Pest management of billbugs in orchardgrass grown in Virginia

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ABSTRACT

The bluegrass billbug (*Sphenophorus parvulus* Gyllenhal) and hunting billbug (*Sphenophorus venatus vestitus* Chittenden) have become important pests of orchardgrass (*Dactylis glomerata* L.) grown in Virginia, causing 40 – 100% stand losses according to a 2005 survey of over 324 ha (800 ac) of orchardgrass. Their sheltered feeding habits combined with a lack of labeled insecticides for orchardgrass make billbug control extremely difficult for this crop.

Over two seasons, orchardgrass fields were surveyed for paired feeding holes caused by feeding of the billbug spring adult. Simultaneously, barrier pitfall traps, a standard method for determining the presence of billbugs in orchardgrass, were used to trap billbug adults in the fields. A comparison of these methods using a Wilcoxon sign-ranked test found no significant differences in the time when paired feeding holes were first observed in fields and when billbug adults were first trapped, showing that the methods are equally satisfactory for determining the presence of billbugs in orchardgrass.

In addition, temperature data from SkyBit E-Weather® service, which are currently used to alert growers and other interested parties of pertinent billbug activity in orchardgrass, was compared to data from a field-based weather data logger over the two seasons. A comparison of these data showed high coefficients of correlation, indicating a close relationship between these two degree-day collection methods. Therefore, the SkyBit system can continue to be used for the alert system.

A field-border application of *Metarhizium anisopliae* (Metschnikoff) Sorokin strain F52 (Met-52), an entomopathogenic fungus, was evaluated against billbug adults as they enter orchardgrass fields in the spring. Randomized pairs of treated and untreated plots were placed along the edge of an orchardgrass field in studies over two seasons. Plots were monitored for billbug adults using barrier pitfall traps, and billbug adults were checked for Met-52 infection. The Met-52 proved unsatisfactory for controlling billbugs in this study.

A field efficacy trial was used to evaluate several insecticides and Met-52 against billbug adults in orchardgrass over two seasons. A randomized complete block design, four insecticide treatments and an untreated control were used in each of two trials. Samples from each treatment plot were dissected and checked for billbug life stages and for injury to orchardgrass plants. In one trial, plants in the Sevin XLR Plus® treatment were found to have a significantly higher percentage of injury to the crowns than all other treatments except Mustang Max. No other significant differences were seen in this study.

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Introduction

The bluegrass billbug, *Sphenophorus parvulus* Gyllenhal and hunting billbug, *S. venatus vestitus* Chittenden (Coleoptera: Curculionidae) are important pests of orchardgrass (*Dactylis glomerata* L.) in Virginia. A survey conducted by R. R. Youngman (personal communication) in 2005 on over 324 ha (800 ac) of orchardgrass in northern Virginia showed losses due to billbugs of 40 – 100%. In 2009, 441,107 ha (1,090,000 ac) of grass hay, excluding alfalfa, were harvested in Virginia with an estimated production value of $302,148,000 (NASS 2010). Orchardgrass can be conservatively estimated to make up 35%, or $105,751,800, of the total production value of grass hay for the commonwealth. The sheltered feeding habits of these species combined with a lack of labeled insecticides for orchardgrass make billbug control extremely difficult in orchardgrass.

The objectives of this project were (1) to investigate whether early season adult billbug feeding (i.e., paired feeding holes) is a timely indicator of when adults are first detected in pitfall traps in orchardgrass; (2) to compare the relationship of temperature data from SkyBit, which are currently used to alert growers and other interested parties of important billbug activity in orchardgrass, to data collected from a field-based weather data logger; (3) to evaluate a field-border application of an entomopathogenic fungus against billbugs in orchardgrass grown in Virginia; and (4) to evaluate selected insecticides and an entomopathogenic fungus against billbugs in orchardgrass grown in Virginia.

Orchardgrass

History & distribution. Orchardgrass, *Dactylis glomerata* (Cyperales: Poaceae), is a cool-season bunch-grass used for forage throughout the world. A native of Eurasia and northern Africa (Smith et al. 1986, Christie and McElroy 1995, Peeters 2004, Allred 2007), it was described by Carolus Linnaeus (L.) in 1753. Orchardgrass was not cultivated until after its introduction into the US, presumably in the 1750s (Miller 1984, Leafe 1988, Christie and McElroy 1995). In 1763, an improved strain from Virginia was re-imported to England (Miller 1984, Leafe 1988, Christie and McElroy 1995). Orchardgrass, named for its propensity to grow in shady areas like orchards, is also called cocksfoot in Europe for the likeness of its inflorescence to the foot of a rooster (Miller 1984, Smith et al. 1986, Christie and McElroy 1995, Balasko and Nelson 2003).

In the US today, various strains of orchardgrass are found in every state, including Alaska and Hawaii, as well as Puerto Rico (NRCS 2010); however, it is predominant in the northeastern, north-central, and Pacific Northwestern US (Smith et al. 1986, Christie and McElroy 1995).

Description. To the untrained eye, orchardgrass (Figure 1.1) closely resembles other grasses; however, it has a few marked characteristics that make it easy to pick out. As a bunch-type grass, orchardgrass plants grow in tight, round clusters (Miller 1984, Smith et al. 1986, Christie and McElroy 1995). The leaves of these clusters have a flacid, almost curly, appearance and tend to be blue-green in color (author's personal observations). Each leaf is folded longitudinally at its base within the bud and has a cross-sectional 'V'-shape (Christie and McElroy 1995, Peeters 2004). Stems can reach 20 – 120 cm in height (Peeters 2004).

Usage & management. Orchardgrass became widely accepted as a forage grass in the US in the 1940s (Miller 1984, Smith et al. 1986, Christie and McElroy 1995) and is now used for pasture, hay, silage (Miller 1984, Christie and McElroy 1995), and green chop (Christie and McElroy 1995). Although it is most recognized for its easy and rapid establishment (Miller 1984, Smith et al. 1986, Borman et al. 1990, Christie and McElroy 1995), early maturation (Miller 1984, Smith et al. 1986, Christie and McElroy 1995), high productivity (Miller 1984, Peeters 2004), shade tolerance (Miller 1984, Smith et al. 1986, Christie and McElroy 1995, Peeters 2004), and heat tolerance (Miller 1984, Smith et al. 1986, Christie and McElroy 1995, Peeters 2004), orchardgrass is also notable for its tolerance to drought (Peeters 2004) and moderate winter-hardiness (Miller 1984, Smith et al. 1986). It responds well to fertilization through direct nitrogen application (Miller 1984, Smith et al. 1986, Christie and McElroy 1995, Peeters 2004), by application of animal manure (Christie and McElroy 1995), or by planting with a legume, such as alfalfa (*Medicago sativa* L.) or ladino clover (*Trifolium repens* L.) (Miller 1984, Smith et al. 1986, Christie and McElroy 1995, Peeters 2004).

The first cutting of an orchardgrass stand is best taken between the boot and full-head stages, as nutritive value decreases at the onset of anthesis (flowering) and seed production (Miller 1984, Christie and McElroy 1995). A healthy stand of orchardgrass can produce up to three cuttings annually (Smith et al. 1986, Christie and McElroy 1995).

Sphenophorus

History. The species of weevils currently belonging to the genus *Sphenophorus* (known as "billbugs"), have a long and confusing nomenclative history, which was explained in detail by Vaurie (1951) and expanded upon by O'Brien and Wibmer (1982). A brief review of this history is needed to avoid confusion with reference to cited literature. The genus currently known as *Sphenophorus* began as *Calendra* Clairville and Shellenberg, 1798 (Schellenberg and Clairville 1798); was subsequently changed to *Calandra* Latreille, 1810 (Latreille 1810); then *Sphenophorus* Schoenherr, 1838 (Schoenherr 1838); reverted to *Calendra* in 1925 (Pierce 1925); and was finally returned to *Sphenophorus* in 1959 (ICZN 1959). The last major revision of the genus was made by Vaurie in 1951, who grouped five previously separate species as subspecies under *S. venatus* Say.

Taxonomic keys. Several taxonomic keys have been written for the identification of various life stages of weevils to the genus- and species-level of *Sphenophorus*, based upon morphology. Anderson (1948) provided a key to larvae of the curculionid subfamily, Calendrinae (now Dryophthorinae), down to the generic level, which included *Calendra.* It should be noted that no species-level key for the larvae of *Sphenophorus* yet exists; however Satterthwait (1931a) devised a species-level key to the pupae of *Calendra* in the US. Species-level keys for *Sphenophorus* adults were written by Blatchley and Leng (1916) for Rhynchophora (now Curculionoidea) of eastern North America, Vaurie (1951) for *Calendra* of the US, Johnson-Cicalese et al. (1990) for those species in this genus that are pests of turfgrass in the US, and Downie and Arnett (1994) for beetles of the US. In his 1931 work, Vaurie also included a key to the five *S. venatus* subspecies.

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Bluegrass billbug

History. The bluegrass billbug (*Sphenophorus parvulus*) was described by Leonard Gyllenhal in 1838 (Schoenherr 1838, p. 961). Since 1838 it has been reported as a pest of many grass species throughout the US (e.g. Bruner 1890, Webster 1892, Forbes 1902, Blatchley and Leng 1916, Tashiro and Personius 1970, Lindgren et al. 1981), particularly corn (*Zea mays* L.), and Kentucky bluegrass (*Poa pratensis* L.). Satterthwait (1931a) compiled a list of 16 additional species of host plants for the bluegrass billbug, which has been augmented by the work of many other authors (reviewed by Johnson-Cicalese (1988, pp. 68-69). It includes barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.), ryegrass (*Lolium* spp.), bentgrass (*Agristis* spp.), and yellow nutsedge (*Cyperus esculentus* L.). Although there are many publications which associate this species with injury of other grasses and plants, only two authors have connected the bluegrass billbug to orchardgrass. Satterthwait (1931a) included orchardgrass as a potential host plant for the species, and Turner (1955) reported it as a pest of orchardgrass in Virginia. Turner's findings are supported in recent observations in Virginia orchardgrass (R.R.Y., unpublished data) and by the author.

Distribution. In Gyllenhal's description of the bluegrass billbug, he presented its home-range vaguely as America "*septentrionalis*" (Schoenherr 1838) meaning *northern* (Marchant and Charles 1917). Satterthwait (1932) later widened this distribution from Canada and the Atlantic coast down to Florida, and west from South Dakota to Texas. Later reports (Blatchley and Leng 1916, Vaurie 1951, O'Brien and Wibmer 1982, Johnson-Cicalese 1988, Watschke et al. 1995) placed the bluegrass billbug in nearly every state of the continental US as well as Ontario, Canada.

Description & life history. Eggs of the bluegrass billbug are off-white and shaped like beans, with a size of approximately 1.6 × 0.6 mm (Tashiro 1987). The larvae (Figure 1.2) are small with soft, white bodies and sclerotized head capsules, which range from yellowish in teneral larvae to dark reddish after maturation. They are legless, somewhat curved, although shorter and stockier than scarabaeiform larvae, and grow to be 4 – 6 mm in length (Bruner 1890, Watschke et al. 1995). Pupae begin off-white in color, darken to a rusty brown, and have a length of 5.5 – 10 mm (Satterthwait 1931a). Bluegrass billbug adults (Figure 1.2 and Figure 1.3) are 5 – 6.5 mm in length (Blatchley and Leng 1916) and range in color from rusty brown, after eclosion, to black in color (Tashiro 1987). This coloration can also be lightened by mud, which frequently covers the body (Tashiro 1987).   The pronotum is covered with tiny, evenly-spaced and uniformly-sized pits, and the elytra have a series of chain-like, deep longitudinal grooves (Bruner 1890, Blatchley and Leng 1916, Watschke et al. 1995). The antennae are geniculate and clavate, and arise near the base of the long slender rostrum (Blatchley and Leng 1916).

Although almost everything that is known about the life history of the bluegrass billbug is based upon its study in turfgrass, its life history appears to be similar in orchardgrass according to observations by Turner (1955) and the author. The bluegrass billbug undergoes a univoltine life cycle over much of its distribution (Forbes 1902), where newly-eclosed adults overwinter (Forbes 1902, Blatchley and Leng 1916) under rocks, boards, sticks, dead leaves, and dead vegetation near its host plants (Bruner 1890, Forbes 1905, Tashiro 1987, Watschke et al. 1995). In Nebraska, however, Bruner (1890) reported a second brood in early fall, which overwintered as pupae. It may be that two generations occur in the warmer regions of its distribution, but thus far in Virginia, only one generation has been observed (author's personal observations).

As the weather begins to warm in the spring and the soil temperature at 2.5 cm below the surface reaches 18.3 – 20.0^{o}C (67 – 69°F) (usually mid-March), adults begin to emerge from their overwintering sites and search for suitable host plants (Watschke et al. 1995). Following a brief period of feeding (Tashiro 1987), oviposition occurs from late May into June and early July (Webster 1892, Blatchley and Leng 1916). At that time, each female chews holes in the host's stems, just above the soil surface, (Webster 1892) and deposits 1 to 3 eggs into each hole (Watschke et al. 1995). Smith (1913) reported one female laying 255 eggs over a three-month period. The egg hatches in about 6 – 7 days (Smith 1913, Watschke et al. 1995) and the larva begins feeding up and down within the stem, filling it with powdery frass (Webster 1892, Satterthwait 1932). Once the larva becomes too large to fit inside the stem, it generally burrows out of the stem, drops to the ground and begins feeding internally and externally on the crown and roots of the plant (Webster 1892, Satterthwait 1932), although some larvae can remain inside the plant throughout the larval stage (Watschke et al. 1995). The larval period has been reported to last from 23 – 60 days (Smith 1913, Satterthwait 1932, Watschke et al. 1995), and is followed by pupation in a soil cell near the host plant (Webster 1892, Forbes 1902). Adults emerge within 8 – 10 days (Smith 1913, Satterthwait 1932, Watschke et al. 1995), and most abundantly in late August through September (Forbes 1902, Blatchley and Leng 1916). New adults feed briefly on nearby host plants, then seek out overwintering sites (Forbes 1902). Although adults are capable of flight, it is rarely seen (Watschke et al. 1995).

Hunting billbug

History. The hunting billbug, *Sphenophorus venatus vestitus*, was described by Frank Hurlbut Chittenden (1904) as *S. vestitus*, and was later placed under *S. venatus* as one of five subspecies (Vaurie 1951). Although much less studied than the bluegrass billbug, it has been repeatedly called a pest of zoysiagrass (*Zoysia* sp.) in the eastern US (Kelsheimer 1956, Kerr 1964, Oliver 1984); in fact it is also known as the zoysiagrass billbug (Kovitvhadi and Kerr 1968). Additional host species include Timothy hay, Bermudagrass (*Cynodon dactylon* (L.)), wheat, yellow nutsedge (Satterthwait 1931a), corn (Satterthwait 1932, Woodruff 1966), sugarcane (*Saccharum officinarum* L.) (Woodruff 1966), et al. (Satterthwait 1931a, LePlante 1966, Woodruff 1966, Oliver 1984). Kamm (1969) reported *S. v.* subspecies *confluens* Chittenden, which is found in the Pacific northwest, to be a major pest of orchardgrass; however, there are no published data that connect *S. v. vestitus* to orchardgrass. Despite this lack of published accounts, unpublished data (R.R.Y.) and observations by the author have shown the hunting billbug to be a pest of orchardgrass in Virginia.

Distribution. The hunting billbug is one of five subspecies designated by Vaurie (1951), the other subspecies being *venatus* Say, *glyceriae* Chittenden., *confluens* Chittenden., and *reticulaticollis* Boheman. These subspecies are geographically distributed across the Atlantic and Pacific coasts and southern US with little overlap between subspecies. In the mainland US, the hunting billbug (*S. v. vestitus*) ranges from Washington, D.C. to Florida and as far west as New Mexico and south-eastern Kansas (Chittenden 1904, Vaurie 1951, O'Brien and Wibmer 1982). Additionally, it has been reported in Hawaii, Puerto Rico, Mexico, the Bahamas, the Dominican Republic, Martinique, and Japan (Marsden 1979, O'Brien and Wibmer 1982, Hatsukade 1997).

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Description & life history. Unfortunately, there are no taxonomic keys to date that differentiate the eggs or larvae of the species of *Sphenophorus*; therefore, it is currently impossible to tell them apart. There are subtle differences between the hunting and bluegrass billbug pupae, which are described in Satterthwait's (1931a) pupal key, but the former is slightly larger, ranging in size from approximately 9 – 12.75 mm. The adults (Figure 1.4) of these two species are also similar except for a few key characteristics. First, the hunting billbug adult is often larger, ranging in size from 6 – 11 mm (Satterthwait 1932). Second, the pronotum has a series of non-uniform pits and raised, smooth areas, which together resemble the letters "Y" or "V" framed by parentheses (Watschke et al. 1995). Third, the marking on its elytra are less distinct than those of the bluegrass billbug.

The hunting billbug, whose life history has been studied far less than that of the bluegrass billbug, appears to share a similar life cycle in northern states with its relative, the bluegrass billbug. However, Watschke et al. (1995) reported that the hunting billbug reproduces continually throughout the year in the southern US, and in places where it overwinters, some may do so as a larva.

Symptoms of billbug injury to orchardgrass

Billbug feeding can take out a stand of orchardgrass or go completely unnoticed, depending upon their population size and weather conditions. In addition to causing direct injury to the plant, feeding can also provide the opportunity for infection by rot-inducing bacteria and fungi (Hanson et al. 1950). Evidence of adult feeding can be seen in late spring as a single, pair, or series of oblong holes, each approximately the size of a typed "o," on the leaves of the host plant. The presence of these characteristic holes have been reported in corn by Forbes (1905) and Satterthwait (1932), and in orchardgrass by Kamm (1969) and Youngman (personal communication). Each set of holes begins as a single hole made by a feeding adult, and then unfurl, as the leaf grows, into a mirrored pair. Damage by billbug larvae appears in mid-summer as irregularly shaped patches of brown grass (Watschke et al. 1995). Damage is often more evident in dry grass than well-watered grass, although they may be equally infested (Bruner 1890). Later in the growing season, infested stems break off easily and the larval frass, which resembles sawdust, spills out of these hollowed stems (Turner 1955, Tashiro and Personius 1970). Feeding by later instars causes the most damage (Turner 1955).

Billbug scouting methods, natural enemies, and control

Scouting methods. Several methods have been devised to sample for billbugs. Tashiro (1987, p. 224) presented a method for extracting billbug eggs from turfgrass by separating the two in a blender and running the resultant mixture through stacked sieves. Methods to sample for larvae in turfgrass include taking soil cores or square-foot samples (Watschke et al. 1995), or checking the thatch layer for larval frass. Adult billbugs may be sampled in turfgrass by drenching the grass (Tashiro 1987); watching for adults as they cross sidewalks and pavement (Tashiro 1987, Watschke et al. 1995); using "suction or vacuum samplers" (Watschke et al. 1995); and using pitfall traps (Tashiro 1987, Johnson-Cicalese et al. 1990, Watschke et al. 1995).

Pitfall traps are generally used to capture surface-dwelling arthropods as they walk along the soil surface and are probably one of the most common methods for billbug sampling. A basic pitfall trap is simply a container buried in the ground so that its rim is flush with the soil surface. The container is partially filled with a killing solution, such as soapy water, ethanol or ethylene glycol, to both prevent captive arthropods from escaping and to preserve them from decay until they can be removed from the trap. Several modifications have been made to the basic trap idea. Linear pitfall traps (Lawrence 1982, Johnson-Cicalese 1988, Johnson-Cicalese et al. 1990) use a piece of buried PVC pipe with a slit cut dorsally along its length to funnel surface-dwelling arthropods into a collection can at one end; while in a barrier pitfall trap (Hansen and New 2005, Laub et al. 2008), a barrier wedged into the ground diverts arthropods into pitfall traps on either of its ends. In the latter method, multiple pitfall traps may be connected by several barriers, or multiple barriers may be employed around a single pitfall trap (Hansen and New 2005). These modified versions of the basic pitfall trap enhance efficiency by increasing the area covered by a trap.

Natural enemies. Predators of billbugs include the adults and larvae of carabid beetles (Bruner 1890); a tachinid fly, *Myiophasia metallica* (Townsend); as well as the American toad, alligators, and 26 species of birds (Satterthwait 1932). Natural parasites and parasitoids include the entomopathogenic fungus, *Beauveria* sp. (Kamm 1969); entomopathogenic nematodes, possibly of the genus *Mermis* Dujardin or *Gordius* Linnaeus (Bruner 1890); mites (Acari) (Forbes 1902); the braconid wasp, *Vipio belfragei* (Cresson) (Satterthwait 1932); and the mymarid wasp, *Anaphes (Patasson) calendrae* (Gahan) (Satterthwait 1931b, Beardsley 2000).

Control methods. A number of cultural, biological, and chemical control methods have been explored and suggested for billbugs in turfgrass and corn, with little focus on orchardgrass. For cultural control, Lugger (1899) reported that spreading "very bad-smelling" hog manure over lawns, followed by a heavy rain, caused a mass emergence of *S. parvulus* from the turf; Lugger (1899), Forbes (1902), and Blatchley and Leng (1916) found fall plowing of old Timothy sod to be effective in preventing billbugs from infesting new plantings of corn. In addition, much research has been invested into the development of billbug-resistant varieties of turfgrass, particularly for the bluegrass billbug. Information about billbug resistance has been reported by Kindler and Kinbacher (1975), and Kindler et al. (1982) in cultivars of Kentucky bluegrass; Ahmad and Funk (1982) in cultivars and selections of perennial ryegrass; Ahmad et al. (1986) in endophyte-enhanced perennial ryegrass; Johnson-Cicalese and White (1990) in *Acremonium* endophyte-infected and endophyte-free tall fescue (*Festuca arundinacea* Schreber); Nielson et al. (1993) in grasses within the tribe Triticeae; and Richmond et al. (2000) in mixtures of Kentucky bluegrass and endophyte-infected perennial ryegrass. Tashiro (1987, pp. 224-25) and Watschke (1995, pp. 256, 258-59) review billbug-resistant varieties of turfgrass.

Biological control has included the introduction of *A. calendrae*, the mymarid egg parasitoid of *Sphenophorus* spp., into Hawaii in 1928 and again in 1963 in an attempt to control the hunting billbug, as well as *S. cariosus* (Beardsley), and the closely related New Guinea sugarcane weevil, *Rhabdoscelus obscurus* (Boisduval). The second introduction proved effective as individuals of *A. calendrae* were found in 1995; however, its efficacy against the target weevil species is unknown (Beardsley 2000). Watschke et al. (1995) recommended the application of various entomopathogenic nematodes (*Steinernema carpocapsae* Weiser, *S. glaseri* Steiner, and multiple *Heterorhabditis* spp.) and the augmentation of soil-borne *Beauveria* spp. by moistening turfgrass in the spring to aid in control of billbug adults and larvae.

Watschke et al. (1995) reported that the most effective means of chemical control for billbugs is to target the egg-laying spring adults. Larvae may also be targeted as they leave the stem and drop to the soil to feed on the roots of the plant; however, some larvae remain within the plant and are therefore never exposed to contact or soil insecticides (Watschke et al. 1995). The current insecticides that are registered for grass hay and pasture, which includes orchardgrass, are listed as follows: beta-cyfluthrin (Baythroid XL® by Bayer CropScience), carbaryl (Sevin 80 WSP®, Sevin SL®, and Sevin XLR Plus® by Bayer CropScience), lambda-cyhalothrin (Karate® and Warrior II® by Syngenta Crop Protection, Inc.), malathion (Malathion 5® by Winfield Solutions), and zeta-cypermethrin (Mustang Max® by FMC Corp.) (VDACS 2003, Youngman 2010). Only lambda-cyhalothrin is currently labeled for use on billbugs in grass hay and pasture in Virginia (Youngman 2010).

Figure 1.1. Orchardgrass (*Dactylis glomerata* L.), showing early-spring regrowth (left), mature plants (center), and a late-summer inflorescence (right). Photographs by W.R. Kuhn.

Figure 1.2. Two billbug *Sphenophorus* sp.) larvae (right and bottom) and bluegrass billbug (*Sphenophorus parvulus* Gyllenhal) adult (center). Black bar equals 1 mm. Photographs by W.R. Kuhn.
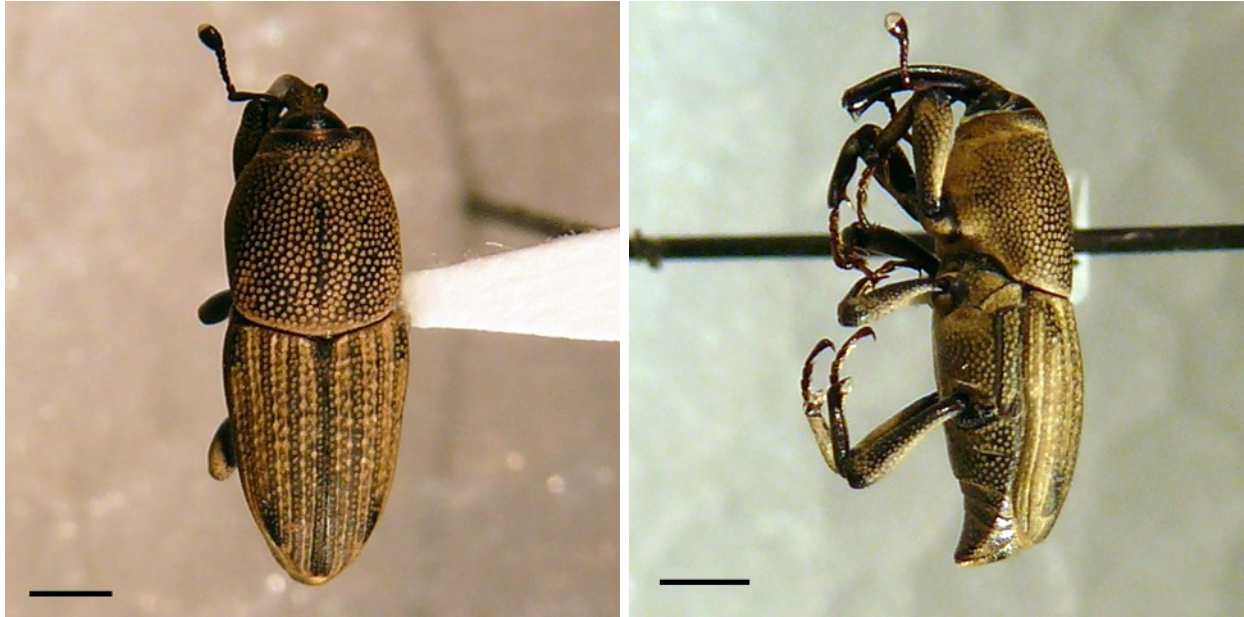
Figure 1.3. Bluegrass billbug (*Sphenophorus parvulus* Gyllenhal) adult, dorsal (left) and lateral (right) views. Black bars equal 1 mm. Photographs by W.R. Kuhn.
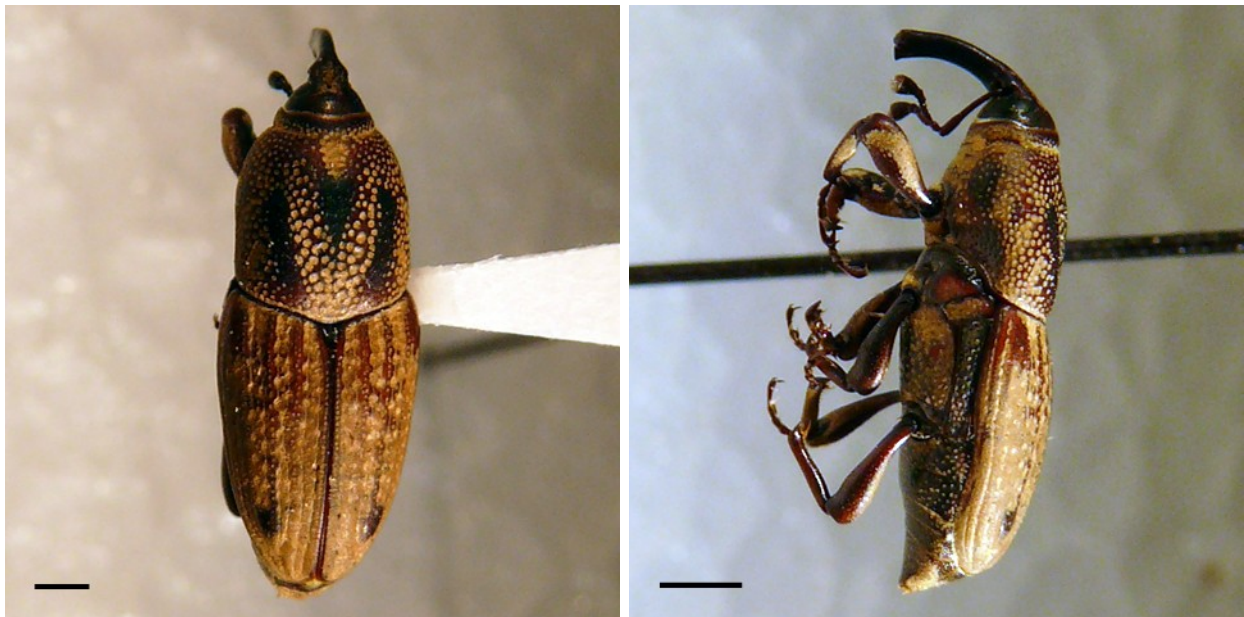
Figure 1.4. Hunting billbug (*Sphenophorus venatus vestitus* Chittenden) adult, dorsal (left) and lateral (right) views. Black bars equal 1 mm. Photographs by W.R. Kuhn.

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Chapter 2: Presence/absence method for detecting activity of billbug adults as they move into orchardgrass fields in the spring in Virginia

Introduction

The bluegrass billbug (*Sphenophorus parvulus* Gyllenhal) and the hunting billbug (*S. venatus vestitus* Chittenden) (Coleoptera: Curculionidae) are important pests of orchardgrass, *Dactylis glomerata* L., in Virginia. Turner (1955) first detected damage to orchardgrass by the bluegrass billbug in Virginia in 1953, whereas the hunting billbug was not detected damaging orchardgrass until 2005 (R.R. Youngman, unpublished data).

The billbug life history has been studied almost exclusively on turfgrass; however, Turner (1955) and Kamm (1969) reported a similar life history in orchardgrass. In late spring, as the soil temperature 2.5 cm below the surface warms to 18.3 – 20.0^{o}C (67-69°F) (Watschke et al. 1995), the adults emerge from overwintering sites in vegetation and debris to seek out nearby host plants (Bruner 1890, Forbes 1905, Tashiro 1987, Watschke et al. 1995). Adults feed briefly (Tashiro 1987) and lay eggs within the stems of the host. The larval stage lasts 23 – 60 days (Smith 1913, Satterthwait 1932, Watschke et al. 1995), beginning with the early instars feeding within the stems of the host, followed by later instars feeding internally and externally on the crowns and roots (Webster 1892, Satterthwait 1932). Larvae pupate in a soil cell (Webster 1892, Forbes 1902), and new adults eclose after 8 – 10 days (Smith 1913, Satterthwait 1932, Watschke et al. 1995). Peak adult emergence occurs from late August through September (Forbes 1902, Blatchley and Leng 1916).

Feeding by different stages during the billbug life cycle is marked by different types of injury to the host plant. In orchardgrass, adult feeding at the base of stems results in holes in the

leaves as they increase in maturity (Kamm 1969). Young larvae feed within orchardgrass stems, hollowing them out, making them brittle, and filling them with sawdust-like frass (Turner 1955). More mature larvae feed on the crowns and roots of the plants, causing severe injury or death (Kamm 1969).

The holes caused by adult feeding are characteristic to billbug feeding and are readily observed. Orchardgrass leaves are folded at the base of the plant (Peeters 2004). As the leaves extend and unfold, the holes become symmetrically mirrored with respect to the center vein of the leaf (Figure 2.1, left). The holes often appear in a series, due to repeated feeding events on a single stem over time (Figure 2.1, center). Matching sets of holes can be seen on multiple leaves arising from the same stem, each equidistant from the stem, as a result of an adult boring through multiple leaves at once at the base of the plant. Leaves weakened by feeding injury often droop at the site of feeding (Figure 2.1, right).

Targeting of the spring, egg-laying billbug adult is currently the most effective means for their control with insecticides. The other stages are less exposed to insecticides (Shetlar 1991, Watschke et al. 1995). Presently in Virginia, barrier pitfall traps (Laub et al. 2008) are used to scout for the adults as they move back into fields in the spring. Orchardgrass growers, however, often find these traps to be impractical. Additionally, their small size and minute defining characteristics make the bluegrass and hunting billbugs difficult for growers to differentiate from other *Sphenophorus* sp. and beetles that may inhabit orchardgrass fields.

Watschke et al. (1995) developed a degree-day (^{o}D) model for the bluegrass billbug in turfgrass in Ohio, which uses a March 1 starting date and a lower activity threshold temperature of 10° C (50° F). The model predicts that first adult activity will occur between 156 – 196

$^o D_{base10C}$ (280 – 352 $^o D_{base50F}$) and 30% first activity (the point at which the last broadcast insecticide would be effective) will occur at 311 – 347 $^o D_{base10C}$ (560 – 624 $^o D_{base50F}$). This model appears to adhere closely to the bluegrass billbug life cycle in orchardgrass, but has not been verified for either the bluegrass or hunting billbug in orchardgrass.

Currently, orchardgrass growers, extension agents, and agribusiness professionals are notified of the best time to scout for billbug adults and apply insecticides through an orchardgrass group email list, (R.R. Youngman (Dept. of Entomology, Virginia Tech)). The notifications are based on the Watschke et al. degree-day model and temperature data collected by SkyBit E-Weather® (SkyBit) (ZedX Inc. 2010). This service provides "automated, simulated [temperature] data" for a site the client has chosen (Skybit.com 2007). The site where temperature data is recorded for use in these notifications is a grassy field located at the Fauquier County Fairgrounds in Virginia. The information from SkyBit is provided daily and is used to inform interested parties on degree-day accumulation for helping pinpoint critical spring billbug activity. However, the temperature data from SkyBit has never been compared with ground-collected weather data from an actual orchardgrass field.

The objectives of this study were: to investigate if early season adult billbug feeding (i.e., paired feeding holes) is a timely indicator of when adults are first detected in pitfall traps in orchardgrass; and to compare the relationship of the temperature data from SkyBit to data collected from a ground-based weather data logger.

Figure 2.1. Feeding holes in orchardgrass caused by billbug adults. The feeding damage is mirrored (left) along the leaf's center vein (black line). These holes often occur in a series (center) and cause the weakened leaf to bend (right). Photographs by W.R. Kuhn.

Materials & Methods

Feeding injury. In spring of 2009 and 2010, eight and ten orchardgrass fields, respectively, were surveyed from Fauquier and Loundoun Counties, VA for feeding holes caused by adult billbugs. Farms were picked based on the availability of cooperators, and fields were chosen with a wide range of orchardgrass-stand ages (Table 2.1). Preference was given to fields that were at least partially surrounded by potential billbug overwintering sites, such as rock walls or woody vegetation. In 2009, fields were selected on four farms. Fields ranged in size from 4.6 – 15.6 ha (mean = 8.9 ha), and were surveyed weekly from 16 March – 11 May, except for two non-consecutive weeks where data were not taken due to inclement weather. In 2010, fields ranged in size from 3.6 to 16.5 ha (mean = 8.8 ha) on three farms. Six of the ten fields sampled in 2010 were the same fields used in 2009. These fields were surveyed weekly between 15 March and 3 May 2010.

The fields were surveyed for feeding holes using a 20.3-cm (8-in) iron square grid. In each field on each sample date, the grid was arbitrarily tossed 10 times approximately 6 m inside the field edge. Feeding holes were counted on the orchardgrass within the grid landed. In 2009, the number of orchardgrass plants within the grid with one or more feeding holes was counted; while in 2010, the total number of feeding holes within the grid was counted. The feeding holes in 2010 were counted based on the following guidelines: (1) holes occurring in a pair, positioned longitudinally along a leaf were counted as 1; (2) multiple sets of holes on one stem (assumed to be caused by one feeding event through multiple leaves at the base of the stem, followed by growth of the leaves) were counted as 1; and (3) holes had to be longitudinally symmetrical to be counted (to prevent counting of feeding injury caused by grazing mammals). The number of plants or holes was recorded for each toss and the mean was calculated for the 10 tosses.

Pitfall traps. Barrier pitfall traps were installed in these fields on 10 March 2009 and

10March 2010 according to the method described by Laub et al. (2008). These traps were

checked for billbug adults on the same sample dates used for feeding injury. These traps used an

aluminum-flashing barrier to divert surface-dwelling insects into pitfall traps at either end. Two

barrier pitfall traps were placed 1 – 5 m inside the edge of each field, so that the barrier of each

trap was parallel to the edge of the field. Pet-safe automotive antifreeze was used as a kill

solution and preservative. On each sample date, the contents of the two traps in each field were

combined, and billbug adults were counted and placed in 70% ethanol. The number of adults

was recorded on each sample date for each field. Adults were later pinned and identified, and

representative specimens were confirmed by J. Prena ((Coleoptera: Curculionidae) Systematic

Entomology Laboratory, Agriculture Research Service, USDA (2010)). The species, location of

capture, and date of capture were recorded for each billbug adult. Voucher specimens are

housed at the Virginia Tech insect collection.

For each of the fields in 2009 and 2010, the sample date on which feeding holes were

first observed was compared to the sample date on which bluegrass and/or hunting billbug adults

were first captured in the pitfall traps.

Degree-day methods. Degree-days were calculated using two collection methods, the

SkyBit system and a field-based system. SkyBit degree-day data were obtained for the Fauquier

County Fairgrounds for 1 March to 14 July 2009 and 1 January to 14 July 2010. SkyBit used the

Modified Arithmetic Method (J. Schlegel, personal communication; McMaster and Wilhelm

1997) to calculate degree-days. This method incorporates an upper developmental threshold,

35°C (95°F), and the lower threshold (10° C (50° F)) used by Watschke et al. (1995).

Temperature data were collected simultaneously using an Onset HOBO® UA-001-64

temperature data logger (Onset Computer Corp., Bourne, MA) (HOBO), which was secured in a sun-shade to prevent false readings from sun exposure, and was mounted onto a PVC pipe approximately 1.5 m above the ground. The HOBO data logger was placed near the edge of a field on Lazy Lane Farm (Upperville, VA) in 2009 and Locust Hill Farm (The Plains, VA) in 2010. Ambient temperature was recorded at hourly intervals for 11 March – 14 July 2009 and 1 January – 14 July 2010. Degree-days were calculated from the HOBO temperature data using the Average Method (Shetlar 1991, Watschke et al. 1995), which is a standard method that uses only the 10° C lower developmental threshold.

In 2009, due to the 10-day lapse in HOBO records between the 1 March SkyBit start date and the 11 March HOBO activation date, only the degree-days for 11 March – 14 July were analyzed. The degree-days accumulated by SkyBit from 1 – 10 March were 27 $^oD_{base10C}$ (48 $^oD_{base50F}$). From 1 January to 28 February 2010, 14 $^oD_{base10C}$ (25 $^oD_{base50F}$) were accumulated by SkyBit and 2 $^oD_{base10C}$ (3 $^oD_{base50F}$) were accumulated by the HOBO data logger.

Statistics. A non-parametric, two-tailed Wilcoxon signed-rank test (Steel and Torrie 1980) was performed to determine if the difference (in days) between the time when feeding holes were first detected and when billbug adults were first trapped was significantly different than zero. The data analysis used $\alpha = 0.05$ on fields where both feeding holes were recorded, and bluegrass and/or hunting billbugs were trapped for both years separately and combined. The coefficient of correlation (r) (Steel and Torrie 1980) was calculated between SkyBit and HOBO degree-day data for 11 March – 14 July 2009 and 1 January – 14 July 2010.

Table 2.1. Location, designated number, and approximate size of orchardgrass fields used in a survey for billbug adult activity over two seasons (spring 2009, 2010)

Year	Study farm and location	Field #[a]	Orchardgrass variety	Approx. field size[b] (ha)	Approx. age of orchardgrass stand at trial start (years)
2009	Lazy Lane Farm, Upperville, VA	1	Benchmark	4.3	1
		2	Benchmark	16.5	2
		3	Benchmark	6.1	<1
	Locust Hill Farm, The Plains, VA	4	?	4.6	<1
		5	Benchmark Plus	6.7	?
	Grasslands Farm, The Plains, VA	6	WP300	11.4	4
		7	WP300	6.0	4
	Glenmore Farm, Marshall, VA	8	Olympia	15.6	?
2010	Locust Hill Farm, The Plains, VA	1	Benchmark Plus & Olympia	9.6	<1
		2	Olympia	9.5	1
		3	Benchmark Plus	3.6	1
	Grasslands Farm, The Plains, VA	4	WP300	11.2	3
		5	WP300	11.4	>6
		6	WP300	6.0	>6
	Lazy Lane Farm, Upperville, VA	7	Benchmark	6.1	1
		8	Benchmark	10.2	?
		9	Benchmark	4.3	2
		10	Benchmark	16.5	3

? Variety or age not known.
[a] Field 1 and 3 from 2009, and fields 7,8 and 9 from 2010 were located in Loudoun Co., VA; all others were located in Fauquier Co., VA.
[b] Field sizes were approximated by tracing the perimeter of each field in Google Earth® (Google 2010) using the Polygon Area Measurement tool.

Results

Feeding injury. From 16 April – 12 May 2009 billbug adult feeding holes were detected on four of the seven sample dates, in seven out of eight fields (Figure 2.2). From 8 April – 4 May 2010, feeding holes were seen on five of the eight sample dates, in nine of the 10 fields (Figure 2.3).

Pitfall traps. In 2009, eight bluegrass billbug adults were captured from four fields on three farms, over two sample dates beginning on 28 April (Figure 2.2, Table 2.2). Three hunting billbug adults were captured in 2 fields, over 2 sample dates starting on 22 April. In 2010, 23 bluegrass billbug adults were captured from seven fields, over five sample dates beginning on 8 April (Figure 2.3; Table 2.2). Three hunting billbug adults were captured in three fields on one farm, over two sample dates beginning on 8 April. Additional *Sphenophorus* species were caught in the pitfall traps in both years (Table 2.2).

A two-tailed Wilcoxon signed-rank test of the difference (in days) between the sample date when feeding holes were first detected and billbug adults were first trapped, by field, showed no significant differences in 2009 fields ($Z = 3.00$; $P = 0.25$) and 2010 fields ($Z = -2.00$; $P = 0.50$). Likewise, combining the data for both years did not result in statistical significance ($Z = 2.50$; $P = 0.69$). In 2009, bluegrass and/or hunting billbug adults were trapped in 5 of the 7 fields where feeding holes were detected (Table 2.3). Of those 5 fields, feeding holes were first detected 0 – 20 days before billbug adults. In one field, no feeding holes or billbug adults were found. In 2010, bluegrass and/or hunting billbug adults were detected in 6 of the 9 fields where feeding holes were detected. In those 6 fields, feeding holes were detected between 12 days after and 7 days before billbug adults. In one field, no feeding holes or billbug adults were found.

Degree-day methods. Coefficients of correlation were calculated to compare SkyBit data and HOBO data for 11 March – 14 July 2009 (Figure 2.4) and 1 January – 14 July 2010 (Figure 2.5). The respective *r*-values for these comparisons were 0.9985 and 0.9949.
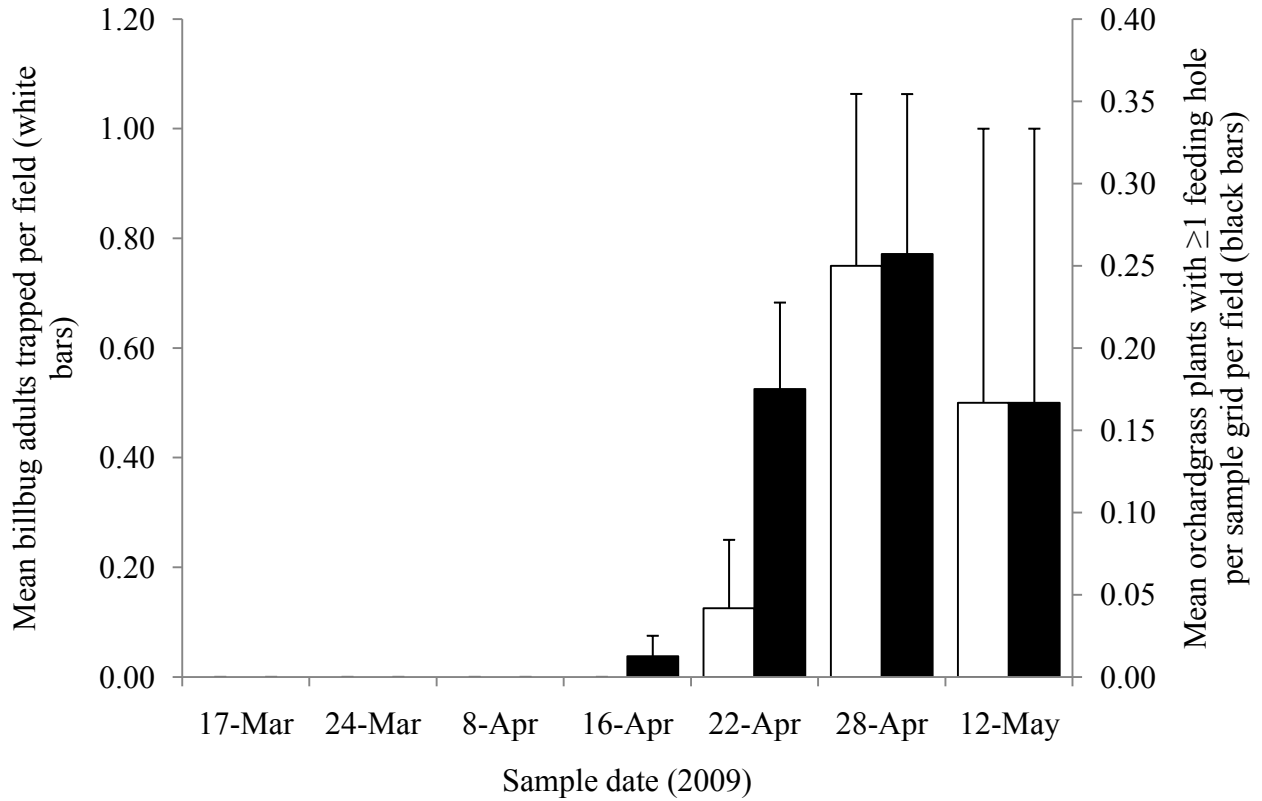
Figure 2.2. Mean (±SEM) number of bluegrass &/or hunting billbug adults collected per field (white bars), and mean (±SEM) number of orchardgrass plants with ≥1 billbug adult feeding holes per 20.3-cm (8-in) square grid per field (black bars) by sample date in a 2009 study of 8 orchardgrass fields in Virginia.
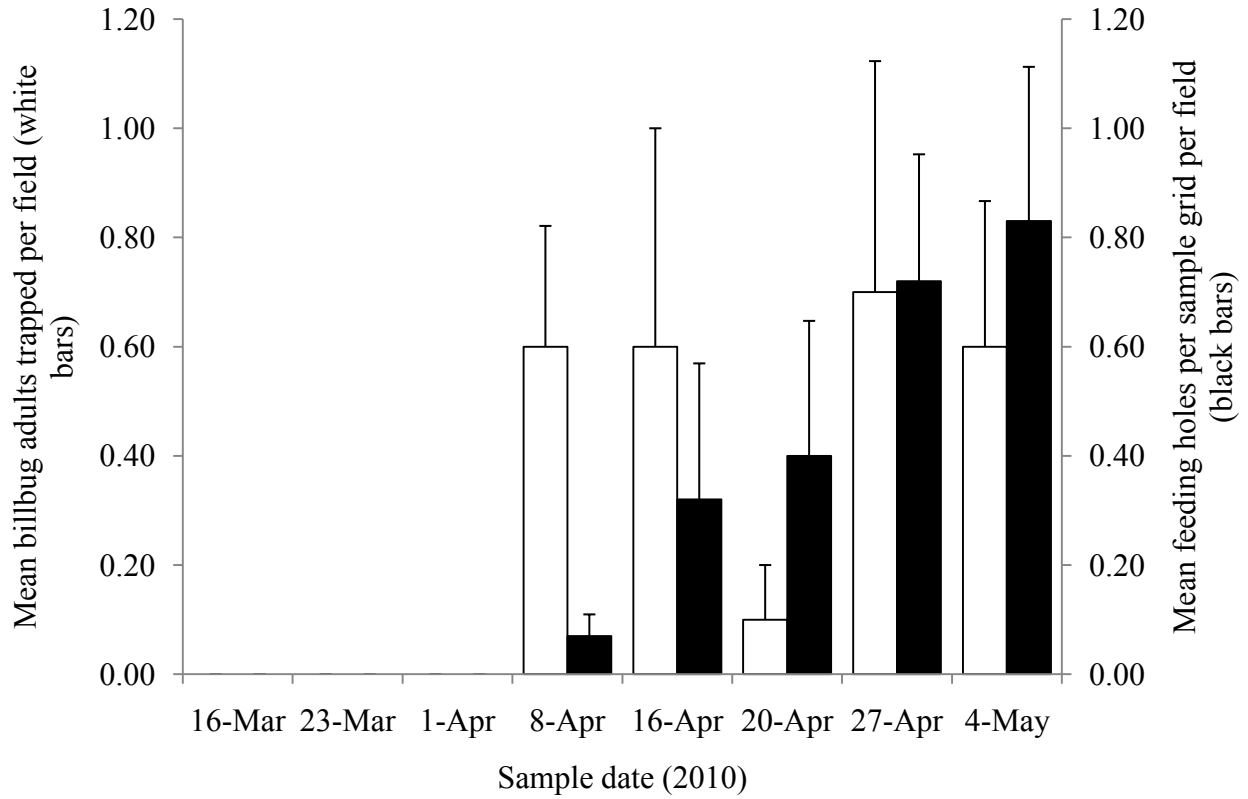
Figure 2.3. Mean (±SEM) number of bluegrass &/or hunting billbug adults collected per field (white bars), and mean (±SEM) number of billbug adult feeding holes per 20.3-cm (8-in) square grid per field (black bars) by sample date in a 2010 study of 10 orchardgrass fields in Virginia.

Table 2.2. Abundances of billbug species adults collected in barrier pitfall traps in a two-season field survey of orchardgrass in northern Virginia

Billbug species[a]	# adults found in 2009	# adults found in 2010
Sphenophorus parvulus Gyllenhal (bluegrass billbug)	8	23
S. venatus vestitus Chittenden (hunting billbug)	3	3
S. callosus (Olivier) (southern corn billbug)	11	12
S. immunis (Say)	1	0
S. minimus Hart (lesser billbug)	2	7
S. zeae Walsh (Timothy billbug)	0	1
S. sp.	1	0

[a] Representative specimens were determined by J. Prena (Coleoptera: Curculionidae), Systematic Entomology Laboratory, Agriculture Research Service, USDA (2010).

Table 2.3. Comparison of the first sample dates on which billbug adult feeding holes were detected and on which bluegrass &/or hunting billbug adults were captured in barrier pitfall traps in a field survey of orchardgrass in northern Virginia over two seasons (spring 2009, 2010).

Year	Field #	Billbug adult feeding holes 1st detected[a]	Billbug adults 1st trapped[a]	Difference[b] (days)
2009	1	-	-	-
	2	22-Apr	12-May	+20
	3	28-Apr	28-Apr	0
	4	22-Apr	-	-
	5	22-Apr	22-Apr	0
	6	16-Apr	28-Apr	+12
	7	22-Apr	-	-
	8	22-Apr	28-Apr	+6
2010	1	-	-	-
	2	20-Apr	-	-
	3	8-Apr	8-Apr	0
	4	20-Apr	8-Apr	-12
	5	8-Apr	8-Apr	0
	6	20-Apr	8-Apr	-12
	7	27-Apr	-	-
	8	16-Apr	-	-
	9	20-Apr	27-Apr	+7
	10	8-Apr	8-Apr	0

No significance was found in the difference between the first dates that feeding holes were detected and adults were trapped for 2009 only ($Z = 3.00$, $P = 0.25$; Wilcoxon signed-rank test (Steel and Torrie 1980)), for 2010 only ($Z = -2.00$, $P = 0.50$) or for both years combined ($Z = 2.50$, $P = 0.69$).

[a] Fields were checked in 2009 on 16,23 March, 8, 16, 22, 28 April, and 12 May; and in 2010 on 17, 24 March, 8, 16, 20, 27 April, and 4 May.

[b] A positive (+) number indicates feeding holes were detected prior to billbugs being trapped; a negative (-) number indicates billbugs were trapped before feeding holes were detected; a zero (0) indicates both were found on the same sample date.
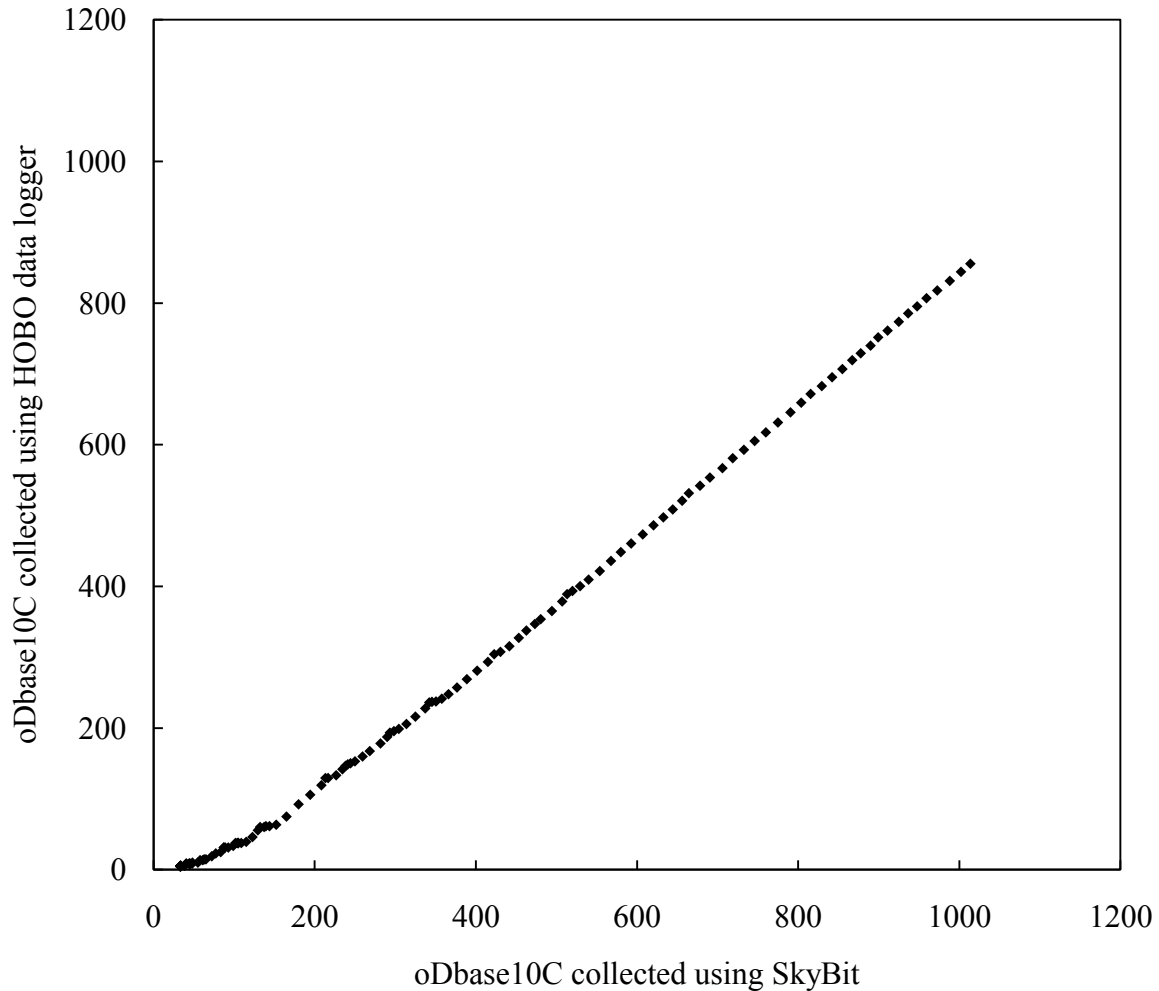
Figure 2.4. Relationship comparison of two degree-day ($^{o}D_{base10C}$) collection methods for 11 March – 14 July 2009: an Onset HOBO® UA-001-64 (HOBO) temperature data logger in an orchardgrass field on Lazy Lane Farm, Upperville, VA (degree-days calculated using Average Method (Shetlar 1991, Watschke et al. 1995)), and SkyBit E-Weather® service (SkyBit) for a grassy field at the Fauquier County Fairgrounds, VA (degree-days calculated using Modified Arithmetic Method (J. Schlegel, personal communication; McMaster and Wilhelm 1997)). A strong relationship is shown by the coefficient of correlation ($r = 0.9985$ (Steel and Torrie 1980)).
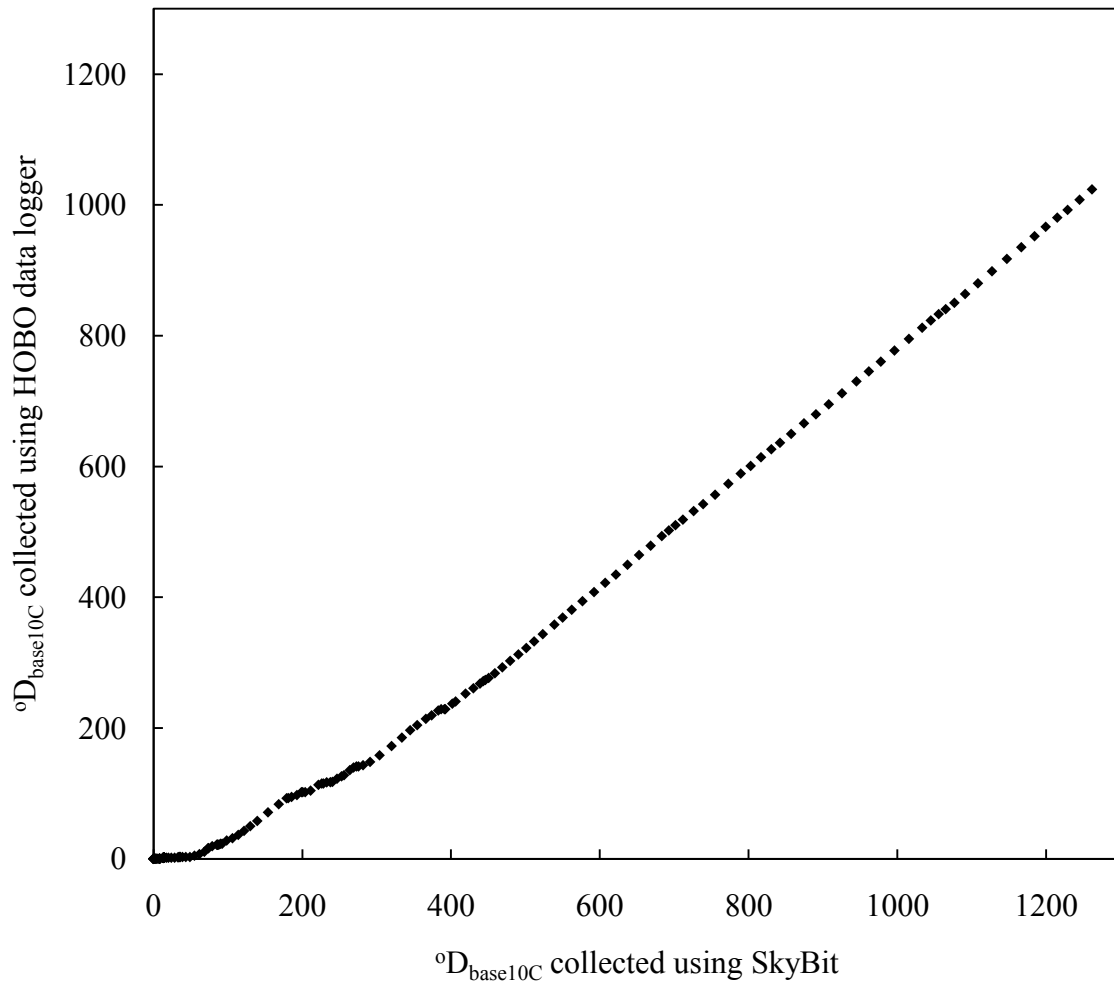
Figure 2.5. Relationship comparison of two degree-day ($^oD_{base10C}$) collection methods for 1 January – 14 July 2010: an Onset HOBO® UA-001-64 (HOBO) temperature data logger in an orchardgrass field on Lazy Lane Farm, Upperville, VA (degree-days calculated using Average Method (Shetlar 1991, Watschke et al. 1995)), and SkyBit E-Weather® service (SkyBit) for a grassy field at the Fauquier County Fairgrounds, VA (degree-days calculated using Modified Arithmetic Method (J. Schlegel, personal communication; McMaster and Wilhelm 1997)). A strong relationship is shown by the coefficient of correlation ($r = 0.9949$ (Steel and Torrie 1980)).

Discussion

Since 2006, this study has used barrier pitfall traps to survey for billbugs in orchardgrass fields in northern Virginia. Orchardgrass growers, however, find this method impractical for their own use. In addition, four non-target species of *Sphenophorus* were found in pitfall traps, of which only *S. minimus* has been reported to use orchardgrass as a host (Satterthwait 1931). While it is possible these other billbug species may feed on orchardgrass, it is more likely they were feeding on other plant species present in the fields at the time of this study. The presence of additional, non-target billbug species in pitfall traps increases the likelihood that pest managers will not identify the proper pest species, which may lead to a negative impact on decision-making.

The present study found no significant differences between the time when billbug adults are first trapped in orchardgrass fields and the time billbug adult feeding holes are first detected. These results suggest that surveying fields for feeding holes is an equally satisfactory indicator of the presence of spring billbug adults as the barrier pitfall traps. Using the paired-feeding holes scouting method should prove useful to growers and crop consultants because it does not require any insect identification skills. The grower or crop consultant should be able to determine immediately if billbug adult feeding activity is present in a field. In addition, it appears that the paired-feeding holes scouting method takes less time an effort compared to the barrier pitfall trap method.

In the barrier pitfall traps used in this study, the bluegrass billbug was the most abundant species captured. The hunting billbug was the fourth most abundant species after the southern corn billbug and the lesser billbug.

Data from the SkyBit system, along with Watschke's degree-day model (1995), are currently being used by Virginia Tech to alert orchardgrass growers, extension agents and crop consultants to the best time to scout and treat for billbugs in orchardgrass. This system was selected because of its daily availability and ease of use; however, until now this automated system had never been checked against field-based temperature data. The results of comparisons of temperature data from the SkyBit system to a field-based HOBO data logger showed high coefficients of correlation, indicating a close relationship between the two systems.

Although unpredictable weather could cause the number of degree-days that occur between 1January and 1 March to fluctuate from year to year, the number of degree-days accumulated for 1 January – 1 March 2010 was miniscule. This suggests that, barring uncharacteristically warm weather in January and February, there may be little difference in the use of a 1 January or 1 March start-date for the Watschke et al. bluegrass billbug degree-day model (1995).

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Chapter 3: Efficacy of field-border trials using an entomopathogenic fungus against billbugs in Virginia orchardgrass

Introduction

Over the last 60 years, two billbug (Coleoptera: Curculionidae) species have become important pests of orchardgrass in northern Virginia; the bluegrass billbug (*Sphenophorus parvulus* Gyllenhal) was first noted as a Virginia pest in 1953 (Turner 1955), while damage from the hunting billbug (*S. venatus vestitus* Chittenden) wasn't reported until 2005 (R.R.Y., unpublished data). The National Agricultural Statistics Service reported for 2008 that 477,500 ha (1,180,000 ac) of grass hay, excluding alfalfa, were harvested in Virginia with an estimated production value of $354,354,000 (NASS 2009). Orchardgrass can be conservatively estimated to make up 35%, or $124,023,900, of the total production value of grass hay for the commonwealth, making control of billbug pests a serious concern for growers and extension services to prevent losses.

Studies of the bluegrass and hunting billbugs have primarily occurred in turfgrass. However, findings by Turner (1955), regarding the bluegrass billbug, and Kamm (1969), concerning *S. venatus confluens* Chittenden, a sister subspecies of the hunting billbug, have shown that the life history of these species is similar in orchardgrass. Adults emerge from overwintering sites under debris and vegetation (Bruner 1890, Forbes 1905, Tashiro 1987, Watschke et al. 1995) in late March to early April, as the weather begins to warm (Watschke et al. 1995), and begin searching for suitable host plants. Once a host has been located, adults feed for a short time (Tashiro 1987) and then the females lay eggs within the base of the plant. After 6 – 7 days, the eggs hatch (Smith 1913, Watschke et al. 1995) and the early instars feed inside the stems. As the larvae grow, they move into the soil to feed within and outside of the crowns

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and roots of the host (Webster 1892, Satterthwait 1932). Larval feeding lasts for 23-60 days (Smith 1913, Satterthwait 1932, Watschke et al. 1995), and is then followed by pupation in the soil, close to the host plant (Webster 1892, Forbes 1902). New adults emerge in 8 – 10 days (Smith 1913, Satterthwait 1932, Watschke et al. 1995).

The entomopathogenic fungus, *Metarhizium anisopliae* (Metschnikoff) Sorokin strain F52 (Met-52), has shown promise for controlling billbugs. *M. anisopliae* is a green muscardine fungus occurring naturally in the soil and is characterized by the green color of its conidia (Zimmermann 2007) (Figure 3.1). Met-52 is manufactured by Novozymes (Salem, VA) and infects a narrow range of insects upon contact with the cuticle of the host. The U.S. Environmental Protection Agency (2008) defines its host range as "primarily affecting coleopterans of the families Elateridae and Curculionidae." Met-52 is not currently labeled for use on grass hay and pasture.

The objective of this study was to determine whether an application of the Met-52 fungus in treated and untreated small plots around part of a border of an orchardgrass field could cause infection of billbug adults as they reenter the field. The ovipositing adult in early spring is the best target for a billbug control program in orchardgrass. A border treatment may be just as effective for controlling those adults as an entire-field treatment, while greatly reducing the cost of treatment and the amount of insecticide application.

Figure 3.1. Bluegrass billbug adult infected with *Metarhizium anisopliae* strain F52, showing the green conidia emerging from the cuticle of the host. Black bar equals 1 mm. Photograph by W.R. Kuhn.

Materials & Methods

Small plot trials were installed in an orchardgrass field at Grasslands Farm (The Plains, VA) in the spring of 2009, and at Lazy Lane Farm (Upperville, VA) in the spring of 2010. Each trial consisted of randomized pairs of adjacent Met-52 treated and untreated plots, with a barrier pitfall trap installed within or near each plot. The Met-52 used in this study contained 5×10^9 conidia per gram, and was provided by Novozymes (Salem, VA). It was kept at $3 - 5^{o}C$ (37 – $41^{o}F$) until use.

In 2009, the trial was placed a 3 m inside the edge of a 6.0-ha (14.9-ac) field of 'WP300' orchardgrass, parallel to a rock wall. Eight randomized pairs of plots were installed. Plots were 3.0 m long by 1.6 m wide (10 ft × 6 ft), and were spaced 3.0 m from one another. The plot pairs were placed end to end and spaced 1.5 m (5 ft) from each other. A barrier pitfall trap was installed 0.3 m (1 ft) inside the field from each plot in the manner described in Laub et al. (2008). These traps used an aluminum-flashing barrier to divert surface-dwelling insects to pitfall traps on either side. Traps were oriented so that the barrier was parallel to the rock wall. Soapy water was used as a killing solution, as it was assumed that standard killing solutions, such as ethanol or ethylene glycol, would interfere with Met-52 infection of billbug adults. It was applied on 31 March 2009 using a CO_2-powered backpack sprayer equipped with four 8002 EVS stainless steel spray tips, calibrated to deliver 187 L/ha (20 gpa) at 276 kPa (40 psi), at a rate of 29.6 ml (1.5 × 10^{11} conidia) per 92.9 m^2 (1 fl oz per 1,000 ft^2).

The 2010 trial was installed in a 3.6-ha (8.9-ac) field of 'Benchmark Plus' orchardgrass. Plots were widened to 5.5 m wide (18 ft), and members of each plot pair were placed directly adjacent to one another. Six randomized pairs were used and were spaced 1.5 m (5 ft) from each other. Met-52 was applied at a rate of 59.1 ml (3.0 × 10^{11} conidia) per 92.9 m^2 (2 fl oz per 1,000

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ft^2) on 9 April. Soapy water was again used as a killing solution. Because Met-52 is not labeled for application on orchardgrass, the Met-52 treated grass was cut and destroyed following each of the trials.

Billbug adults were collected from barrier pitfall traps on four samples dates from 2009 and four dates from 2010. Traps were checked weekly for billbug adults from 16 April – 12 May 2009, except for two sample dates where data were not taken due to inclement weather, and from 16 April – 4 May 2010. On each sample date, billbug adults were collected and placed in sample cups filled with soapy water, and traps were refilled with fresh soapy water. Sample cups were then placed cold cooler, and transported to the laboratory at Virginia Tech. There, billbugs were identified to species. The collection location and date, and billbug species were recorded for each plot on each sample date.

Specimens from 2009 were checked for Met-52 infection by J. Leland at Novozymes. I checked the 2010 specimens for Met-52 infection. The 2009 specimens were sent to Leland in a cold cooler, and were then surface sterilized (Lacey and Kaya 2000), plated on a sterile growth medium and observed for Met-52 sporulation. I used the following procedure to check the 2010 billbug adults for infection. Specimens were surface sterilized as in the previous year and each specimen was placed on sterile filter paper in a Petri dish. Approximately 0.5 ml of distilled water was applied to the paper, then the dishes were closed and sealed with Parafilm (Pechiney Plastic Packaging Company, Chicago, IL). The dishes were placed in a growth chamber, which was kept at 20°C (68°F) with a photoperiod of LD 13:11 and an average RH of 90%. Samples were checked every 2-3 days for Met-52 sporulation, and distilled water was added as needed to keep the paper moist. If Met-52-like sporulation was observed on a specimen, it was recorded and sent to Leland to be confirmed as Met-52; otherwise samples were discarded after June 16.

In addition to the billbug specimens from the trial, 3 live bluegrass billbug adults were hand collected from a field on Grasslands Farm (The Plains, VA) on 27 April 2010. These billbugs were each surface-sterilized and placed in Petri dishes, as above. One dish was treated with 0.5 ml distilled water; one with 0.5 ml of a 1:58.77 solution of Met-52 and distilled water (equivalent to 1 oz Met-52 per 1,000 ft^2 at 187 L/ha), and the last with 1:29.38 solution of Met-52 and distilled water (equivalent to 2 oz Met-52 per 1,000 ft^2 at 187 L/ha). The specimens were then checked for sporulation according to the aforementioned procedures.

Results

In 2009, 21 bluegrass billbug adults were collected from barrier pitfall traps over 3 of the 4 sample dates; 12 of those came from control plots and 9 from Met-52 treated plots. Eleven hunting billbug adults were collected over all 4 of the sample dates; 7 and 4 of those were found in control and treated plots, respectively.

In 2010, 5 bluegrass billbugs were trapped over 3 sample dates; 3 came from control plots, while 2 were from treated plots. Nine hunting billbugs, 5 from control plots and 4 from treated plots, were trapped over all 4 sample dates.

No billbug specimens from 2009 were found to be infected with Met-52. In 2010, Met-52 sporulation was seen on 1 hunting billbug adult, trapped on 4 May in an untreated plot. The specimen died 31 days after capture (4 June) and green Met-52 sporulation was first observed on the specimen after 36 days (9 June).

Of the additional bluegrass billbug adults captured on 27 April 2010, Met-52 sporulation was observed in the 2 billbugs that were treated with Met-52, but not in the specimen treated with distilled water only. Both treated specimens died 17 days after capture and Met-52 fungus was first observed on each of them after 31 days (28 May).

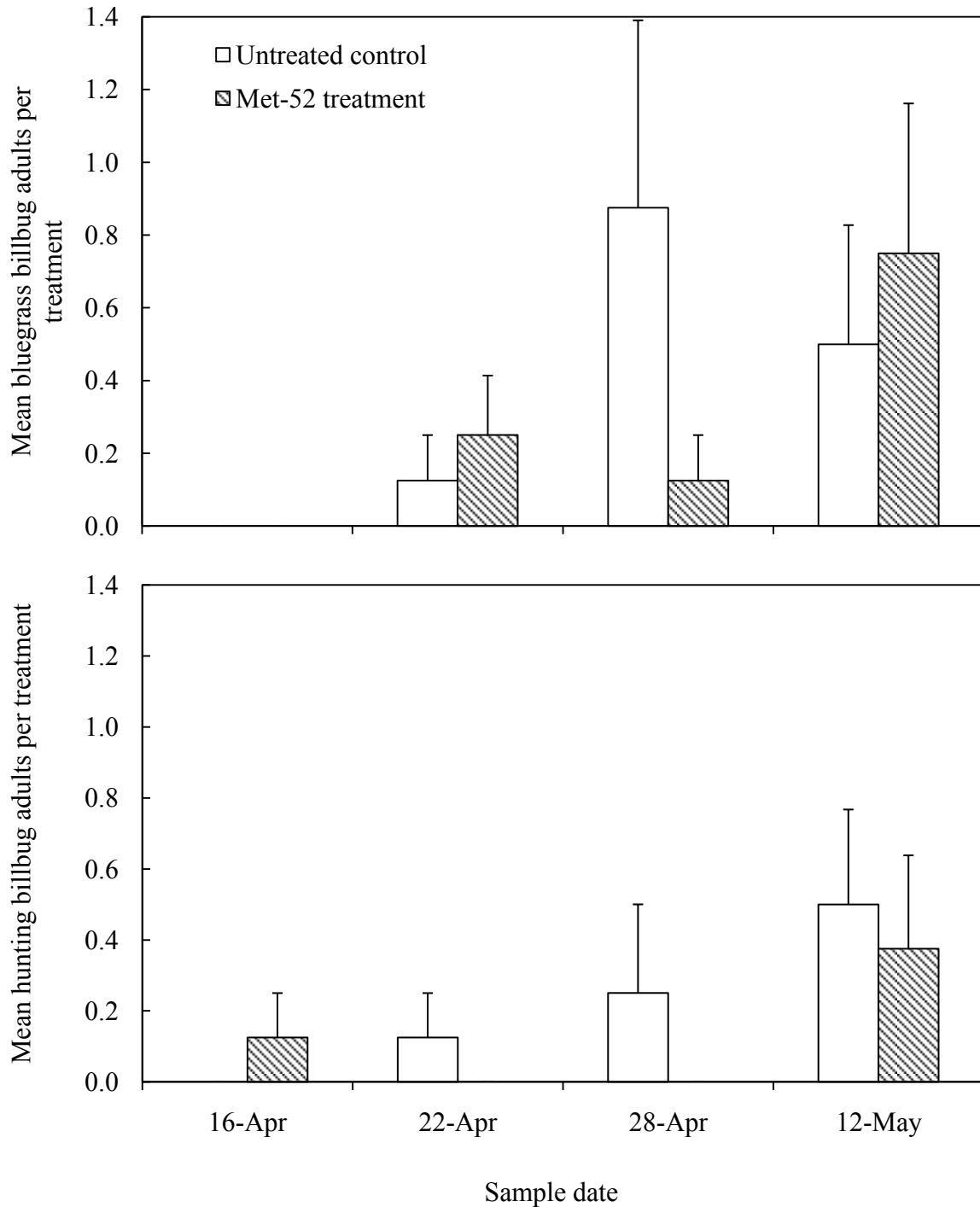**Figure 3.2.** Mean (±SEM) number of billbugs (n = 8 blocks) collected from barrier pitfall traps in a 2009 orchardgrass field border trial in northern Virginia against billbug spring adults. Treatments were applied on 31 March 2009. Met-52, *Metarhizium anisopliae* strain F52 (not labeled for use on orchardgrass). None of the billbug adults were infected with Met-52.

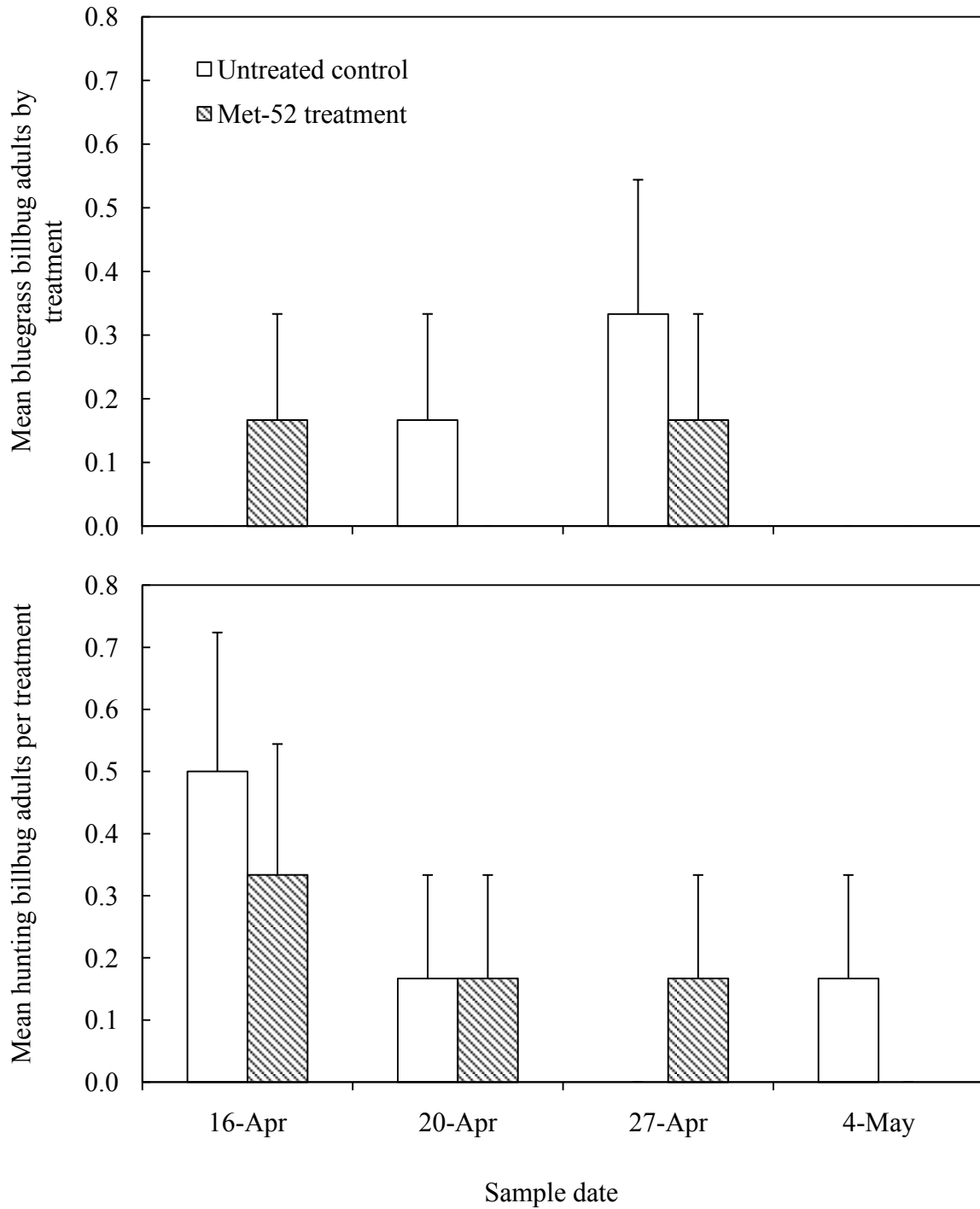**Figure 3.3.** Mean (±SEM) number of billbugs (*n* = 6 blocks) found in barrier pitfall traps in a 2010 orchardgrass field border trial in northern Virginia against billbug spring adults. Treatments were applied on 9 April 2010. Met-52, *Metarhizium anisopliae* strain F52 (not labeled for use on orchardgrass). One hunting billbug adult collected from an untreated control plot on May 4, 2010 was infected with Met-52.

Discussion

In this study, one hunting billbug from an untreated plot was found to be infected with Met-52. Infection was also found in two bluegrass billbug adults that were hand-inoculated with Met-52 spores. These observations show that it is possible for the Met-52 to infect hunting and bluegrass billbug adults. However, it is likely that the orchardgrass foliage present at the time of Met-52 application interfered with the number of spores to which the soil-surface dwelling billbug adults were exposed.

Met-52 did not perform satisfactorily as an infection agent of billbug adults in orchardgrass in either year of this study. However, a field-border application using a conventional insecticide may provide economic control of billbug adults, while at the same time markedly reducing the amount of insecticide applied to a field in addition to the costs associated with applying an insecticide to the entire field.

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   anisopliae*. Biocontrol Sci. Technol. 17: 879 - 920.

Chapter 4: Efficacy of selected insecticides and an entomopathogenic fungus against billbug adults in orchardgrass grown in Virginia

Introduction

Over the last 60 years, two *Sphenophorus* spp. (Coleoptera: Curculionidae) have become important pests of orchardgrass in Virginia: the bluegrass billbug (*Sphenophorus parvulus* Gyllenhal) and the hunting billbug (*S. venatus vestitus* Chittenden). Turner (1955) first reported that the bluegrass billbug was damaging orchardgrass in Virginia. The hunting billbug was not known to be a pest on orchardgrass in Virginia until 2005 (R.R.Youngman, unpublished data). In 2008, approximately 441,107 ha (1,090,000 ac) of grass hay, excluding alfalfa, were harvested in Virginia with an estimated production value of $302,148,000 (NASS 2010). Assuming conservatively that orchardgrass makes up 35% of the grass hay harvested in Virginia, its value for the commonwealth would be $105,751,800, making control of billbug pests a critical concern for growers and those who manage these pests.

Although studies of the bluegrass and hunting billbug have primarily taken place in turfgrass, their life histories appears to be similar in orchardgrass, as reported by Turner (1955) for the bluegrass billbug, and Kamm (1969) for a sister subspecies of the hunting billbug, *S. venatus confluens* Chittenden. As the weather warms in late March to early April, billbug adults emerge from overwintering places under vegetation and debris, and walk to find suitable host plants (Bruner 1890, Forbes 1905, Tashiro 1987, Watschke et al. 1995). A short period of feeding (Tashiro 1987) is followed by egg laying within the stems of the host plant. The eggs hatch after 6 – 7 days (Smith 1913, Watschke et al. 1995), and the new larvae begin to feed. Early instars feed internally within the stems, and as they grow, drop out to feed internally and externally on the crowns and roots of the plant (Webster 1892, Satterthwait 1932). The larvae

feed for 23 – 60 days (Smith 1913, Satterthwait 1932, Watschke et al. 1995), then pupate in a soil cell near the host plant (Webster 1892, Forbes 1902). Eclosion of new adults occurs after 8 – 10 days (Smith 1913, Satterthwait 1932, Watschke et al. 1995).

The billbug adult, early instar and later instar each cause a different type of feeding injury to the host plant. Adults feed at the base of the stem, causing characteristic holes in the leaves as they elongate (Forbes 1905, Satterthwait 1932, Kamm 1969). By feeding inside the stems, the early instar causes them to become brittle, hollow, and filled with sawdust-like frass (Turner 1955, Tashiro and Personius 1970). The later instar causes the most injury as it feeds on the crowns and roots of the plant, which is manifested as irregularly shaped patches of dead grass (Watschke et al. 1995).

The objective of this two year study was to determine the efficacy of selected insecticides and an entomopathogenic fungus against ovipositing billbug adults as they walk back into orchardgrass fields in the spring.

Materials & Methods

The insecticides that are currently registered for use on grass hay and pasture include one carbamate, carbaryl (Sevin 80 WSP®, Sevin SL®, and Sevin XLR Plus® by Bayer CropScience); three pyrethroids, beta-cyfluthrin (Baythroid XL® by Bayer CropScience), lambda-cyhalothrin (Karate® and Warrior II® by Syngenta Crop Protection, Inc.), and zeta-cypermethrin (Mustang Max® by FMC Corp.); and an organophosphate, malathion (Malathion 5® by Winfield Solutions) (Ware 1993, VDACS 2003, Youngman 2010). In addition, the entomopathogenic fungus, *Metarhizium anisopliae* (Metschnikoff) Sorokin strain F52 (Met-52) (Novozymes, Salem, VA), has shown promise as a biological insecticide, although it is not labeled for grass hay and pasture. This fungus predominantly infects elaterids and curculionids (EPA 2008) upon contact with the cuticle of its host.

An efficacy trial was installed in spring of 2009 in Fauquier County, VA and in spring of 2010 in Loudoun County, VA. The treatments in both trials were replicated four times and arranged in a randomized complete block design (RCBD) (Table 4.1). Treatments were applied using a CO_2-powered backpack sprayer equipped with four 8002 EVS stainless steel spray tips, calibrated to deliver 187 L/ha (20 gpa) at 276 kPa (40 psi).

The 2009 trial was installed at Grasslands Farm, The Plains, VA on 8 April in a 6.0 ha (14.9 ac) field containing a 4-5-yr old stand of 'WP300' orchardgrass. The trial site was situated approximately 6 m (20 ft) inside the edge the field and parallel to a rock wall. Plots were 2.44 m long by 1.83 m wide (8 ft × 6 ft). Blocks were placed end to end so that the whole trial was 48.80 m long by 1.83 m wide (160 ft × 6 ft).

The 2010 trial was installed at Lazy Lane Farm in Upperville, VA on 9 April in a 6.1-ha (15.0-ac) field containing a 1-year-old stand of 'Benchmark' orchardgrass. The trial site was situated approximately 15 m (50 ft) inside the edge of the field and parallel to a rock wall. Plots were 3.05 m long by 2.44 m wide (10 ft × 8 ft), and only the inner 1.83 m (6 ft) was treated along the width of each plot. Blocks were arranged so that the trial was 15.24 m long by 9.75 m wide (50 ft × 32 ft). Met-52 was removed as a treatment and Baythroid XL was added (Table 4.1).

The 2009 trial was assessed on 29 July at the site of the trial, while samples were taken from the 2010 trial on 22 July and assessed in a laboratory setting between 23 and 24 July. In each trial, two 20.3-cm square by 7.6-cm deep (8-in × 8-in × 3-in depth) samples were dug from the center of each treatment plot; samples were then checked for billbug life stages (larvae, pupae, and adults) and orchardgrass plants were checked for injury. In 2009, samples were placed on a trash bag on the ground and examined for billbug larvae, pupae, and adults, which were recorded, placed in 70% ethanol, and later identified. Orchardgrass plants were teased apart and checked for feeding injury, which was categorized into 3 types: injury to leaves and/or stems, injury to crowns, and injury to roots. The total number of plants and the number of plants with each type of injury was recorded, and the percentage of plants with each type of injury was calculated for each sample. Values from the two samples in each plot were averaged together. Hay from the Met-52 treatments was then removed and destroyed. Yield data were not taken.

In 2010, each sample was sealed in a plastic freezer bag and kept on ice in an ice chest to minimize decomposition. The samples were then assessed in a laboratory at Virginia Tech. Similar to the 2009 study, billbug larvae, pupae, and adults were recorded, placed in 70% ethanol, and later identified. Because it was determined during assessment of the 2009 trial that crowns and roots were sometimes difficult to differentiate, only two plant injury categories were

used for the 2010 trial assessment: injury to leaves and/or stems, and injury to crowns and/or roots. The same calculations from the 2009 data were then made for the 2010 data.

  Statistics. All data were transformed using a square root transformation ($x' = (x + 0.5)^{1/2}$) for billbug life stage count data, and an arcsine transformation ($x' = \arcsine(x^{1/2})$) for plant injury data (Little and Hills 1978). A two-way ANOVA and Fisher's LSD ($\alpha = 0.05$) were used to analyze for differences among treatments in billbug counts and plant injury (Ott and Longnecker 2008).

Table 4.1. Treatments used in 2009 and 2010 orchardgrass field efficacy trials against billbugs in Virginia

Treatment (active ingredient)	Rate in 2009 trial		Rate in 2010 trial	
Untreated control	-		-	
Baythroid XL (beta-cyfluthrin)	not applied		204.60 ml/ha	(2.8 fl oz/ac)
Mustang Max (zeta-cypermethrin)	292.29 ml/ha	(4.0 fl oz/ac)	292.29 ml/ha	(4.0 fl oz/ac)
Sevin XLR Plus (carbaryl)	3.51 L/ha	(1.5 qt/ac)	3.51 L/ha	(1.5 qt/ac)
Warrior II (lambda-cyhalothrin)	140.30 ml/ha	(1.92 fl oz/ac)	140.30 ml/ha	(1.92 fl oz/ac)
Metarhizium anisopliae (Metschnikoff) Sorokin strain F52[a]	3.18 L/ha	(43.56 fl oz/ac)	not applied	

[a] Material provided by Novozymes (Salem, VA); not labeled for use on orchardgrass.

Results

In the 2009 trial, the plant injury data in two of the four blocks were not used due to a sampling error. However, the billbug life stage count data for all blocks were used for analysis.

In the 2009 trial, 9 billbug larvae, 0 billbug pupae, 1 hunting billbug adult, and 1 *S. callosus* (Olivier) adult were found. The number of larvae per sample ($n = 4$) ranged from $0 - 2$ (mean = 0.23), and adults per sample ($n = 4$) ranged from $0 - 1$ (mean = 0.05). No significant differences were detected in billbug life stages according to ANOVA (larvae, $F = 1.87$; df = 4, 12; $P = 0.18$; adults, $F = 1.00$; df = 4, 12; $P = 0.44$; total life stages, $F = 1.07$; df = 4, 12; $P = 0.41$) (Figure 4.1).

In 2009, the number of orchardgrass plants in the samples ($n = 4$) ranged from $1 - 16$ (mean = 6.70). The number of plants showing injury in the stems and/or leaves ranged from $0 - 5$ (mean = 0.95); plants with injury to the crown ranged from $0 - 3$ (mean = 1.10); and no plants were found with injury to the roots. Treatment means for injury to orchardgrass stems and/or leaves ranged from $6.3 \pm 2.0\%$ in the untreated control to $33.3 \pm 33.3\%$ in the Sevin XLR Plus treatment; and treatment means for crown injury ranged from $20.5 \pm 6.2\%$ in the untreated control to $33.3 \pm 0.0\%$ in the Sevin XLR Plus treatment. No significant differences were detected among treatments in injury to stems and/or leaves ($F = 0.27$; df = 4, 4; $P = 0.88$). However, the Sevin XLR Plus treatment showed significantly higher injury to crowns than all other treatments except Mustang Max ($F = 6.96$; df = 4, 4; $P = 0.04$) (Figure 4.2).

In the 2010 trial, 6 billbug larvae, 0 billbug pupae, 8 bluegrass billbug adults, 2 hunting billbug adults, and 1 *S. minimus* Hart adult were found. Overall, samples ($n = 4$) had between $0 - 2$ (mean = 0.92) billbug larvae and between $0 - 3$ (mean = 1.09) adults. No significant differences

were detected in billbug life stages according to ANOVA (larvae, $F = 1.87$; df = 4, 12; $P = 0.18$; adults, $F = 1.62$; df = 4, 12; $P = 0.23$; total life stages, $F = 1.11$; df = 4, 12; $P = 0.40$) (Figure 4.3).

In the 2010 trial, the number of orchardgrass plants with injury to stems and/or leaves ranged from 0 – 7 (mean = 2.36) in the samples ($n = 4$). Likewise the number of orchardgrass plants with injury to the crown and/or roots ranged from 0 – 10 (mean = 2.47). No significant differences were detected among treatments in injury to stems and/or leaves ($F = 0.01$; df = 4, 12; $P = 1.00$) or crowns and/or roots ($F = 0.84$; df = 4, 12; $P = 0.53$) (Figure 4.4).
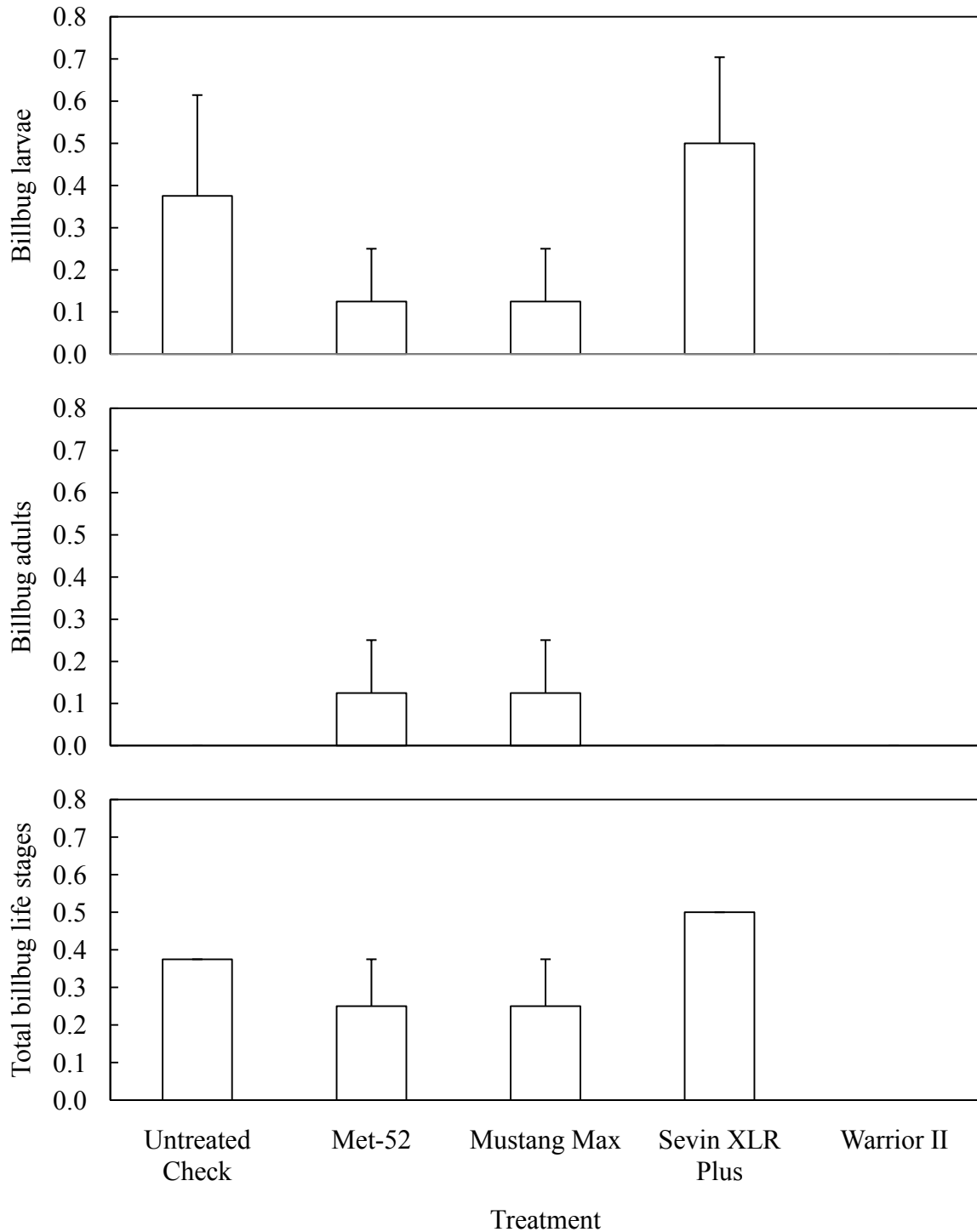
Figure 4.1. Mean (±SEM) number of billbug life stages per 20.3-cm square by 7.6-cm depth (8" × 8" × 3" depth) (*n* = 4 blocks) in a 2009 orchardgrass field efficacy trial in Virginia against billbugs. Treatments were applied 8 April 2009; no pupae were found. Met-52, *Metarhizium anisopliae* (Metschnikoff) Sorokin strain F52 (not labeled for use on orchardgrass). No significant differences were found between treatments ($P < 0.05$; two-way ANOVA of square-root transformed data (Little and Hills 1978)).
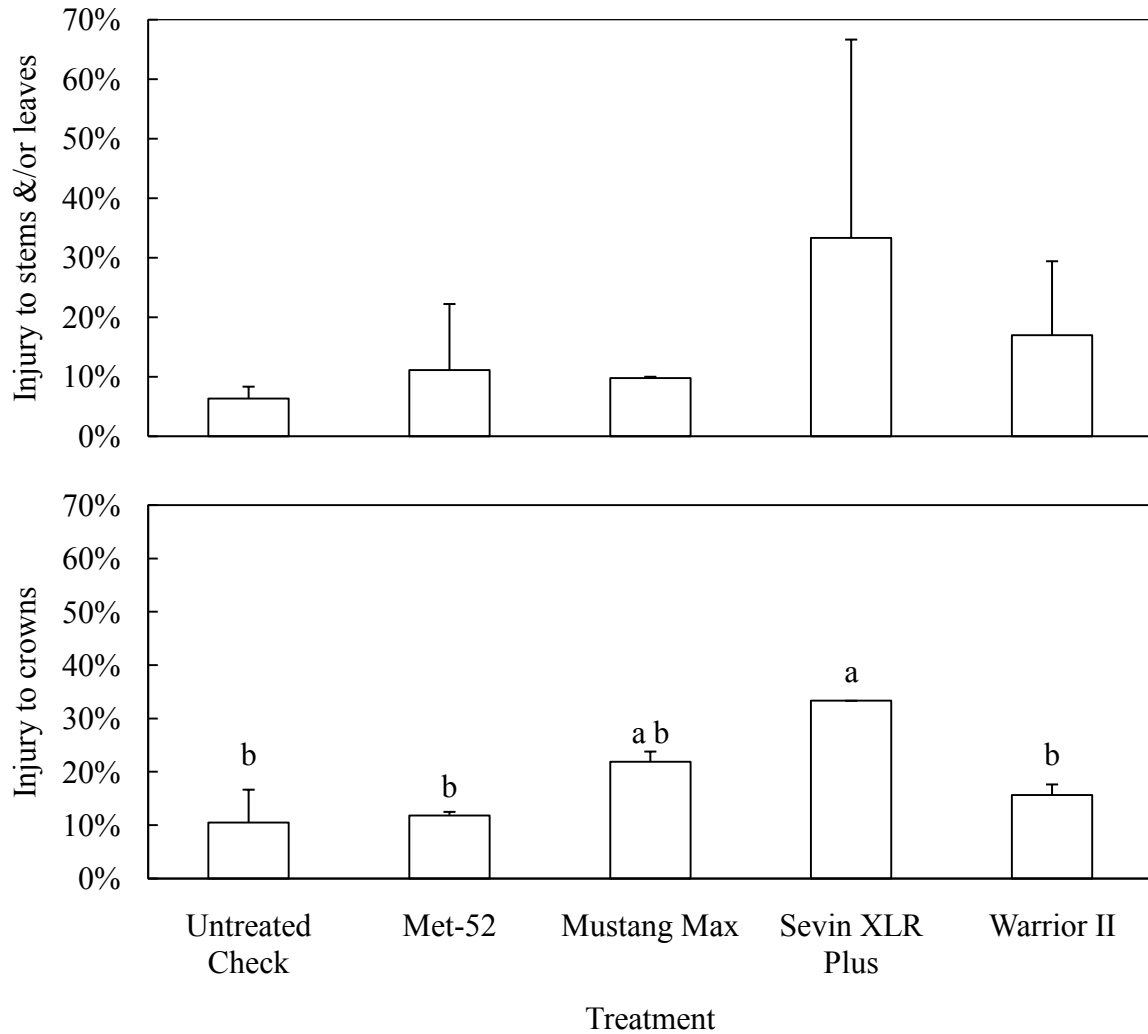
Figure 4.2. Mean (±SEM) percent of orchardgrass plants (*n* = 2 blocks) with different categories of injury (no root injury was found) from a 2009 orchardgrass field efficacy trial in Virginia against billbugs. Treatments were applied on 8 April 2009. Met-52, *Metarhizium anisopliae* (Metschnikoff) Sorokin strain F52 (not labeled for use on orchardgrass). No significance was found between treatments in injury to stems and/or leaves; for injury to crowns, bars with the same letter above them are not significantly different ($P < 0.05$; two-way ANOVA of arcsine transformed data (Little and Hills 1978) and Fisher's protected LSD).
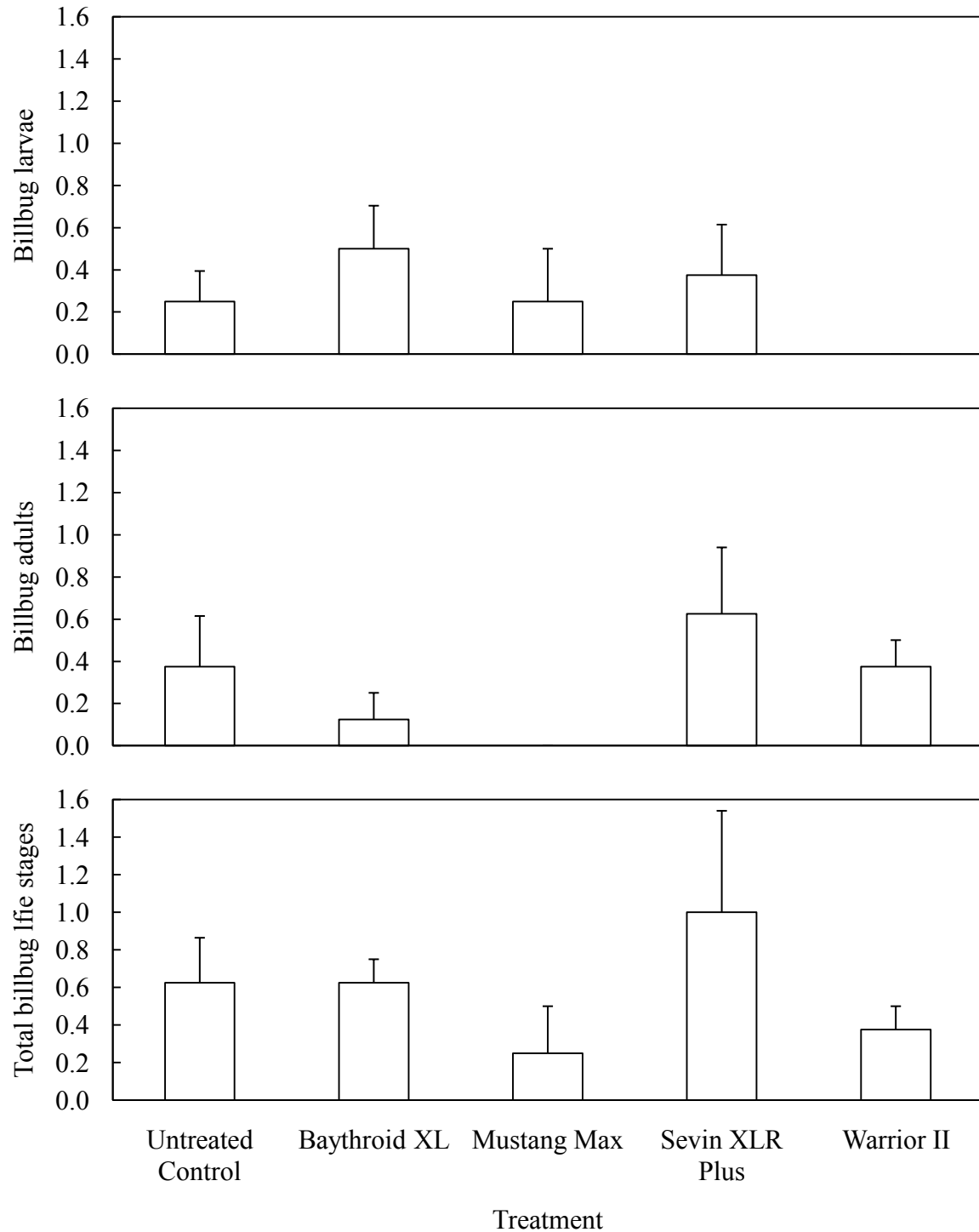
Figure 4.3. Mean (±SEM) number of billbug life stages per 20.3-cm square by 7.6-cm depth (8" × 8" × 3" depth) (*n* = 4 blocks) in a 2010 orchardgrass field efficacy trial in Virginia against billbugs. Treatments were applied 9 April 2010; no pupae were found. No significant differences were found between treatments (*P* < 0.05; two-way ANOVA of square-root transformed data (Little and Hills 1978)).
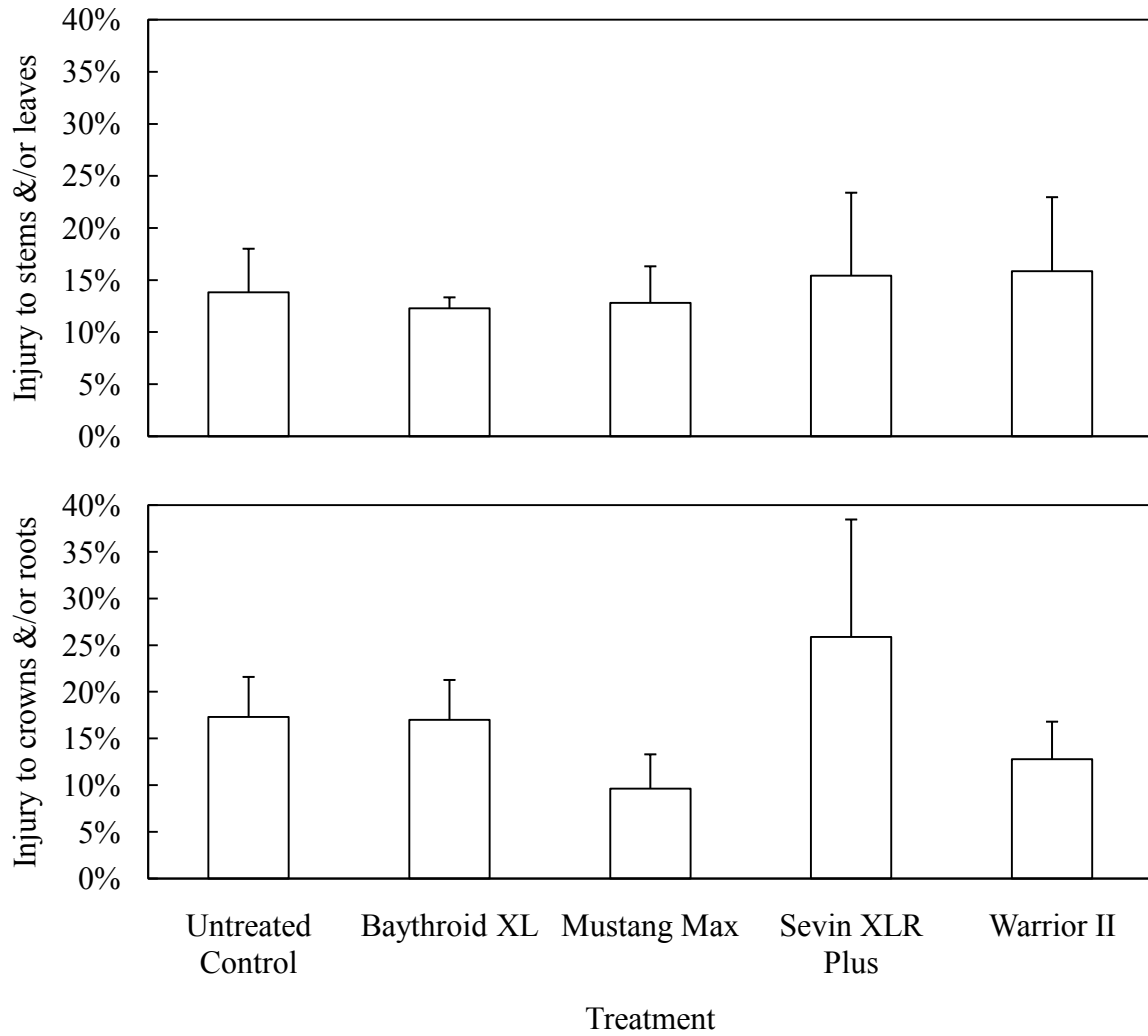
Figure 4.4. Mean (±SEM) percent of orchardgrass plants ($n = 4$ blocks) with different categories of injury from a 2010 orchardgrass field efficacy trial in Virginia against billbugs. Treatments were applied 9 April 2010. No significant differences were found between treatments ($P < 0.05$; two-way ANOVA of arcsine transformed data (Little and Hills 1978)).

Discussion

No significant differences were found between treatments in billbug life stage counts in either 2009 or 2010; however, Sevin XLR Plus performed the poorest of all treatments except Mustang Max in percentage of crown injury in 2009. Injury was not significantly different in any other category in 2009 or 2010. Of the insecticides labeled for grass hay and pasture, Warrior II is the only one to have billbugs included on its label; however, it is for suppression only (Youngman 2010).

The method for evaluating samples, described for the 2009 trial, where samples were dissected in the field on dark plastic by many samplers, has been used for several years in efficacy trials. In 2010, the same sampling technique was used, but the evaluation method was modified. All samples were placed in individual bags in an ice chest and taken back to a lab at Virginia Tech. Within 48 hr, all samples were carefully sifted through and broken apart to detect plant injury and billbug life stages. The latter method proved to be more thorough in regards to the number of billbug life stages found, and in better assessment of injury to orchardgrass. Additionally, the possibility of sampling error was minimized by having fewer samplers.

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Chapter 5: Summary

The bluegrass billbug (*Sphenophorus parvulus* Gyllenhal) and hunting billbug (*Sphenophorus venatus vestitus* Chittenden) (Coleoptera: Curculionidae) are important pests in orchardgrass (*Dactylis glomerata* L.). The adults of these species walk into orchardgrass fields in the spring from nearby overwintering sites to feed and lay eggs (Bruner 1890, Forbes 1905, Tashiro 1987, Watschke et al. 1995), beginning the next generation of these pests. The time between when billbug adults first begin to enter the fields and before peak egg laying has occurred is a crucial point for billbug control in orchardgrass. At this time, the adults are exposed to insecticides. However, once eggs are laid, they are protected from insecticides within the plant, as are the subsequent larvae and pupae protected in the plant and surrounding soil. My project focused on the three tenets of billbug pest management in orchardgrass: timing of scouting and control, scouting methods, and control options.

First, scouting and control methods are useless unless they are properly timed to coincide with important events in the life history of a pest. Currently, degree-days from the SkyBit E-Weather® service and a degree-day model (Watschke et al. 1995) designed for the bluegrass billbug are used to alert growers and other interested parties of the proper timing for billbug scouting and control practices. My study compared the SkyBit degree-day data to field collected data taken from orchardgrass fields. I found that these two collection methods followed each other very closely, indicating that SkyBit can continue to be used in the billbug-orchardgrass notification.

Furthermore, in any proper pest management strategy, the target pest must be identified in the crop system. Barrier pitfall traps (Laub et al. 2008) are currently recommended for billbug scouting in orchardgrass. However, growers find these traps tedious to install and maintain. I

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found four additional billbug species occurring in orchardgrass in Virginia, which may be easily confused with the target billbug species. The presence of these additional species could cause growers and crop consultants to misidentify billbugs present in barrier pitfall traps. I found that a novel method, scouting for feeding holes on orchardgrass leaves caused by the billbug adults (Kamm 1969), was an equally good indicator of the presence of billbugs in orchardgrass as using barrier pitfall traps. This new scouting method is easy to perform, provides instant results, and can be immediately implemented on farms where orchardgrass is grown.

Finally, control options must be explored to find the best and most efficient strategy for controlling a particular pest in a particular system. In this project, a field-border treatment of *Metarhizium anisopliae* (Metschnikoff) Sorokin strain F52 (Met-52), an entomopathogenic fungus, was tested against billbug adults as they walk into orchardgrass fields in the spring. Met-52 did not perform adequately to control billbugs in this study. However, the field-border treatment concept, which would require less insecticides compared to an entire-field application, thereby reducing costs, should be considered in future studies using labeled insecticides. Additionally, several labeled insecticides and Met-52 were tested against billbugs in efficacy trials in orchardgrass. Although little impact was demonstrated by the insecticidal agents used in this study, a better method for assessing the trial samples was developed.

This project impacts billbug pest management for orchardgrass in the following ways. The timing system used to determine when to scout and control for billbugs has been verified. A novel scouting method has been developed and tested against the current scouting method. The concept for a new control method, field-border treatment, has been developed and should be tested using labeled insecticides. The traditional assessment method for testing insecticidal control has been improved upon.

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