass, only a few studies have been conducted to better understand the

life history and behavior of billbugs in warm season turfgrasses (Huang and Buss 2008, Dosekocil et al. unpublished b). In those studies, it was found that hunting billbugs can have two to six overlapping generations per year, with both larvae and adults overwintering. Additionally, adults were found to be nocturnal in activity (Huang and Buss 2008) and capable of causing damage to warm season turfgrasses (Dosekocil et al. unpublished b). No work has been conducted on describing hunting billbug movement and due to adults spending the majority of their time deep within the thatch layer, finding and observing them is difficult (Potter 1998). For effective pest management strategies to be developed, a better understanding of this pest's movement and behavior is needed.

Several experiments, involving insects, have been conducted using Radio Frequency Identification (RFID) technology for tracking the movement of insects. The wireless sensor technology is based on the detection of electromagnetic signals. The effective read range between antenna and tag varies depending upon power output, radio frequency used, and the size of the tag (Domdouzis et al. 2007). Each of these experiments was conducted using social insects in high visibility environments (Streit et al. 2003, Sumner et al. 2007, Robinson et al. 2009). Recent work was conducted using RFID technology in the lab to test the utility of this technique in mark-relocation experimentation of surface and subterranean insects (Dosekocil et al. unpublished a). The objective of this study was to continue that work in the field by monitoring and quantifying the movement of hunting billbugs in bermudagrass *Cynodon dactylon* (L.) Pers.

MATERIALS AND METHODS

Field experiments were conducted at the North Carolina State University Faculty Club golf course in Raleigh, NC. The course consisted of bermudagrass *Cynodon dactylon* (L.) Pers, fairways and rough, with creeping bentgrass *Agrostis stolonifera* L., greens. Adult hunting billbugs were collected by hand at night. Each beetle was placed in a numbered, plastic graduated 30- ml plastic medicine cup (Solo®, Highland Park, IL) and a utility flag with the corresponding number was placed at the site of collection. Beetles were taken immediately to the lab for tagging. Forceps were used to restrain the beetle, on either side of the abdomen and thorax, while a drop of quick-set cyanoacrylate adhesive, 1/2 the size of the beetle pronotum, (Gorilla Glue Co., Cincinnati, OH) was placed on the dorsal side of the thorax. An 8 mm x 2.12 mm, 67mg, RFID tag, TXP148511B (Biomark®, Inc., Boise, ID), was balanced lengthwise on top of the beetle and the glue was allowed to dry. Additional glue was added to the top and sides of the tag, encasing it to ensure its attachment. A wooden toothpick was used to manipulate the glue before it dried (Doskocil et al. unpublished a).

Tagged beetles were returned to the location of their original collection within the same night. Ten different beetles were tagged, five male and five female, on 10 and 25 May, 29 Jun, 14 Jul, 4 Aug, 25 Aug, 14 Sep, and 6 Oct 2009. The location of collection (green, collar or rough) and number of beetles collected from each location varied by date. Ten beetles were collected from and returned to the rough on 10 and 25 May, and 29 Jun. On 14 Jul four beetles from the green and six from the collar were tagged. On 4 and 25 Aug, and 6 Oct, five beetles from the rough, three from the green and two from

the collar, and on 14 Sep five from the rough, two from the green and three from the collar were tagged. The unique ID from each RFID tag, and the sex of the beetle, was recorded to ensure proper identification of each beetle so that individual movement could be monitored. Using a BP Portable Antenna System and FS2001F-ISO Reader (Biomark®, Inc., Boise, ID), beetles were located the morning immediately following tagging and at 24 hour intervals for two weeks there after. When relocating a beetle, the last known point was scanned first; if the tag was not located, sweeping movements in 2 to 3 m arcs were made from the last known point out in every direction up to 15 m away. Once the tag was located, the numbered utility flag was moved to the new position and the previous position was marked with turfgrass paint. Movement was measured by taking straight line distance (cm) and direction from the previously known position to current position. Direction was recorded from a compass with readings broken down into quadrants for data analysis: quadrant 1, 315-45 degrees; quadrant 2, 45-135 degrees; quadrant 3, 135-225 degrees; and quadrant 4, 225-315 degrees. Individuals which made no recorded movement during the two week observation period were excluded from the data set. Distance data were transformed (*square root X + 0.5*) and analyzed to determine the effect of Date, Location, Sex, and interaction between Date and Sex, Location and Sex, and Date, Location and Sex using Proc GLM in the Statistical Analysis System version 9.1 (SAS Institute 2003), comparisons were attained using Tukey's HSD values. Directional data were analyzed to determine effect of Location, Direction, and Date with Proc GLMMIX.

RESULTS

Sixty eight billbugs were tagged over the course of six months, 34 of each sex. Fifty seven of the 68 (84%) tagged individuals were located the following morning and 35 of those (61%) moved at least once over the two week monitoring period (Table 1). There was no statistical difference in the average daily movement between males (30.2 cm) and females (48.6 cm) ($F=2.18$; $df=1, 58$; $p < 0.05$) (Table 1). Average billbug movement varied from month to month through the course of the season but did not statistically differ ($F = 1.03$; $df = 5, 95$; $p < 0.05$) (Figure 3). Location effect was significant with individuals tagged then released on greens moving greater average distances than those in the rough ($F = 3.69$; $df = 2, 95$; $p < 0.05$) (Figure 1). Assessment of directional movement provided that neither time of the year ($F= 1.55$; $df = 15, 74$; $p = 0.108$) (Figure 4) nor initial location ($F= 0.92$; $df = 6, 74$; $p = 0.48$) (Figure 2), had a significant effect on the direction an individual traveled.

DISCUSSION

In an attempt to capture behavior, without disrupting the billbug, no effort was made to visually confirm beetle location as indicated by the tag during the course of the experiment. Beetle location was not visually confirmed until the conclusion of the monitoring period. Since there were no practical means to determine if inactivity was attributed to natural behavior, lost tag, or mortality, tags which apparently did not move, during the duration of the monitoring period, were left out of the data set. The objective of this study was to quantify the movement of adult hunting billbugs. We determined

that there was no difference between the movement of male and female beetles; the number of individuals which moved, nor the average distance traveled over the course of the six month monitoring period (Table 1). The average movement of beetles was less than 0.5 meter per day with the maximum distance traveled just over 3 meters (Table 1). Individuals rarely moved during consecutive days. This would suggest that individuals stay in localized areas through the course of a season. These data support observations, made in the field, of damage occurring in the same localized areas year after year with little movement from one year to the next (R.L.B. personal observation).

Adult movement by location was found to be significant with individuals on greens moving greater average distances than those in the rough (Figure 1). This difference may be attributed to several factors. The environments of these two locations differ greatly. Beetles on greens are subject to predation and desiccation since the short height of the turfgrass canopy and limited thatch layer do not offer much protection. This may prompt more activity on the beetle's part in an effort to find suitable cover during the daylight hours. Beetles in the rough not only had cover available, but the height and density of the turfgrass canopy could have presented an obstacle to movement with the attachment of the tag, thus resulting in smaller average movements. The interaction between sex and location was not found to be significant.

Females appear to travel greater distances than males on greens and collars but the small number of observations from both sexes prevent any statistical significance from being found (Figure 1). Average movement from month to month was not found to be significant. The same is true for the interaction between sex and time of the year.

Females appear to move longer distances than males during the months of May, Aug, and Sep, but unbalanced data and lack of robustness prohibited statistical confirmation (Figure 3). Directional movement by whether by time of year or location showed no variation (Figure 2 and 4).

Ten beetles were tagged during each two week monitoring period. Each monitoring period varied in the number of beetles tagged by location with as few as two from any given location. From these beetles only those which recorded movement were included in the analysis. However, this study has laid the ground work to better understanding the movement and behavior of an insect which was previously unknown. We have been able to relocate, quantify, and characterize the movement of adult hunting billbugs from May through Oct. Trends in directional movement and the movement of male versus female beetles were identified, but additional experimentation is needed to provide a data base that can be used to better understand seasonal movement, overwintering sites, and recurring infestations.

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Footnotes

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Table 1: The number of beetles tagged, recovered, moved and the minimum, maximum, and average distances traveled (cm). Data were transformed (*square root of $X + 0.5$*) prior to analysis for treatment effect using Proc GLM and means were separated using Tukey's HSD, ($F=2.18$; $df=1, 58$; $p < 0.05$). Sex effect on average distance traveled in 24hrs not significant (a).

Insect	No. of Individuals			Distance Traveled (cm)		
	Tagged	Recovered	Moved	Min.	Max	Average
Male	34	28	16	4	222	30.2 (a)
Female	34	29	19	5	316	48.6 (a)

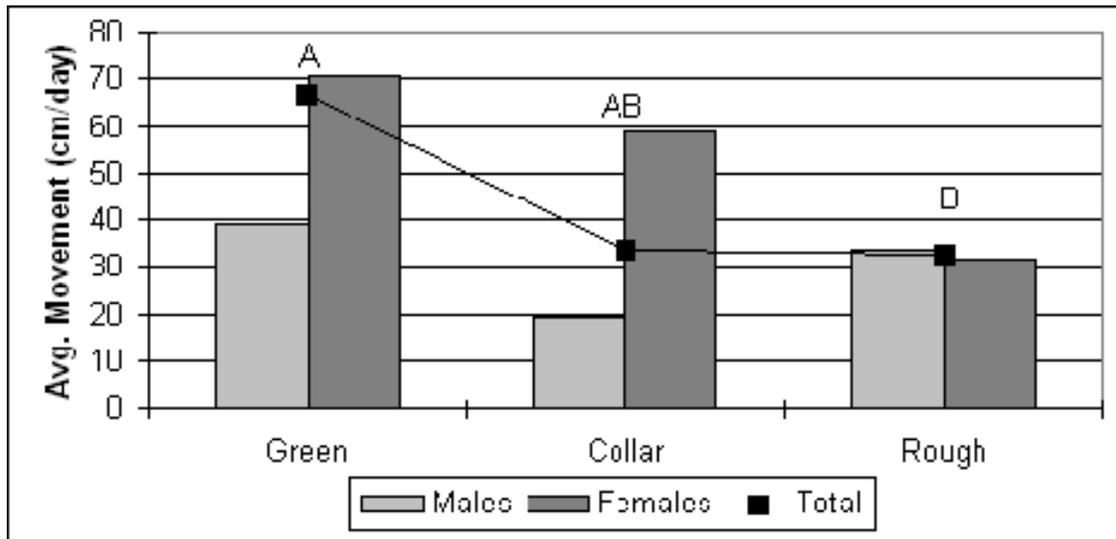


Figure 1: The average movement of male and female beetles by location (cm/day). Total is the average movement of all beetles within a location. Data were transformed (*square root of $X + 0.5$*) prior to analysis for Location effect and Sex by Location interaction analyzed with Proc GLM, Tukey's HSD at $p < 0.05$, $F = 3.69$; $df = 2, 95$. Location effect significant, interaction not significant.

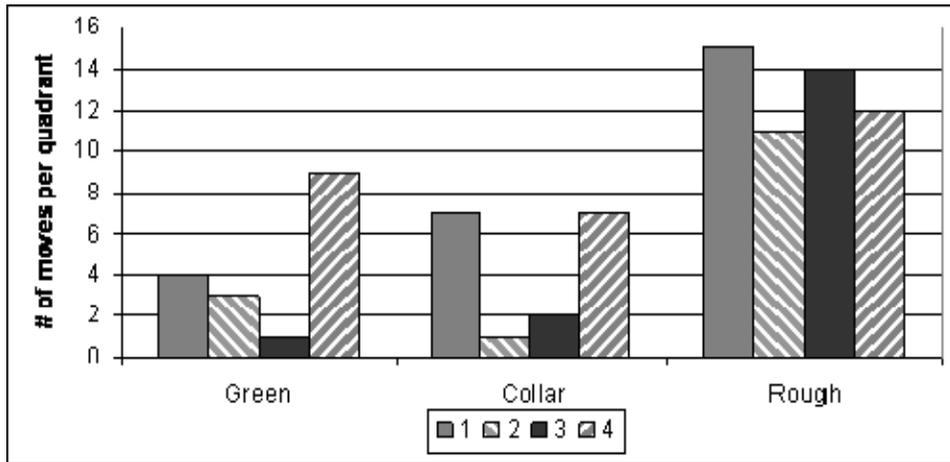


Figure 2: The directional movement of all beetles by location. Analyzed with Proc GLMMIX, $p = 0.48$; $F = 0.92$; $df = 6, 74$. Location effect not significant.

Quadrant 1: $315^\circ - 45^\circ$

Quadrant 2: $45^\circ - 135^\circ$

Quadrant 3: $135^\circ - 225^\circ$

Quadrant 4: $225^\circ - 315^\circ$

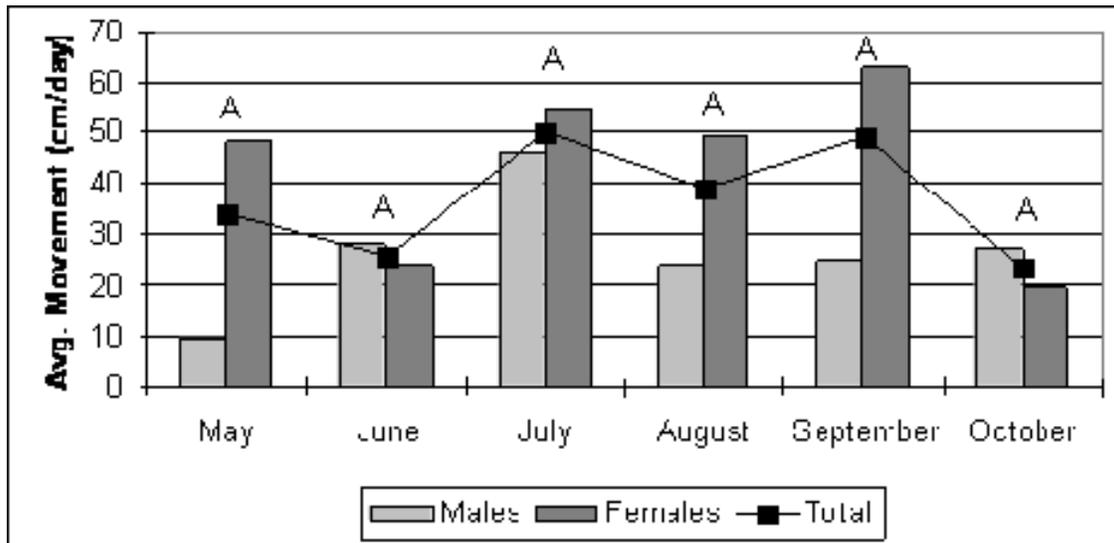


Figure 3: The average movement of male and female beetles by month (cm/day). Total is the average movement of all beetles within a month. Data were transformed (*square root of $X + 0.5$*) prior to analysis for Date effect and Sex by Date interaction analyzed with Proc GLM, Tukey's HSD at $p < 0.05$, $F = 1.03$; $df = 5, 95$. Neither was found to be significant.

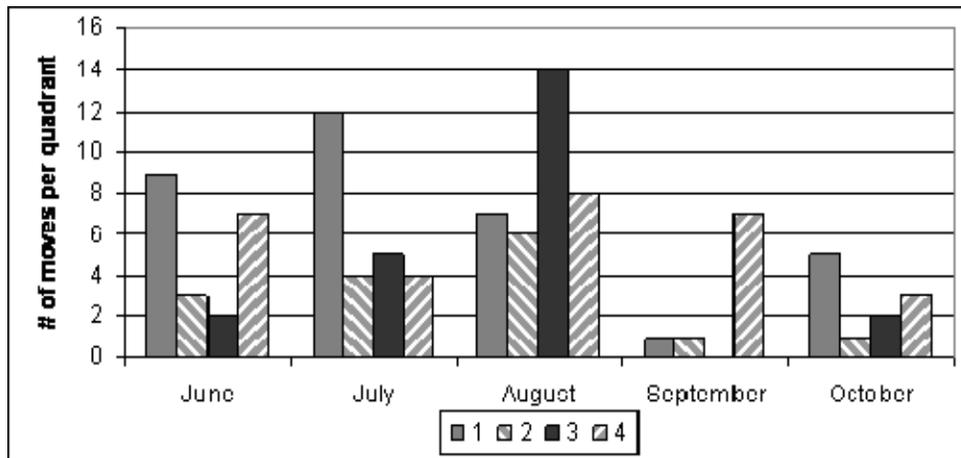


Figure 4: The directional movement of all beetles by month. Analyzed with Proc GLMMIX, $p = 0.108$, $F = 1.55$, $df = 15, 74$. Month effect not significant.

Quadrant 1: $315^\circ - 45^\circ$

Quadrant 2: $45^\circ - 135^\circ$

Quadrant 3: $135^\circ - 225^\circ$

Quadrant 4: $225^\circ - 315^\circ$

Journal of Economic Entomology
Horticultural Entomology
Doskocil et al.: Compound Activity
against Hunting Billbug

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Evaluation of Insecticides for Lethal Dose, Lethal Concentration, and Field Activity on
Hunting Billbug, (Coleoptera: Curculionidae) in Warm-Season Turfgrass.

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ABSTRACT

The response of hunting billbug, *Sphenophorus venatus vestitus* Chittenden, (Coleoptera: Curculionidae), to insecticides was tested in field and laboratory bioassays. Field bioassays evaluated treatment by timing and compound. Laboratory bioassays evaluated lethal dose and lethal concentration. The field evaluation of a bifenthrin and imidacloprid combination, Allectus™, suggests that it is effective at reducing adult billbug populations when applied in Sep and May/Sep. LD₉₅ and LC₉₅ data collected in the laboratory showed significant differences between lethal doses of neonicotinoids and pyrethroids or combination products which contain pyrethroids. Pyrethroid containing products required less material to achieve mortality than neonicotinoids.

Keywords: *Sphenophorus venatus vestitus*, LC₅₀, LD₅₀.

INTRODUCTION

Billbugs (Coleoptera, Curculionidae) are well known insect pests of turfgrass in many parts of the world (Tashiro 1987). In the United States, billbug activity has historically been in areas where cool season turfgrasses predominate. Our most complete knowledge of billbugs in turfgrass is from studies on bluegrass billbug, *Sphenophorus parvulus* Gyllenhal, in cool season turfgrasses, where research has been extensive and the insect's biology and ecology are well understood. The bluegrass billbug has one

generation per year, overwinters as the adult, and the larvae are the damaging stage (Johnson-Cicalese et al. 1990).

In regions where warm season turfgrasses are grown, the hunting billbug, *Sphenophorus venatus vestitus* Chittenden, is an emerging pest. In Florida, where ten different billbug species have been recorded, the hunting billbug was the predominate species collected, and was found to have up to six overlapping generations per year (Huang and Buss 2008). In other southeastern states, hunting billbug adults are frequently observed, but our knowledge of their biology and ecology is limited, and our ability to effectively manage them is poor.

Insecticides have traditionally been the primary tool for the reduction of subterranean insect pests in turfgrass systems (Shetlar 1995). Numerous studies have evaluated chemical control of *Sphenophorus* spp. in field situations (Anderson et al. 2003, Heng-Moss et al. 2005, Eickhoff et al. 2006, Pierson et al. 2007, Toda et al. 2007, Heller et al. 2008), but only two investigations have been conducted in warm season turfgrass (Walker and Royer 2001, Buss et al. 2003) and only those conducted by Buss et al. (2003) occurred in the southeastern United States. Furthermore, no work has been published reporting LD₅₀ or LC₅₀ values for any of the insecticides recommended for managing *Sphenophorus* spp. The objectives of this study was to determine optimal timing of insecticide treatments, compare insecticides used for billbug management under field conditions, and determine the lethal dose and lethal concentration of five insecticidal compounds for *S. venatus vestitus* adults.

MATERIALS AND METHODS

Field Evaluation - A field of 'Meyer' zoysiagrass, *Zoysia japonica* L., located at Quality Turf Sod Farm in Burgaw, NC with a history of hunting billbug damage was selected for evaluation of chemical control methods. In 2008, application timing was evaluated using a bifenthrin and imidacloprid combination, Allectus™ GCSC (Bayer Environmental Science, Research Triangle Park, NC). Treatment plots were 6.1 m x 6.1 m, arranged in RCB design and replicated four times. A single application at 398 g AI/ha, was made with a CO₂ pressurized backpack sprayer operating at 2.81 kgf/cm² delivering approximately 550 L/ha using two 8003 flat fan TeeJet® nozzles (TeeJet Technologies, Wheaton, IL) mounted on a one m boom. Applications were made on 13 May, 24 Jun, 5 Aug, 16 Sep, and on 13 May with re-treatment on 16 Sep. Irrigation, 1.27 cm, was applied to the entire study within 48 h of each application. Plots were evaluated twice for adult and immature presence, 30 Sep 2008 and 13 May 2009. Four, 10.2 cm dia x 10.2 cm depth, cup cutter samples were taken per plot. Samples were processed by sifting soil and tearing apart the thatch and sod in a tub to locate immature billbugs. The number of live larvae and pupae per core were recorded. Adult presence was evaluated at night with the use of LED headlamps. A 3.05 m x 3.05 m area of turfgrass in the middle of each plot was searched in its entirety for approximately 15 min. The number of live adult billbugs found on the turfgrass surface and thatch within this area was recorded.

In 2009, multiple products were evaluated. Treatment plots were 3.05 m x 3.05 m with a 0.6 m border around each plot, arranged in a RCB design and replicated four times. A bifenthrin and imidacloprid combination, (Allectus™ GCSC at 398 g AI/ha),

bifenthrin (Talstar® F at 123 g AI/ha), (FMC Corp., Philadelphia, PA), imidacloprid (Merit™ 2F at 448 g AI/ha), (Bayer CropScience, Kansas City, MO), and chlorantraniliprole (Acelepryn® at 234 g AI/ha), (Dupont, Wilmington, DE) were applied once on 22 Apr and, in additional previously untreated plots, on 2 Sep. Applications were made with the same equipment and parameters as in 2008. Plots treated in Apr were evaluated on 20 May, and those treated on 2 Sep were evaluated 24 Sep 2009, with the same techniques as in 2008. All data were analyzed for treatment effect using Proc GLM and Tukey's HSD, differences evaluated at $p < 0.05$ (SAS Institute 2003). Immature data were transformed prior to analysis ($\log y + 1$).

LD₅₀ and LD₉₅ - values were determined in the laboratory using technical grade material and field collected insects. Adult hunting billbugs were collected from North Carolina State University Faculty Club, Raleigh, NC. This location was selected because there was no history of insecticide use for management of white grubs or billbugs and was therefore believed to harbor a susceptible hunting billbug population.

Technical grade imidacloprid (Bayer CropScience, Kansas City, MO), bifenthrin (Arysta LifeScience, Cary, NC), clothianidin (Arysta LifeScience, Cary, NC), chlorantraniliprole (Dupont, Wilimington, DE), and mixtures of bifenthrin/imidacloprid (1:2.5) and bifenthrin/clothianidin (1:2) (all >95% purity) were evaluated to determine topical toxicity. Stock solutions of each material or combination of materials were made by weighing out desired amounts of technical grade material and combining with ethanol in glass scintillation vials. Care was taken to ensure that all technical grade material went into solution before diluting. An initial dilution was made from the stock solution to

attain the desired initial treatment concentration and serial dilutions were made thereafter to obtain subsequent treatment concentrations.

Specimens were hand collected at night from the turfgrass surface with the aid of an LED headlamp and held in empty plastic graduated 29.6 mL (1 oz) diet cups (Solo[®], Highland Park, IL) in groups of ten. The following day, beetles were separated and placed individually into empty diet cups for treatment and monitoring. Each beetle received a 1 ul dose applied directly on the ventral side of the thorax delivered via micropipettor (Metcalf 1958, Perez-Mendoza 1999, Ramoutar et al. 2009). Two pieces of paper towel 2 cm x 2 cm, moistened to saturation, were added to each diet cup as a source of moisture to prevent beetle desiccation, and each cup was covered and labeled. Individual trials consisted of six treatments; a range of five concentrations of the technical grade material and an ethanol control. Each treatment was delivered to ten specimens. Treated beetles were held in the laboratory at approximately 23 °C. After 24 h beetles were probed to assess mortality; if no movement occurred when probed, a beetle was recorded as dead. Each insecticide was replicated with varying concentrations until mortality of 50% and 100% was achieved within the range of tested concentrations. A minimum of five trial replicates for each insecticide was executed. If 50% mortality or better was not achieved for an insecticide after five trial replications, experimentation was halted. LD₅₀ and LD₉₅ values were attained through use of the Statistical Analysis System version 9.1 program PROC PROBIT (SAS Institute 2003).

LC₅₀ and LC₉₅ - values were determined in the laboratory using formulated insecticidal products and field-collected insects. A bifenthrin and imidacloprid

combination (Allectus[™] GCSC), bifenthrin and clothianidin combination (Aloft[®] GCSC), (Arysta LifeScience, Cary, NC) imidacloprid (Merit[™] 2F), bifenthrin (Talstar[®] F), clothianidin (Arena[™] WDG), (Valent Biosciences, Libertyville, IL), and chlorantraniliprole (Acelepryn[®]), were evaluated to determine their toxicity to adult billbugs. Stock solutions of each formulated product were made by measuring desired amounts of each material and combining them with water in a diet cup. Care was taken to ensure that all material was well mixed before diluting to test concentrations. An initial dilution was made from the stock solution to attain the desired initial treatment concentration and serial dilutions were made thereafter to attain subsequent treatment concentrations.

Adult specimens were collected in the same manner as the LD_{50/95} experimentation. The following day, beetles were placed in pairs in empty diet cups for treatment and monitoring. Tidi[®], #2 medium dental roll (0.95cm x 15.24 cm) (Tidi Products, Neenah, WI), cotton dental wick was cut into 2 cm pieces and placed into a test solution until saturated. Excess solution was removed by touching the gauze to the sides of the container before placing it into a diet cup containing beetles. Individual trials contained six treatments; a range of five concentrations of the insecticide and a water control. Each treatment was delivered to ten specimens. Beetles were held in the laboratory at approximately 23 °C; after 120 h beetles were prodded with a probe to assess mortality. Experiments were repeated until mortality of 50% and 100% was achieved within the range of tested concentrations. A minimum of five trial replicates for each compound was executed. If 50% mortality or better was not achieved for a

compound after five trial replications, experimentation was halted. LC₅₀ and LC₉₅ values were attained through use of Probit analysis as above.

RESULTS

Field Evaluation - In 2008, control of adult billbugs varied among insecticide application times. The Sep evaluation showed that an application made in Sep, and an application in May followed by a second treatment in Sep, significantly reduced the number of adults when compared to the control, while those made in May, Jun, or Aug did not (Figure 1) ($F = 3.78$; $df = 5, 18$; $p < 0.05$). All applications significantly reduced the immature population when compared to the control ($F = 1.85$; $df = 12, 27$; $p < 0.05$) (Figure 2). When evaluated the following May, all applications significantly reduced the number of adults except for the Jun application (Figure 1).

In 2009, adult billbug susceptibility to different insecticides applied in the field did not vary regardless of application timing or product (Figure 3 & 4). All products failed to reduce the number of adult billbugs in comparison to the control ($F = 2.96$; $df = 12, 27$; $p < 0.05$).

LD₅₀ and LD₉₅ - Susceptibility of *S. venatus vestitus* adults in the lethal dose bioassay differed between neonicotinoids, and all compounds versus bifenthrin and combination products containing bifenthrin (Table 1). The test for probit curve prediction strength was significant for all compounds (Wald chi-squared test; $p > 0.05$). Mortality within controls was negligible with only 5 of 290 individuals dying and no more than one individual dying in any given trial replication. Adult hunting billbugs

were most susceptible to bifenthrin and combination products containing bifenthrin; higher doses of the neonicotinoids were required to obtain the same mortality (Table 1). Potency of compounds in the lethal dose bioassay at 95% mortality ranked: bifenthrin/clothianidin = bifenthrin/imidacloprid = bifenthrin > imidacloprid > clothianidin (95% Feducial confidence intervals).

LC₅₀ and LC₉₅ - Susceptibility of *S. venatus vestitus* adults in the lethal concentration bioassay differed between insecticide classes. The test for probit curve prediction strength was significant for all compounds except chlorantraniliprole (Wald chi-squared test; $p > 0.05$) (Table 2). Values determined for chlorantraniliprole were not used in further comparisons. Mortality within controls was minimal with only 12 of 290 individuals dying and no more than one individual dying in any given trial replication. Adult hunting billbugs were most susceptible to products containing bifenthrin; higher doses of the neonicotinoids were required to obtain the same mortality (Table 2). Potency of compounds in the lethal concentration bioassay at 95% mortality ranked: bifenthrin/clothianidin = bifenthrin/imidacloprid = bifenthrin > clothianidin = imidacloprid (95% Feducial confidence intervals).

DISCUSSION

Results from the timing evaluation with the bifenthrin and imidacloprid combination product conducted in 2008, suggest that optimal timing for short-term adult and immature hunting billbug suppression with this material is Sep (Figure 1). May, Aug, Sep, and May/Sep 2008 timing of the bifenthrin and imidacloprid combination

product also provided long-term suppression of adults (Figure 1). This could be attributed to two factors; the initial reduction in adult population or the reduction of the larval population. These factors could also have had a cumulative effect on the subsequent adult population.

The reduction in immature populations (Figure 2) in the Sep 2008 evaluation was significant when compared to the control. No difference was found between application timings. This suggests activity of the compounds on the billbugs present, but these data could also be attributed to the overall scarcity of immatures in the field at the time of evaluation; an average of fewer than 3 immatures were found per control plot and fewer than 0.5 immatures per treated plot. Data from the May 2009 immature evaluation are not presented as no immature individuals were found in either the control or the treated plots.

All products failed to reduce the number of adult billbugs in 2009 (Figures 3 & 4). The average number of individuals recorded in each treatment suggests that a sufficient population was present for evaluation. Inconsistency in results from 2008 to 2009 for the bifenthrin and imidacloprid combination product could be attributed to several factors. In 2008, irrigation was applied to plots within 48 h of treatment to ensure movement of the product into the thatch and soil, but this was not done in 2009. Due to a forecast of precipitation shortly after application, no irrigation was applied to either treatment timing in 2009. However, only 0.25 cm of precipitation was recorded within 48 h of application on 22 Apr, while 0.5 cm was recorded within 48 h of 2 Sep. It is likely these amounts

were not adequate to move the applied products into the thatch and soil profile where they could potentially have been more effective.

In addition, the adult population was active earlier in 2009 than 2008 (Dorskocil and Brandenburg *Submitted*). Therefore, application timing in 2009 took place two weeks earlier than in 2008. This change in application timing could explain the inconsistency from 2008 to 2009 in the bifenthrin and imidacloprid combination product, and the lack of efficacy in the additional products added to the field trial in 2009. This wide variation in results between years indicates a sound understanding of billbug biology, monitoring of adult activity, and the use of irrigation after treatment are critical factors in effective management.

Evaluation of the lethal dose of compounds to adult billbugs suggests no difference in activity between bifenthrin and combination products containing bifenthrin. The presence of neonicotinoids in combination with bifenthrin did not significantly enhance the toxicity of bifenthrin. When evaluated alone, imidacloprid exhibited greater toxicity than clothianidin (Table 1). The lethal concentration bioassay was initiated to provide more complete information on the activity of those compounds with limited contact toxicity. Although not significant, there appeared to be a trend for greater toxicity in the combination products which contain both bifenthrin and a neonicotinoid when compared to bifenthrin alone. Lethal concentrations of neonicotinoids necessary to elicit 95% mortality differed significantly from those products containing bifenthrin (Table 2). Due to the contact activity of bifenthrin the mortality which occurred during

this experiment cannot be separated from any oral toxicity as mortality due to contact and ingestion could not be distinguished.

The $LC_{50/95}$ determination technique was not able to provide consistent mortality with chlorantraniliprole to obtain a functioning curve (Table 2). The irregularity in our results would suggest that consistent concentrations of compound were not available for consumption. It is possible that this formulation bound to a component of the cotton gauze making it unavailable to the feeding insect.

This study provides insight into the lethal doses and lethal concentrations of specific insecticides for field populations of hunting billbugs. Field evaluations provided information regarding product application timing and efficacy which had been lacking in literature. Future work is needed to better characterize the optimal window of application and to determine lethal doses and lethal concentrations of these insecticides for immature billbugs.

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Footnotes

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Table 1. LD_{50/95} values for compound toxicity to adult hunting billbug. Letter in parenthesis next to combination compound name indicates compound for which values are calculated. Values reported in mg/kg obtained through PROC Probit analysis. Asterisk (*) indicates probit curve prediction strength significance ($P > 0.05$).

Compound	N	LD₅₀ (95% FL)	LD₉₅ (95% FL)	X² Wald
Bifenthrin	360	542 (457 -942)	4,599 (2,599-12,485)	45.3 *
Bifenthrin/ Imidacloprid (B)	300	648 (428-828)	3,742 (2,657-6,714)	41.5 *
Bifenthrin/ Imidacloprid (I)	300	1,468 (971-1,942)	9,257 (6,428-16,999)	41.7 *
Imidacloprid	420	5,714 (3,542-8,799)	127,426 (58,370-7.2 e 5)	39.4 *
Bifenthrin/ Clothianidin (B)	300	454 (342-571)	2,508 (1,828-4,057)	66.8 *
Bifenthrin/ Clothianidin (C)	300	908 (714-1,114)	4,765 (3,542-7,457)	68.9 *
Clothianidin	360	22,285 (8,114-1.6 e 5)	89,827,224 (2.9 e 6- 5.7 e 12)	14.5 *

Table 2. LC_{50/95} values for compound toxicity to adult hunting billbug. Letter in parenthesis next to combination compound name indicates compound for which values are calculated. Values reported in mg/L obtained through PROC Probit analysis. Asterisk (*) indicates probit curve prediction strength significance ($P > 0.05$).

Compound	N	LC₅₀	LC₉₅	X²
		(95% FL)	(95% FL)	Wald
Chlorantraniliprole	300	21500	69000	0.09
		N/A	N/A	
Bifenthrin	360	0.38	2.4	79.2 *
		(0.3-0.4)	(2-4)	
Bifenthrin/ Imidacloprid (B)	300	0.24	1.5	78.5 *
		(0.2-0.3)	(1-2)	
Bifenthrin/ Imidacloprid (I)	300	0.61	3.9	78.5 *
		(0.5-0.7)	(2-6)	
Imidacloprid	300	4.7	97	44.9 *
		(3-6)	(51-299)	
Bifenthrin/ Clothianidin (B)	420	0.04	1.3	46.7 *
		(0.02-0.07)	(0.7-4.2)	
Bifenthrin/ Clothianidin (C)	420	0.09	2.6	46.7 *
		(0.05-0.1)	(1-8)	
Clothianidin	360	0.11	17.2 mg/L	27.6 *
		(0.03-0.2)	(7-114)	

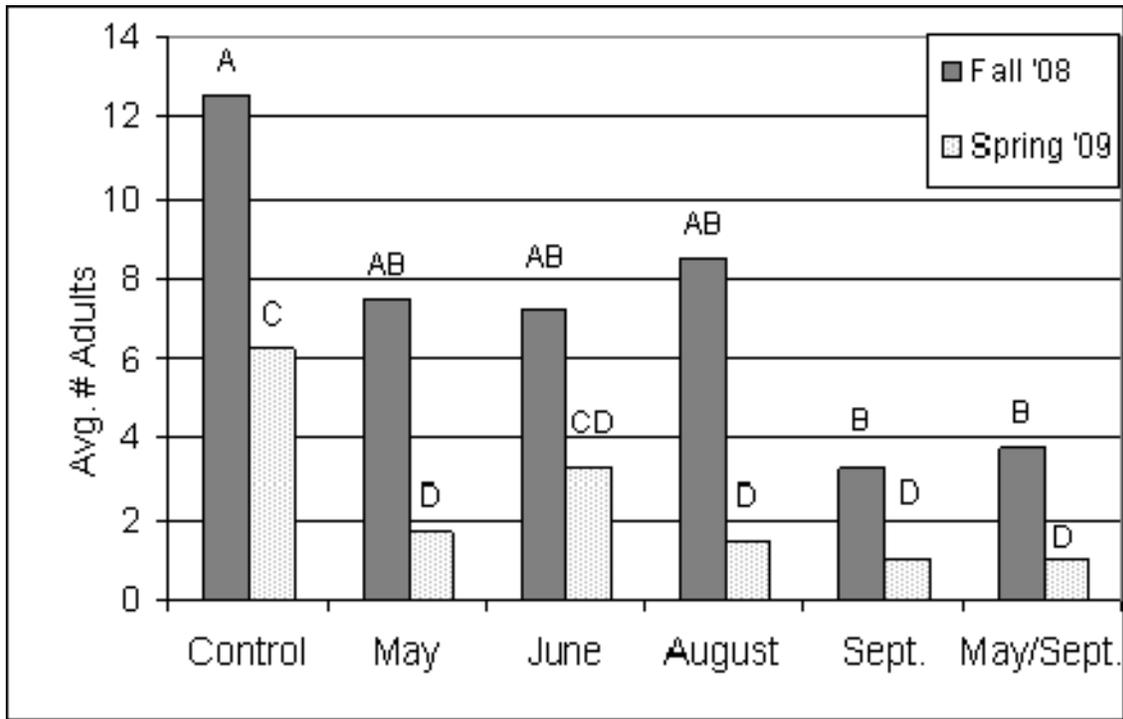


Figure 1. Evaluation of application timing of Allectus in reducing field populations of adult hunting billbug. Applications occurred 2008 with two evaluation periods. Fall and spring evaluations analyzed separately with Proc GLM and means were separated using Tukey's HSD ($F = 3.78$; $df = 5, 18$; $p < 0.05$) (Treatments with the same letter designation not significantly different from one another.)

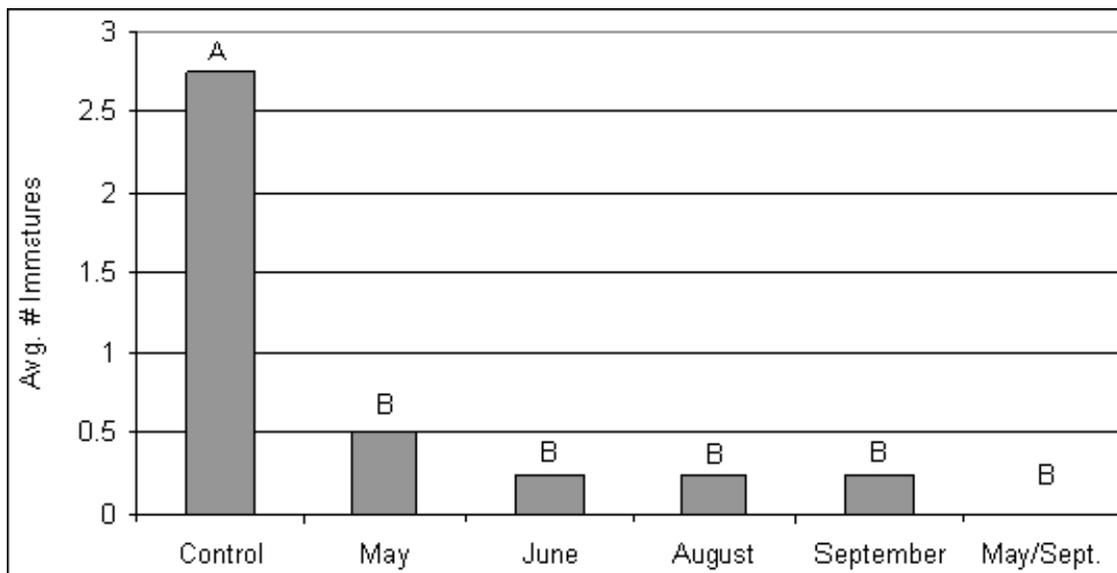


Figure 2. Evaluation of application timing of Allectus in reducing field populations of immature hunting billbug. Applications occurred 2008 with evaluation in Sep 2008. Data were transformed ($\log y+1$) prior to analysis with Proc GLM and means were separated using Tukey's HSD ($F = 1.85$; $df = 12, 27$; $p < 0.05$). (Treatments with the same letter designation not significantly different from one another.)

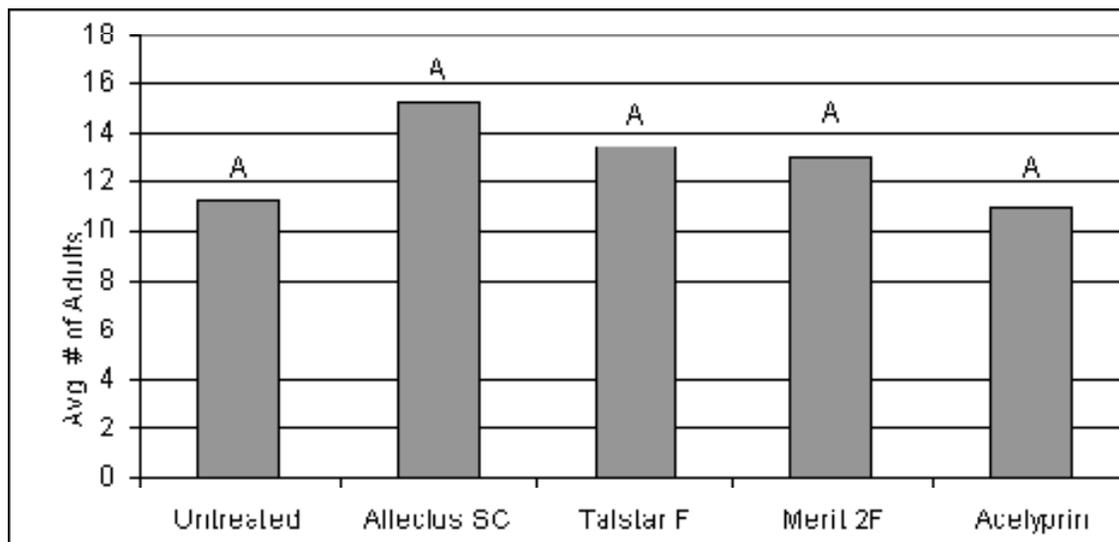


Figure 3. Evaluation of product and timing in reducing field populations of adult hunting billbug. Application occurred 22 Apr 2009 with evaluation three weeks post application 13 May 2009. Treatments analyzed with Proc GLM and means were separated using Tukey's HSD ($F = 2.96$; $df = 12, 27$; $p < 0.05$) (Treatments with the same letter designation not significantly different from one another.)

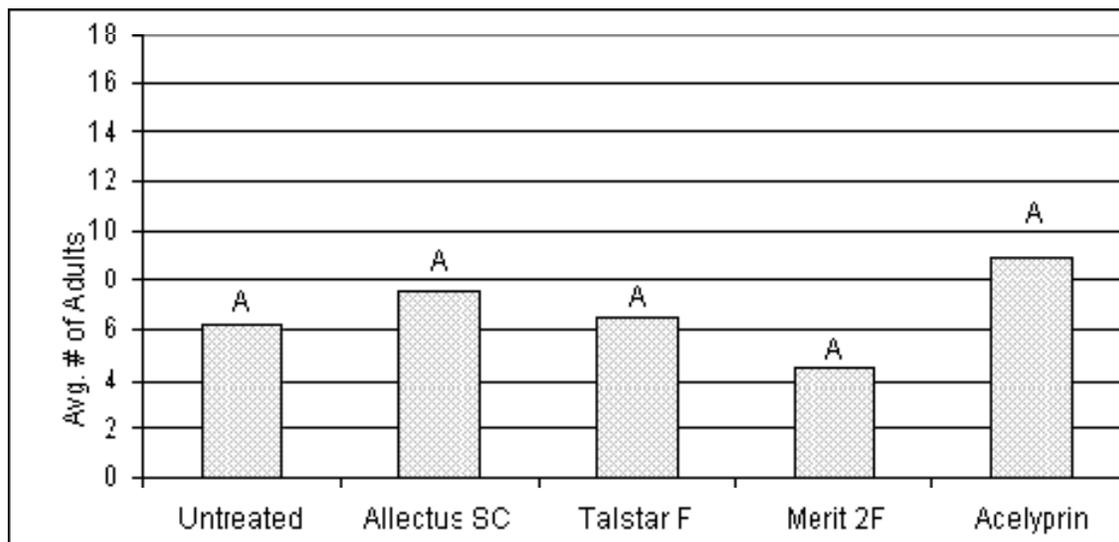


Figure 4. Evaluation of product and timing in reducing field populations of adult hunting billbug. Application occurred 2 Sept 2009 with evaluation three weeks post application 23 Sept 2009. Treatments analyzed with Proc GLM and means were separated using Tukey's HSD ($F = 2.96$; $df = 12, 27$; $p < 0.05$) (Treatments with the same letter designation not significantly different from one another.)

Hunting Billbug Attraction to
Weevil Pheromones

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Evaluation of Weevil Aggregation Pheromones for Attraction of Hunting Billbug
(Coleoptera: Curculionidae)

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Hunting billbugs, *Sphenophorus venatus vestitus* Chittenden, are an emerging pest of turfgrasses in the southeastern United States, and other regions where warm-season turfgrasses are grown. Billbugs cause damage that result in initial yellowing of turfgrass that can spread and coalesce into patches of brown or tan turfgrass (Shetlar 1995). This damage is often misdiagnosed as disease, drought, fertility, or other factors (Potter 1998). Adult billbugs are inconspicuous and their presence is difficult to detect with current methodology, including pitfall traps (Lawrence 1982), soapy water flushes, and vacuum sampling of turfgrass and surrounding paved areas (Watschke et al. 1995). Furthermore, effectiveness of these techniques varies widely. There is a need for an efficient means to monitor billbug populations.

The use of pheromones and host-derived kairomone lures is a proven, effective tool for monitoring economically-important Curculionidae species (Rameriz-Lucas et al. 1996 and Alpizar et al. 2002). Weevil pheromones are often not specific and can attract other species (Giblin-Davis et al. 1995). Our study investigated the potential of pheromone and/or kairomonal compounds which are attractive to the hunting billbug. We evaluated individual adult billbug responses of either sex to putative attractant compounds (pheromones or kairomones) alone or in combination with host plant feeding. We also tested commercially-available aggregation pheromone blends of closely related Curculionidae (*Rhynchophorus* and *Metamasius* spp.). Beetles used in laboratory experiments were collected from bermudagrass *Cynodon dactylon* (L.) Pers. roughs and fairways of the North Carolina State University Faculty Club Golf Course in Raleigh,

NC. Adult hunting billbugs were collected by hand at night. Each beetle was placed in an empty graduated 30 ml plastic medicine cup (Solo®, Highland Park, IL) and taken immediately to the lab for use in bioassays.

Billbugs are nocturnal (Huang and Buss 2009); therefore, all bioassays were conducted at night, in complete darkness, with observation aided by red LED light HDL33A2 (Energizer®, St. Louis MO). Beetles were sexed and inspected for deformities which might hinder their mobility. Only visually healthy beetles were used in these experiments. Beetles were individually placed in a corner of a tin foil (testing) arena, 49.5 x 29.2 x 8.9 cm Hefty® EZ Foil® Pasta Pan (Pactiv Corp., Lake Forest, IL) directly opposite a potential attractant source. This was done so beetle would have to traverse the length of the arena to encounter the potential attractant.

Billbug attraction due to pheromones or kairomones was tested via responses of individual insects (10 females, 10 males) to: 1) a 10.2 cm dia bermudagrass plug, taken from the beetles' native environment, alone (control), 2) a plug plus four male hunting billbugs, 3) a plug plus four female hunting billbugs, or 4) a plug plus two male and two female hunting billbugs. These potential attractant combinations were placed individually in 3.78 L plastic paint cans (EZX10032, E-Z Mix®, Chicago, IL) (staging arena) which were filled to a depth of 9 cm with concrete sand. Grass plugs and treatment beetles were placed on top of the sand 48 hrs prior to beetle exposure to allow for any potential host plant or beetle produced volatiles to diffuse into the clear plastic staging arena. Each test beetle was released on the side of the tin foil testing arena opposite the treatment and was observed for 15 min or until it encountered the treatment, at which

point the replicate was terminated. Data were recorded binomially to denote contact or no contact with the attractant. The effect of treatment and beetle sex on response was analyzed using Proc GLIMMIX (SAS Institute 2003). Means were separated with a significance level of $p < 0.05$.

A second experiment was designed to test four commercial aggregation pheromone blends including Ferrolure+700mg (P028) for *Rhynchophorus ferrugineus*, Metalure (P044) for *Metamasius hemipterus*, *Rhynchophorus cruentatus* (P010), and *Rhynchophorus palmarum* (P058) (Chemtica Internacional, San Jose, Costa Rica). Treatments consisted of 1) individual lures alone, 2) lure plus bermudagrass plug, 3) lure plus plug, plus four males, 4) lure plus plug, plus four females, and 5) lure plus plug, plus two beetles of each sex. Bermudagrass plugs and plugs plus beetles were added to the clear plastic staging arena as previously described. Lures were introduced into the tin foil testing arena 30 min prior to test beetle introduction to allow for diffusion throughout the arena. One lure was used per treatment and each treatment was replicated 10 times with each sex (20 insects per treatment). Bioassay set-up, number of replicates, data collection and analysis were as previously described.

Billbug responses did not differ significantly among treatments of host plant and beetles combined, or by sex ($F = 0.4$; $df = 4, 75$; $p = 0.75$)(Table 1). Billbug responses to treatments of a lure in combination with bermudagrass plugs and beetles were found to be significant ($F=2.64$; $df=23, 455$; $p<0.001$) (Figure 1). Commercial aggregation pheromone lures alone had a low percentage of encounters with $\leq 40\%$ (Figure 1). Lure combinations with sod plugs increased billbug responses to the various treatments

(Figure 1). This is consistent with the results reported for these lures in studies performed with the target species for these lures (Giblin-Davis et al. 1995, Perez et al. 1997, Cerda et al. 1999). We found no effect of sex or interaction with treatments on billbug responses to lure/turfgrass/billbug combinations.

This report contains the results from studies to determine if an aggregation pheromone is produced by either sex of the hunting billbug, the assessment of the attractiveness of host-plant produced volatiles, and the feasibility of using a synthetic weevil aggregation pheromone for attracting adult hunting billbugs. These data suggest that the use of a sex or aggregation pheromone produced by adult hunting billbugs is not feasible as a monitoring tool, but Metalure (90% ♀ response) and *R. palmarum* (80% ♀ and ♂ response) pheromone blends when combined with bermudagrass have potential as an attractant for hunting billbug adults.

SUMMARY

The presence of an aggregation pheromone produced by either sex of the hunting billbug, *Sphenophorus venatus vestitus* Chittenden, host-plant induced volatiles, and the potential activity of synthetically-produced aggregation pheromones of *Rhynchophorus* spp. and *Metamasius* spp. weevils were tested toward both sexes of hunting billbugs. Treatments included: bermudagrass, bermudagrass plus either or both sexes, pheromone alone, pheromone plus bermudagrass, and pheromone plus bermudagrass plus either or both sexes of weevils. Weevil responses to host plant volatiles and potential sex or aggregation pheromones produced by the beetles themselves was minimal but

synthetically produced aggregation pheromones in combination with bermudagrass were significant with multiple combinations receiving 80% response or better.

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Table 1. The percentage of male and female hunting billbug encounters after 15 minutes of exposure to plugs of bermudagrass which had been exposed for 48 h to either or both sexes. Data in total column represent responses of 20 insects (10 per gender). No treatment or sex effect, data analyzed using GLIMMIX, means separation with a significance level of $p < 0.05$ ($F = 0.4$; $df = 4, 75$; $p = 0.75$)

Treatment	Response (%)		
	Female	Male	Total
Grass (alone)	60	50	55
+ ♂	50	50	50
+ ♀	30	70	50
+ ♂ + ♀	60	70	65

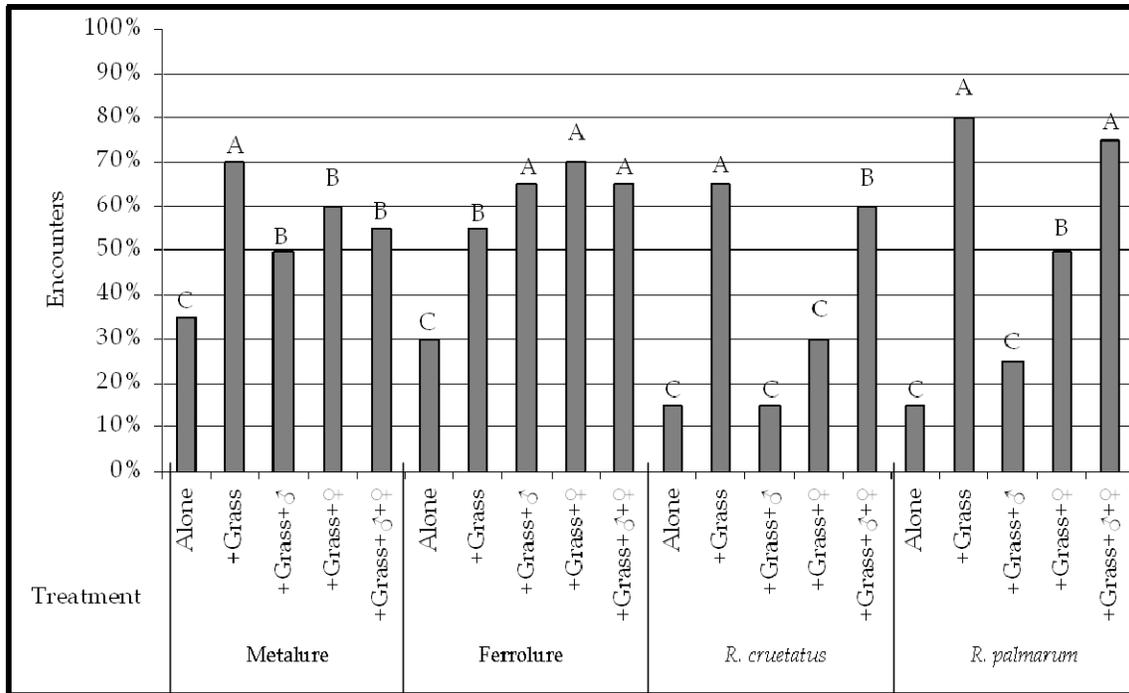


Figure 1. The percentage of hunting billbugs out of 20 exposed which encountered each treatment within 15 min. Treatment effect significant for letters A and C, data analyzed using GLIMMIX, means separation with a significance level of $p < 0.05$ ($F = 2.64$, $df = 23, 455$, $p < 0.001$) (SAS Institute 2003).